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Desarrollo de un Modelo Predictivo para la Incidencia de Demencia en Adultos Mayores con Diabetes Mellitus tipo 2

TESIS

QUE PARA OPTAR POR EL GRADO DE
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Dedicatorias

A mis padres Perfecto y Lucía y mi hermana Aymara. Por su cariño incondicional, infinita paciencia e indicional apoyo. Por formarme y apoyarme en mi crecimiento, en mis planes profesionales y personales. No entiendo lo que fui, soy y seré sin ellos.

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Resumen

La diabetes mellitus tipo 2 (DM2) es una enfermedad crónico-degenerativa de presentación clínica heterogénea caracterizada por diversos fenotipos metabólicos que a largo plazo devienen en el desarrollo de complicaciones. La DM2 se ha asociado con en el desarrollo de demencia aunque los mecanismos fisiopatológicos y los factores de riesgo que favorecen su desarrollo no están completamente descritos. Considerando que la mayor parte de los factores de riesgo asociados a demencia no son modificables, los esfuerzos en su estudio se han enfocado en la identificación de casos para desacelerar la progresión de la enfermedad. A pesar de que previamente se han desarrollado puntajes de riesgo, el control de los factores predictores no ha demostrado tener beneficios significativos en la reducción de riesgo.
El objetivo general de este proyecto fue el desarrollar modelos predictores de la incidencia de demencia por cualquier causa, así como de las complicaciones metabólicas asociadas que sea específico para pacientes con DM2 en pacientes adultos mayores con DM2. En el transcurso del rpoyecto doctoral se desarrollaron herramientas para evaluar diferentes fenotipos metabólicos asociados con alteraciones neurológicas y cognitivas en pacientes con DM2 en diferentes grupos de estudio con perfiles de riesgo muy diversos. Además, se establecieron colaboraciones para el desarrollo de modelos utilizando datos prospectivos de estudios longitudinales de envejecimiento poblacional para el desarollo de una nueva propuesta para la predicción del riesgo de demencia en DM2.
Los objetivos específicos del proyecto fueron:

- Desarrollar herramientas metabólicas útiles para la predicción de desenlaces adversos en pacientes con DM2 para su aplicación en estudios epidemiológicos.
- Validar el modelo de predicción de demencia específico para DM2 previamente desarrollado, así como el fenotipo que caracteriza a los pacientes tamizados por ésta herramienta.
- Identificar factores de riesgo asociados a la incidencia de demencia en pacientes adultos mayores con DM2.
- Desarrollar un modelo integral para la predicción para la incidencia de demencia, específico para pacientes con DM2.

Para alcanzar los objetivos específicos se siguió la siguiente estrategia:

1. Se desarrollaron herramientas para la evaluación de resistencia a la insulina y tejido adiposo visceral comparado con mediciones altamente específicas para su aplicación en estudios epidemiológicos a bajo costo.

2. Se realizó una validación fenotípica de la herramienta de riesgo *Diabetes-Specific Dementia Risk Score* (DSDRS, por sus siglas en inglés) para identificar factores relacionados al riesgo de demencia en población mexicana.

3. Se realizó un modelaje de riesgo con las herramientas desarrolladas para identificar factores diferenciales asociados a demencia incidente en sujetos con y sin DM2 en al cohorte de las 3 ciudades (3C) de Bordeaux, Francia.

4. Finalmente, se evaluaron los factores de riesgo de forma simultánea en la población completa de sujetos con DM2 de las 3 ciudades del estudio 3C para la identificación de factores predictores.

Se concluyó que una menor proporción de masa magra, la obesidad visceral caracterizada por acumulación de tejido adiposo visceral, la enfermedad vascular cerebral, la discapacidad motora y el haplotipo para riesgo de demencia en APOE-ϵ4 se asociaban con un mayor riesgo de demencia incidente a tras 14 años de seguimiento en el estudio 3C. Una mayor escolaridad, sexo femenino y el desarrollo de actividad física se asociaban con un menor riesgo de demencia incidente en DM2. Las herramientas desarrolladas permiten la evaluación de riesgo de fenómenos metabólicos y dianas con potencial para su intervención. Es necesaria la realización de estudios independientes para evaluar el efecto de la modificación de los factores de riesgo identificados.

Abstract

Type 2 diabetes mellitus (T2D) is a chronic degenerative disease with a heterogeneous clinical presentation characterized by various metabolic phenotypes that in the long term lead to the development of complications. T2D has been associated with an increased risk of incident dementia, although the pathophysiological mechanisms and risk factors that favor its development are not completely understood. Considering that most of the risk factors associated with dementia are non-modifiable, the efforts in their study have focused on the identification of cases to slow the disease progression. Although diabetes-specific dementia risk scores have previously been developed, the control of the identified predictive factors has not had specific benefits in risk reduction.

The general objective of this project was the development of predictive models of any-cause dementia incidence, as well as the associated metabolic complications that are specific for patients with T2D in older adults with T2D. During this doctoral project, we developed tools to evaluate different metabolic phenotypes associated with neurological and cognitive alterations in patients with T2D in different study settings with very diverse risk profiles. In addition, collaborations were established for the development of predictive models using prospective data from longitudinal studies of population aging for the development of a new proposal for the prediction of dementia risk in T2D.

The specific objectives of the project were:

- To develop metabolic tools useful for predicting adverse outcomes in patients with T2D for application in epidemiological studies.
- To validate the previously developed T2D-specific dementia risk prediction model as well as the phenotype that characterizes patients screened as having high risk by this tool.
- To identify risk factors associated with the incidence of dementia in older adults with T2D.
- To develop a comprehensive model for predicting the incidence of dementia, specific for patients with T2D.

To achieve these specific objectives, we had the following strategy:

1. We developed tools for the evaluation of insulin resistance and visceral adipose tissue compared to gold-standard measurements for application in epidemiological studies at low cost.

2. We carried out a phenotypic validation of the *Diabetes Specific Dementia Risk Score* (DSDRS) to identify factors related to dementia risk in Mexican population.

3. We performed risk modeling using with the developed metabolic tools to identify differential factors associated with incident dementia in subjects with and without T2D in the 3-city study (3C) of Bordeaux, France.

4. Finally, risk factors we evaluated all identified risk factors simultaneously in the entire population of subjects with T2D from the three cities of the 3C study for identification of predictive factors for incident dementia.

We concluded that lower lean mass, visceral obesity characterized by accumulation of visceral adipose tissue, cerebral vascular disease, motor disability and positivity for the haplotype for risk of dementia in APOE-ϵ4 were associated with a higher risk of incident dementia after 14 years of follow-up in the 3C study. More years of schooling, female sex and performing physical activity were associated with a lower risk of incident dementia incident in T2D. The developed tools allowed for risk assessment of different metabolic phenomena and identified targets with potential for intervention to emaliorate dementia risk. Independent studies are necessary to evaluate the effect of the modification of the identified risk factors in dementia risk.

Índice general

Capítulo 1

Introducción

1. Marco teórico

1.1. Particularidades de la diabetes mellitus tipo 2 en el paciente adulto mayor

La diabetes mellitus tipo 2 (DM2) es una enfermedad crónico-degenerativa de presentación clínica heterogénea caracterizada por fenotipos de resistencia a la insulina, obesidad abdominal, deficiencia de funcionamiento de la célula β-pancreática e hiperglucemia persistente y que a largo plazo deviene en el desarrollo de complicaciones micro y macrovasculares (1). En México, la DM2 representa una importante causa de morbi-mortalidad, presentando una elevada prevalencia en la población general y en particular en individuos 65 años de edad, como ha sido reportada en las la Encuestas Nacionales de Salud y Nutrición (ENSANUT) 2012 y 2016 (1; 2). Evidencia reciente demuestra que la expresión clínica de la diabetes es heterogénea y que la variabilidad en su presentación clínica tiene implicaciones pronósticas y terapéuticas (3). Es por esto que la presentación de la DM2 en el paciente geriátrico es heterogénea. En ENSANUT 2016, la mediana del tiempo de evolución de DM2 en pacientes de 65 años o más fue de 10 años (IQR 5.0-20), lo que representa la dispersión más alta de exposición a la enfermedad con respecto a otros grupos etáreos (2). Ésta variabilidad hace que el manejo de la DM2 en éste grupo sea particularmente complicada pues se combina además con un mayor número de comorbilidades crónicas y asociadas a la edad que pueden devenir en perfiles de riesgo diferenciales para complicaciones agudas y crónicas relacionadas a diabetes (3; 4).

La DM2 puede presentarse en el paciente adulto mayor como una enfermedad de aparición temprana, lo que lleva a una larga exposición a la enfermedad y una alta susceptibilidad al desarrollo de complicaciones crónicas. La acumulación de complicaciones crónicas y la falta de control metabólico devienen en el desarrollo de dependencia, alteraciones cognitivas y funcionales (3; 4). Por otra parte, existe un grupo de pacientes con DM2 diagnosticados en después de los 65 años, que presentan una baja prevalencia de complicaciones microvasculares y que pueden alcanzar metas de tratamiento con uno o dos fármacos orales (3). Un esfuerzo reciente de clasificación

de diabetes basado en características metabólicas específicas agrupó estos casos de aparición tardía como una *diabetes relacionada a la edad*. Ésta heterogeneidad en la presentación de la DM2 ha devenido en que su manejo en éste grupo poblacional sea complejo y generalmente requiera de un abordaje multidisciplinario (4).
La prevalencia de complicaciones relacionadas a DM2 en el paciente adulto mayor también ha demostrado tener una amplia heterogeneidad (3). Se ha descrito que los pacientes con DM2 que adquieren el padecimiento a edad avanzada tienen un menor riesgo de complicaciones micro y macrovasculares, además de un mejor control metabólico que reduce la posibilidad de generar discapacidad o dependencia (5; 6). Sin embargo, además de las complicaciones microvasculares y aquellas que dependen de un control metabólico óptimo el paciente geriátrico con DM2 presenta un elevado riesgo de complicaciones que no han demostrado beneficio con el control glucémico, incluyendo complicaciones macrovasculares, elevado riesgo cardiovascular, depresión, fragilidad, deterioro cognitivo y demencia (6; 7). Ésta última ha sido de particular interés debido a que evidencia reciente demuestra riesgo elevado de demencia aún en adultos mayores con diagnóstico reciente de diabetes, sugiriendo que factores independientes a la edad de diagnóstico y el tiempo de evolución subyacen a los mecanismos que vinculan a la diabetes con un mayor riesgo de demencia (7).
El impacto de la diabetes en el desarrollo de deterioro cognitivo y demencia ha sido ampliamente estudiado, aunque los mecanismos fisiopatológicos se mantienen aún sólo parcialmente descritos (5). En población Mexicana, se han descrito asociaciones de deterioro cognitivo en pacientes con DM2 con la presencia de síndromes geriátricos, incluyendo al síndrome fragilidad e incontinencia urinaria (6). Además, el estudio 10/66 demostró por primera vez que la DM2 es un factor de riesgo para el desarrollo de demencia en población Mexicana (8). A pesar de ésta evidencia, el estudio de los diferentes fenotipos metabólicos en la DM2 presentes en el paciente adulto mayor y su riesgo asociado no ha sido motivo de estudio en los estudios epidemiológicos.

1.2. Riesgo de demencia asociado a diabetes

La carga de morbi-mortalidad asociada a diabetes ha presentado un acelerado crecimiento en los últimos años (9). Esto ha ido en paralelo con una tendencia de envejecimiento poblacional a nivel mundial con un progresivo crecimiento del grupo de adultos >60 años de edad. Se estima que para el año 2030 habrá cerca de 690 millones de adultos mayores (11). Debido al envejecimiento poblacional, se ha dado un aumento en la prevalencia de enfermedades relacionadas a la edad y una acumulación de población susceptible con multicomorbilidad de enfermedades crónicas que ocasionan acumulación de factores que aumentan el riesgo de dependencia, discapacidad y deterioro funcional. La demencia es un síndrome de deterioro neurológico causado por afectaciones graduales en dominios cognitivos que incluyen memoria, comportamiento, pensamiento y la capacidad para llevar a cabo actividades de la vida diaria (5; 10). Además de los factores de riesgo clásicamente descritos para el desarrollo de demencia que incluyen edad, sexo y escolaridad, diversos estudios epidemiológicos han demostrado de forma consistente un aumento en el riesgo de demencia asociado enfermedades crónicas, entre las que se encuentran la hipertensión arterial sistémica,

la enfermedad cardiovascular y, de forma consistente, la DM2 (10). En contraste con algunos datos sugieren una disminución en la incidencia de de casos de demencia por mejoras en la atención a adultos mayores, los casos de DM2 han ido en aumento (11). Esto ha atraído particular atención a la asociación entre DM2 y demencia pues basado en proyecciones epidemiológicas, se espera que cerca de 552 millones de personas padezcan DM2 para el año 2030, con una predominancia de casos en países de mediano y bajo ingreso, donde la incidencia de DM2 ha ido en aumento debido a cambios en estilos de vida poco saludables (12; 13; 14). En México, se estima que la prevalencia de DM2 en adultos mayores de 50 años pueda hasta duplicarse para el año 2050 si las políticas públicas encaminadas a reducir la incidencia de DM2 no resultan exitosas (15).

Diversos estudios epidemiológicos, revisiones sistemáticas y meta-análisis han corroborado en diferentes grupos étnicos, con diversos tiempos de evolución de la enfermedad, grupos de tratamiento y tiempos de seguimiento, que la DM2 es un factor de riesgo para el desarrollo de demencia por cualquier causa, demencia vascular y demencia de tipo Alzheimer (16). Sin embargo, el efecto deletéreo de la DM2 sobre la función y arquitectura cerebral se ha posicionado sobre todo en la edad adulta. De hecho, al evaluar mediante métodos de componentes principales los principales determinantes de la prevalencia actual de demencia, se determinó que el 35 % de los casos de demencia se deben a una combinación de 9 factores de riesgo incluyendo educación máxima de 11-12 años, hipertensión, obesidad, pérdidas auditivas, depresión, diabetes, inactividad física y tabaquismo. Se ha estimado además que podría prevenirse hasta un 21.7 % de los casos de deterioro cognitivo hacia demencia si se eliminan hábitos dietéticos adversos, DM2 y síntomas neuropsiquiátricos (11).

1.3. Factores de riesgo asociados al desarrollo de demencia en diabetes

Un meta-análisis reciente de estudios longitudinales confirmó que la DM2 aumenta el riesgo de enfermedad de Alzheimer hasta en un 50 % y hasta dos veces para el caso de demencia de tipo vascular (10). Los factores de riesgo que se han estudiado en la mayor parte de los estudios son resultado de análisis secundarios, con tamaños de muestra y definiciones operacionales inconsistentes. Algunos factores de riesgo comunes para el desarrollo de demencia en DM2 incluyen edad avanzada, historia familiar de demencia, tabaquismo y enfermedades crónicas entre las que se incluyen hipertensión y obesidad en la vida adulta, dislipidemia, enfermedad vascular cerebral y depresión en la vida adulta tardía (17). Además, se ha identificado que una proporción significativa del riesgo de demencia podría atribuirse a perfiles genéticos, como la positividad para el alelo de riesgo en *APOE*-ϵ4. Otros factores de riesgo específicos incluyen alteraciones del sueño y antecedentes de episodios de trastorno depresivo mayor (18; 19). Algunos factores protectores que se han investigado para DM2 incluyen años de educación formal, actividad física y ocupación, aunque la evidencia en DM2 no es concluyente (20).

Como se discutirá en la sección de Resultados, algunos estudios han vinculado fac-

tores de riesgo específicos a la DM2 que aumentan el riesgo de demencia incidente incluyendo control glucémico, años de exposición a DM2, uso de insulina exógena, hiperinsulinemia endógena, resistencia a la insulina e hipoglucemia (7). Sin embargo, se ha demostrado que la DM2 es un factor de riesgo para el desarrollo de demencia independientemente del tiempo de evolución (21), lo que sugiere que la resistencia a la insulina, obesidad abdominal y el síndrome metabólico podrían ser mediadores tempranos de las alteraciones metabólicas que vinculan a la DM2 con deterioro cognitivo y demencia, en particular con la enfermedad de Alzheimer (17; 22).

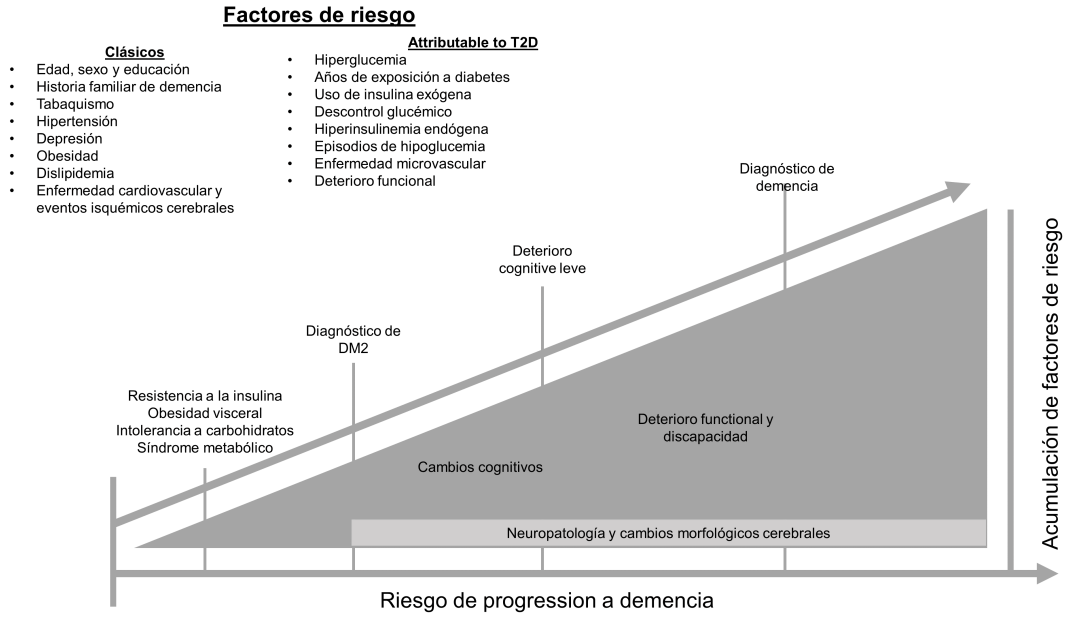

Figura 1.1: Modelo de riesgo de demencia en diabetes. La acumulación de factores de riesgo conforme evolucionan las alteraciones metabólicas asociadas a diabetes aumentan la progresión de deterioro neurológico y funcional. Adaptado de Bello-Chavolla et al (7).

En la **Figura 1.1** se describe un modelo de riesgo acumulado propuesto para la relación observada entre DM2 y demencia. Se ha propuesto que el riesgo de cambios cognitivos y estructurales del sistema nervioso central (SNC) ocurre desde estados pre-diabéticos con una compleja interacción entre resistencia a la insulina, obesidad abdominal y en particular visceral, intolerancia a carbohidratos y síndrome metabólico (23). Estas alteraciones metabólicas inducen cambios neuropatológicos y morfológicos como se describirá en la sección siguiente, con consecuentes cambios cognitivos conductuales que aunados al deterioro funcional secundario a multicomorbilidad y complicaciones crónicas de DM2 podría favorecer el desarrollo de demencia en ésta población. Por tanto, es factible concluir que la acumulación de factores de riesgo específicos y no específicos de la DM2 podría devenir en una aceleración del deterioro, explicando la asociación de menor edad de demencia incidente en pacientes con DM2 (7). Dado que la mayor parte de las complicaciones metabólicas y microvasculares de

la DM2 pueden prevenirse, el desarrollo de modelos de cálculo de riesgo en pacientes con DM2 ha ganado atención reciente en diferentes entornos de investigación.

1.4. Efectos neurológicos de alteraciones metabólicas en pacientes con diabetes

El establecimiento de la demencia como una complicación cónica de la DM2 se mantiene como un tema controversial. La correlación fisiopatológica entre diabetes y demencia desde el punto de vista de la enfermedad microvascular ha arrojado resultados prometedores. Se ha demostrado que la presencia de retinopatía diabética o enfermedad renal relacionada a diabetes son predictores independientes del riesgo de demencia por cualquier causa (24; 25). Además, como se comentará en la sección siguiente, un puntaje de riesgo desarrollado por un grupo del Kaiser Permanente Institute demostró que la enfermedad microvascular era predictora significativa de demencia en pacientes con diabetes (26). La hipótesis del daño microvascular ha sido cuestionada por el hecho de que se han observado cambios neurológicos significativos en pacientes sin alteraciones microvasculares de importancia; además, el deterioro funcional y la discapacidad asociada a complicaciones crónicas de diabetes podría ser un mecanismo mediador que explique la relación entre ambas (27). Finalmente, evidencia del estudio ACCORDION-MIND ha demostrado que el estricto control glucémico reduce la carga de morbilidad asociada a complicaciones crónicas sin beneficios aparentes en cognición (28).

Como se ha comentado, los mecanismos que subyacen a la asociación de DM2 y demencia no han sido completamente elucidados (5). Por un lado se ha propuesto que el daño micro y macrovasculares que caracteriza a las complicaciones de la DM2 podría inducir daños neuronales que devengan en un aceleramiento del deterioro cognitivo. Sin embargo, evidencia reciente ha demostrado un papel de alteraciones en la señalización neuronal de la insulina, llevando al desarrollo de la hipótesis de resistencia central a la insulina, que deviene en daños en materia gris, materia blanca y neurodegeneración (29). Esta evidencia es concordante con observaciones de alteraciones en la abundancia y función de la enzima degradadora de insulina y alteraciones en la señalización del factor de crecimiento similar a insulina 1 (IFG-1) con alteraciones cognitivas en modelos animales (5; 29; 7). Algunos autores han propuesto que en realidad una combinación de ambos mecanismos es responsable de la manifestación insidiosa de demencia en los pacientes con DM2, aunque los mecanismos fisiopatogénicos que vinculan DM2 con las alteraciones cognitivas y funcionales derivadas de resistencia a la insulina no han sido completamente descritas (5; 7).

Otro mecanismo fisiopatológico de relevancia que ha vinculado alteraciones metabólicas con modificaciones en la función y morfología del SNC es la obesidad abdominal (23). Estudios recientes vinculan una relación entre la acumulación de tejido adiposo visceral, un compartimento de tejido adiposo asociado con mayor riesgo cardiometabólico, con disminución de la covarianza de redes neuronales en el SNC, daño microvascular y alteraciones en memoria (30). Además, se ha demostrado que el estradiol tiene un efecto neuroprotector para el efecto deletéreo que tiene el tejido adiposo

visceral sobre la estructura y función cerebra, con un marcado deterioro tras la instauración de la menopausia (31). El papel de la obesidad en alteraciones cognitivas y de la estructura cerebral ha sido motivo de controversia, pero su predencia en la edad adulta ha demostrado ser un factor de riesgo para el desarrollo de demencia (32; 33). Dado que la diabetes es un factor de riesgo independiente para la acumulación de tejido adiposo visceral, sería razonable esperar que la acumulación de tejido adiposo visceral y su perfil metabólico y pro-inflamatorio adverso asociado, pudiera tener un papel importante en la fisiopatología del deterioro neurológico asociado a DM2 (34).

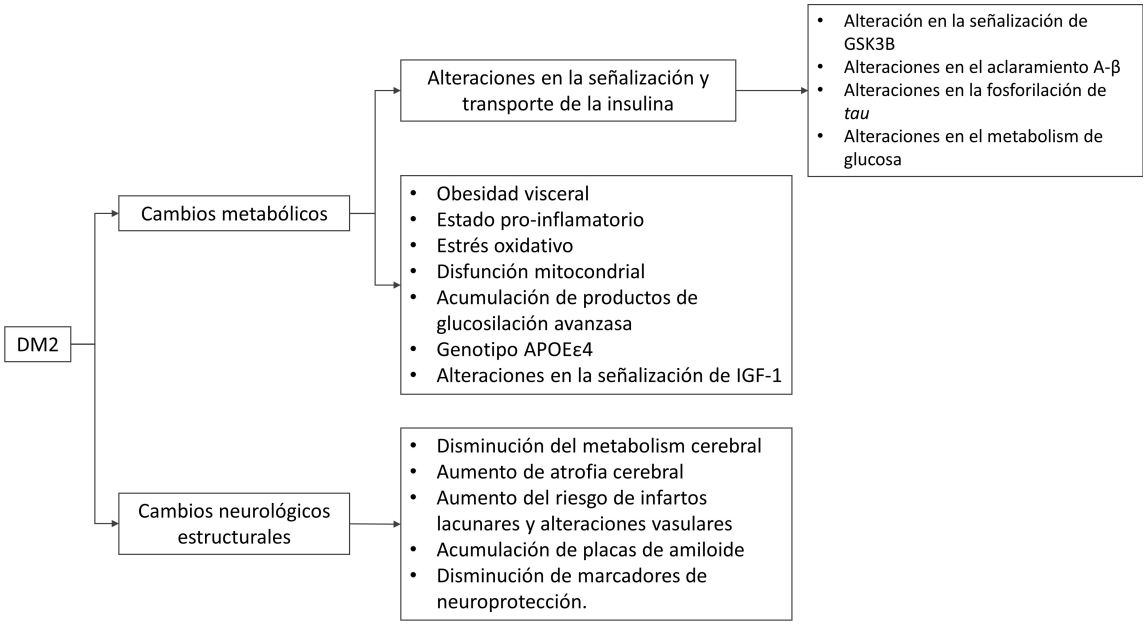

Figura 1.2: Alteraciones metabólicas y estructurales en el sistema nervioso central asociadas a la diabetes mellitus tipo 2. Adaptado de Bello-Chavolla et al (7).

En la **Figura 1.2** se detallan los principales mecanismos identificados en DM2 que podrían vincular a la enfermedad con deterioro en la estructura y funcionamiento cerebral. Ante la insuficiencia de un mecanismo fisiopatológico integral que explique la asociación de DM2 y demencia en humanos, se llevado a cabo evaluaciones epidemiológicas en búsqueda de factores de riesgo que permitan postular mecanismos que devengan en estudios funcionales. Sin embargo y a pesar de la consistencia de la evidencia epidemiológica sobre los factores de riesgo identificados, algunas diferencias entre estudios incluyendo el tiempo de seguimiento, las estrategias de análisis estadístico, el control de variables confusoras y las definiciones operacionales de las variables entre estudios ha llevado a que la identificación de factores de riesgo asociados a demencia en pacientes con diabetes sea heterogéneo y carezca de consistencia en los reportes.

1.5. Aproximaciones al modelaje de riesgo

Considerando que la mayor parte de los factores de riesgo asociados a demencia en la población general no son modificables, los esfuerzos en el estudio de la demencia se han enfocado en la identificación temprana de casos para desacelerar o prevenir la progresión de la enfermedad. En pacientes con DM2, Exalto y colaboradores desarrollaron una herramienta predictiva denominada Diabetes Specific Dementia Risk Score (DSDRS), utilizando información del estudio Kaiser Permanente Northern California (KPNC) Diabetes Registry (26). El modelaje para riesgo de demencia a 10 años se hizo para demencia por todas las causas utilizando modelos de riesgos proporcionales de Cox, ajustados por edad, sexo y escolaridad. El puntaje final fue del 1 al 12 con un incremento progresivo de riesgo de demencia por cualquier causa a 10 años, representando el primer puntaje de riesgo para pacientes ancianos con diabetes. Entre las limitaciones del puntaje se encuentran el importante riesgo atribuible a la edad, la estrategia de modelaje estadístico y el enfoque en patología vascular, lo cual hace que sea una herramienta que podría sobreestimar el riesgo de demencia en ésta población. Además, la falta de especificidad etiológica para los distintos tipos de demencia la hacen una herramienta que sesga el diagnóstico hacia el riesgo de demencia de tipo vascular por sobre las demencias no vasculares.

2. Planteamiento del problema de investigación

Los adultos mayores con DM2 son una población con presentación clínica y perfiles de riesgo heterogéneos, con elevada susceptibilidad al desarrollo de complicaciones, deterioro cognitivo, funcional y demencia. La emergencia epidemiológica por el aumento en la prevalencia e incidencia de DM2 y la acumulación de población con multicomorbilidad y riesgo de demencia hace que la identificación de pacientes con DM2 y alto riesgo para el desarrollo de demencia pueda permitir la investigación con enfoque preventivo para retrasar la instauración o progresión de la enfermedad. El único modelo actual para estimar el riesgo de demencia, a pesar de su fortaleza metodológica, presenta limitaciones prácticas importantes que hacen necesario el desarrollo de alternativas pronosticas para la predicción de demencia en pacientes con DM2. Además, el modelo no contempla algunas comorbilidades metabólicas frecuentes de la DM2 como resistencia a la insulina y adiposidad visceral, factores que han demostrado alterar el funcionamiento y estructura del SNC.

2.1. Pregunta de investigación

¿Qué factores predictores clínicos y metabólicos se asocian al desarrollo de incidencia de demencia vascular, no vascular y por cualquier causa en pacientes adultos mayores con DM2 al seguimiento a 10 años?

2.2. Hipótesis

Como han sugerido diversos estudios epidemiológicos, se espera identificar algunos factores como el descontrol metabólico, manejo farmacológico inadecuado, tiempo de exposición de DM2, positividad para el genotipo de riesgo en *APOE*-ϵ4. También esperamos identificar que las alteraciones funcionales y neuropsiquiátricas como dependencia, depresión e inmovidilidad sean predictoras para la incidencia de demencia, posiblemente como resultado de una acumulación adversa de comorbilidades y complicaciones microvasculares crónicas asociadas a DM2. Finalmente, esperamos identificar un papel predictor para fenotipos de diabetes con resistencia a la insulina y acumulación de tejido adiposo visceral, factores que han demostrado conllevar alteraciones estructurales y funcionales en estudios epidemiológicos previos.

3. Objetivos del proyecto

- **Objetivo principal:** Desarrollar modelos predictores de la incidencia de demencia vascular, no vascular y por cualquier causa, así como de las complicaciones metabólicas asociadas que sea específico para pacientes con DM2 a partir de datos obtenidos en una cohorte prospectiva.
    - **Objetivo específico 1:** Desarrollar herramientas metabólicas útiles para la predicción de desenlaces adversos en pacientes con DM2 para su aplicación en estudios epidemiológicos.
    - **Objetivo específico 3:** Validar y calibrar el modelo de predicción de demencia específico para DM2 desarrollado por Exalto et al. (26), así como el fenotipo de riesgo que caracteriza a los pacientes tamizados por ésta herramienta.
    - **Objetivo específico 2:** Identificar factores de riesgo asociados a la incidencia de demencia en pacientes adultos mayores con DM2.
    - **Objetivo específico 4:** Desarrollar un modelo integral para la predicción para la incidencia de demencia, específico para pacientes con DM2.

Capítulo 2

Metodología General

1. Desarrollo de herramientas para estimación de riesgo metabólico en estudios epidemiológicos

Para la investigación de alteraciones metabólicas ligadas a DM2 incluyendo resistencia a la insulina y adiposidad visceral en cohortes con datos epidemiológicos similares a los contenidos en la Cohorte de *Three-City study* (38) se llevó a cabo la generación de indicadores que estimaran con mayor precisión la sensibilidad periférica a la acción de la insulina sin requerir la medición de insulina sérica de ayuno mediante inmunoensayo.

- **Metabolic Score for Insulin Resistance, METS-IR**: Fue desarrollado en sujetos Mexicanos con y sin DM2 en quienes se realizó una pinza euglucémica hiperinsulinémica. El modelo desarrolló a partir de un modelo de regresión lineal múltiple y contempla la estimación de resistencia a la insulina utilizando glucosa de ayuno, colesterol de muy alta densidad (HDL-C), índice de masa corporal (IMC) y triglicéridos séricos en ayuno. En nuestro estudio (35) se demostró que METS-IR además predice acumulación de grasa intrahepática, grasa subcutánea y grasa visceral, además se asociarse con hiperinsulinemia endógena. Finalmente, demostramos que METS-IR podría ser una herramienta útil para la predicción de riesgo de diabetes y en un estudio subsecuente (36) demostramos que era un predictor de hipertensión arterial sistémica y rigidez arterial.

- **Metabolic Score for Visceral Fat, METS-VF**: Se desarrolló en una cohorte diversa de sujetos Mexicanos en quienes se realizó medición de tejido adiposo visceral (TAV) utilizando Absorciometría Dual por rayos X (DXA) utilizando métodos de regresión no lineal considerando al índice METS-IR, el índice cintura-estatura (ICE), sexo y edad (37). El score se validó contra DXA, medición de TAV con resonancia magnética, con bioimpedancia eléctrica y en obesos metabólicamente sanos, un fenotipo de obesidad sin alteraciones metabólicas significativas en quienes la estimación de TAV es de interés fisiopatológico. Se identificó además que el score METS-VF se asociaba con un perfil adverso de adipocinas caracterizado por adiponectina baja y FGF-21 elevado por compen-

sación. Finalmente, se demostró que el score era predictor de DM2 e hipertensión incidente independientemente de la categoría de IMC a la que pertenezcan.

2. Identificación de factores de riesgo para demencia en la literatura

Previo al modelaje de riesgo, se llevó a cabo una revisión sistemática de la literatura para identificar factores de riesgo asociados a la incidencia de demencia en pacientes con DM2 en estudios de cohortes prospectivos utilizando los motores de búsqueda Medline PubMed, Medline Ovid, Cochrane y Scielo. El desenlace primario de búsqueda fue demencia incidente por cualquier causa, demencia no vascular, vascular o enfermedad de Alzheimer al seguimiento. También se estableció que el diagnóstico de DM2 debe darse durante la evaluación basal de acuerdo a cualquier criterio aceptado (glucosa plasmática de ayuno, hemoblogina glucosilada, curva de tolerancia a la glucosa oral) y que el estado cognitivo debe ser verificado con instrumentos aceptados para su evaluación por perfonal calificado.

Metodología de revisión sistemática y meta-análisis

Los criterios de búsqueda incluyeron “dementia”, “vascular dementia”, “non-vascular dementia”, “frontotemporal dementia”, “Alzheimer disease”, “Lewy body”, “type 2 diabetes mellitus”, “diabetes mellitus”, "diabetes complications", "multi-infarct dementia", ïmpaired glucose tolerancez “diabetes-related dementia” con filtros de fecha adicional entre Enero 1 de 2014 y Abril 30 de 2019. Se incluyeron además resultados identificados de meta-análisis previos que abarcaban hasta Enero de 2014. Se consideraron estudios epidemiológicos longitudinales con cocientes de riesgos (HR) disponibles, que tuvieran como desenlace primario incidencia de demencia por cualquier causa, vascular o enfermedad de Alzheimer y que evalúen la asociación de con DM2 con un mínimo de seguimiento de 2 años. Estudios con datos insuficientes, que no reporten HR o intervalos de confianza, con diseño retrospectivo, seguimiento <2 años o que no tengan definiciones operacionales claras del desenlace fueron excluidos. Se realizó una revisión de los títulos y resúmenes relevantes a los criterios de inclusión y se seleccionaron los que se consideraron más pertinentes para la evaluación. Además se realizó una evaluación de calidad de los manuscritos utilizando el Newcastle-Ottawa Quality Assessment. Se incluyeron estudios que evaluaran solo población diabética para la identificación de factores de riesgo y se registró el protocolo en extenso (PROSPERO 2017:CRD42017065676). Los estudios con desenlaces comparables que reportaran HR se incluyeron en meta-análisis de efectos aleatorios para calcular HR e intervalos de confianza al 95 %. La heterogeneidad entre estudios se evaluó utilizando la prueba de χ^2 y el estadístico I^2; un valor $> 50\,\%$ se consideró como evidencia sustancial de heterogeneidad. Todos los análisis se llevaron a cabo en el paquete `meta` de `R` (Versión 3.6.1).

3. Modelaje de factores de riesgo en la cohorte de las Tres Ciudades (Three-City study)

Población estudiada

La evaluación inicial se llevó a cabo utilizando de la cohorte de las Tres Ciudades (3C, (38)). La cohorte 3C es un estudio prospectivo diseñado para evaluar el riesgo de demencia y deterioro cognitivo atribuible a factores de riesgo vasculares que incluyó 9,294 individuos ancianos con edad 65 años de tres ciudades francesas, incluyendo Bordeaux, Dijon y Montpellier. La información basal fue recolectada por personal de enfermería e incluyó información demográfica, reporte de enfermedades crónicas y estilo de vida en entrevistas cara a cara, así como evaluación por exploración física, antropometría, medición de presión arterial y evaluación bioquímica en ayuno que incluyó mediciones de glucosa, perfil de lípidos y genotipificación para apolipoproteína E (ApoE$-\epsilon 4$). A la fecha, se han realizado 7 seguimientos cada 2 años para un total de 14 años de seguimiento.
El diagnóstico de demencia se estableció por un algoritmo de tres pasos:

- Inicialmente se llevó a cabo una batería de evaluación neuropsicológica para evaluación de memoria, atención, lenguaje y habilidades visuoespaciales.
- A aquellos sujetos en quienes se identificasen alteraciones se realizó una evaluación cognitiva complementaria por parte de neurólogos de los tres centros evaluados, quienes administraron las pruebas de Mini-Mental State Examination, retención visual de Benton y la prueba de Isaacs.
- Finalmente, un comité de neurólogos expertos independientes del comité de 3C determinó el diagnóstico de demencia por consenso basado en los criterios del DSM-IV. Además, se emitió un juicio por consenso sobre la posible etiología del padecimiento incluyendo Enfermedad de Alzheimer, demencia frontotemporal, demencia por cuerpos de Lewy y demencia vascular.

Definición de predictores

Para el modelaje de riesgo en la cohorte de 3C fue de nuestro interés evaluar mecanismos fisiopatológicos previamente estudiados en función de su efecto sobre la estructura y funcionamiento cerebral. Además, se decidió estimar la masa libre de grasa o masa magra como un subrogado para estudiar deterioro funcional (También evaluado por la escala de Rosow-Breslau) y sarcopenia.

- Resistencia a la insulina. La resistencia a la insulina fue estimada utilizando el Metabolic Score for Insulin Resistance (METS-IR), desarrollado por nuestro grupo (35). Un valor de METS-IR >50 fue considerado como resistencia a la insulina para ésta evaluación.
- Obesidad visceral. La acumulación excesiva de tejido adiposo visceral fue estimada utilizando el Metabolic Score for Visceral Adiposity, desarrollado por

nuestro grupo (37). Un valor de METS-VF >7.18 se consideró diagnóstico de obesidad abdominal para ésta evaluación.

- Masa libre de grasa (MLG). Se utilizó el modelo desarrollado por Janmahasatian y colaboradores (39)estandarizado para hombres y mujeres. El índice de MLG (IMG) se obtuvo dividiendo el estimado de MLG entre el cuadrado de la estatura en metros. Para definir disminuciones en IMLG se utilizaron los puntos de corte propuestos por Schutz y colaboradores, como un IMLG $<16.7 kg/m^2$ en hombres y $<14.6 kg/m^2$ en mujeres (41).

- Discapacidad motora. Se definió mediante la escala de Rosow-Breslau para detectar limitaciones en actividades cotidianas. La discapacidad para al menos uno de los tres componentes de la escala fue considerada para determinar la presencia de discapacidad motora.

Estrategia de análisis estadístico

Las variables cuantitativas continuas fueron evaluadas para normalidad utilizando la prueba de Anderson-Darling. Para evaluaciones entre grupos, se llevó a cabo la prueba t de Student para muestras independientes o la prueba U de Mann-Whitney, según la distribución de la variable. Las variables categóricas fueron analizadas utilizando la prueba de Chi cuadrado o la prueba exacta de Fisher según fuera necesario. Un valor de $p < 0.05$ fue considerado como estadísticamente significativo. Los análisis estadísticos se realizaron utilizando los software estadísticos SPSS (Versión 23.0) y R (Versión 3.6.1).

Establecimiento del riesgo de demencia por diabetes

Inicialmente se modeló el riesgo de desarrollar demencia incidente asociado a DM2 utilizando la evaluación basal de la cohorte de 3C en Burdeos ($n = 2,104$). La tasa de incidencia de demencia se estimó dividiendo el número de eventos observados durante el seguimiento entre los años persona de los individuos observados estimado para 1,000 años persona y se categorizó de acuerdo a variables de interés; se calcularon intervalos de confianza al 95 % assumiendo una variable con distribución Poisson. Para evaluar el efecto de los comparadores sobre el tiempo libre de demencia se utilizaron modelos de Kaplan-Meier, evaluando las comparaciones mediante la prueba de log-rank.
Para el modelaje de riesgo, se utilizaron tres abordajes estadísticos para reducir la incertidumbre asociada al modelaje de riesgo de patologías asociadas a la edad.

- La primera se llevó a cabo mediante regresión de riesgos proporcionales de Cox, utilizando como variable desenlace la incidencia de demencia por cualquier causa, demencia de tipo Alzheimer y demencia vascular o censura si el paciente no completó el seguimiento o lo completó pero no desarrolló el evento.

- El segundo modelaje se llevó a cabo mediante una regresión semi-paramétrica utilizando el modelo de riesgos competitivos de Finn y Gray, utilizando como desenlace la incidencia de demencia, como riesgo competitivo la mortalidad por

cualquier causa durante el seguimiento o la censura si el paciente no completó el seguimiento o no desarrolló el padecimiento tras el seguimiento.

- Los modelos fueron además ajustados por entradas tardías, utilizando la edad de entrada a la cohorte y su edad de terminación como tiempo de seguimiento en el modelo; las co-variables de ajuste incluyeron edad, sexo, escolaridad y genotipificación de ApoE-ϵ4.

Factores de riesgo para demencia en DM2

Se llevó a cabo una comparación de las variables estudiadas en la evaluación basal de 3C en Bordeaux, comparando a sujetos que desarrollarían demencia con y sin DM2, para identificar potenciales predictores. Para establecer el papel de las variables basales en la predicción demencia se realizó una evaluación de la tasa de incidencia de demencia para las variables previamente reportadas en la literatura y nuevas evaluaciones utilizando variables evaluadas en la cohorte de 3C utilizando regresión de riesgos proporcionales de Cox, ajustado por edad, sexo y escolaridad para evaluaciones de demencia por cualquier causa para variables no cardiovasculares y adicionalmente por colesterol total, tabaquismo, antecedente personal o heredofamiliar de enfermedad cardiovascular y presión arterial para las variables cardiovasculares.

3.1. Modelaje predictivo

Para evaluar la factibilidad de utilizar el modelo de Cox de riesgos proporcionales en las variables candidatas de la cohorte de pacientes con DM2 de 3C (N=908), se utilizó la prueba de residuales de Schoenfeld modificada por Grambsch y Therneau. Una vez confirmado el supuesto de riesgos proporcionales, se utilizaron los predictores identificados para modelaje primero utilizando las variables en su codificación original, ajustadas por edad, sexo y escolaridad. Para ajustar por variabilidad inducida en parámetros bioquímicos o cifras de tensión arterial por el uso de fármacos, se realizaron ajustes por medicamentos hipolipemiantes, ácido acetil-salicílico y antihipertensivos.
Para estimar puntos de corte para las variables cuantitativas continuas, se realizó una simulación utilizando el modelo con el paquete `simPH` de `R` para estimar HR para cada punto de corte de la variable. Además, se seleccionaron puntos de corte para realizar la categorización de las variables en el modelo de riesgos proporcionales de Cox. Finalmente, se utilizaron los coeficientes $\hat{\beta}$ de las regresiones de Cox para estimar el puntaje de cada individuo y su riesgo atribuible de demencia incidente por cuartiles del puntaje de riesgo fue calculado utilizando curvas de Kaplan Meier. Todos los modelos el cual fueron validados utilizando validación cruzada k-veces ($k = 10$) y validación cruzada por bootstrap ($B = 1,000$) y las mediciones del modelo fueron corregidas por el optimismo generado por validación en la cohorte de generación del modelo. La concordancia de los modelos se evaluó utilizando el estadístico-c de Harrel y el $D_{xy} de Sommers$.

Capítulo 3

Manuscritos relevantes

La presente sección recopila algunos de los manuscritos desarrollados en el contexto de mi trabajo doctoral. A continuación, se introducen los resultados del presente trabajo doctoral-tesis así como su pertinencia al campo de investigación. Los resultados de la tesis doctoral en proceso de publicación se presentarán en la siguiente sección **Resultados Adicionales**.

1. Relación entre demencia y diabetes

1.1. Epidemiologia de Diabetes Mellitus tipo 2 en México

Bello-Chavolla OY, Rojas-Martinez R, Aguilar-Salinas CA, Hernández-Avila M. Epidemiology of diabetes mellitus in Mexico. Nutr Rev. 2017 Jan;75(suppl 1):4-12

En éste manuscrito se describen los datos epidemiológicos más recientes al momento de su escritura a finales del año 2016 sobre la diabetes mellitus tipo 2 en México, las principales características de su distribución etárea y regional. También se discuten las principales barreras para el logro de metas de tratamiento y mejora en la calidad de la atención de la diabetes mellitus en México.

1.2. Diabetes en el aduto mayor

Bello-Chavolla OY, Aguilar-Salinas CA. Management of type 2 diabetes in the elderly patient. J Lat Am Geriat Med. 2017; 3:26-36

Éste manuscrito presenta el conocimiento más reciente a la fecha de su escritura sobre las particularidades de la diabetes mellitus tipo 2 en el adulto mayor y su manejo farmacológico y no farmacológico. Además, se discuten algunas particularidades en relación a funcionalidad y cognición que deben tomarse en cuenta al momento de la elección de tratamiento para un paciente adulto mayor con diabetes mellitus tipo 2.

1.3. Fisiopatología de la demencia relacionada a diabetes

Bello-Chavolla OY, Antonio-Villa NE, Vargas-Vázquez A, Ávila-Funes JA, Aguilar-Salinas CA. Pathophysiological mechanisms linking type 2 diabetes and dementia: Review of evidence from clinical, translational and epidemiological research. Curr Diabetes Rev. 2019 doi: 10.2174/1573399815666190115151500

En éste manuscrito se recopila toda la investigación bibliográfica pertinente a la pregunta de investigación de mi tesis doctoral; desde el abordaje de factores de riesgo clásicos para demencia hasta aquellos que han sido explorado desde diferentes abordajes específicos para el paciente con diabetes mellitus tipo 2. El manuscrito también reflexiona sobre las áreas de oportunidad pertinentes a la pregunta de investigación que deben abordarse para profundizar y diversificar el conocimiento generado sobre demencia en pacientes con diabetes mellitus tipo 2.

2. Desarrollo de indicadores metabólicos

2.1. Metabolic Score for Insulin Resistance

Bello-Chavolla OY, Almeda-Valdes P, Gomez-Velasco D, Viveros-Ruiz T, Cruz-Bautista I, Romo-Romo A, Sánchez-Lázaro D, Meza-Oviedo D, Vargas-Vázquez A, Campos OA, Sevilla-González MDR, Martagón AJ, Hernández LM, Mehta R, Caballeros-Barragán CR, Aguilar-Salinas CA. METS-IR, a novel score to evaluate insulin sensitivity, is predictive of visceral adiposity and incident type 2 diabetes. Eur J Endocrinol. 2018;178(5):533-544.

Para estudiar el fenómeno de resistencia a la insulina en el contexto de estudios epidemiológicos en los que la medición sérica de concentraciones de insulina en ayuno no se encuentran disponibles, como es el caso de la cohorte del *Three-City Study*, se desarrolló una ecuación para la estimación de resistencia a la insulina validado contra la pinza euglucémica hiperinsulinémica, el estándar de oro para evaluación de resistencia a la insulina. El indicador *Metabolic Score for Insulin Resistance* (METS-IR, por sus siglas en inglés) utiliza mediciones en ayuno de glucosa, triglicéridos séricos, colesterol de alta densidad (HDL-c) y el índice de masa corporal (IMC) para su estimación.

2.2. Metabolic Score for Visceral Fat

Bello-Chavolla OY, Antonio-Villa NE, Vargas-Vázquez A, Viveros-Ruiz T, Almeda-Valdes P, Gomez-Velasco D, Mehta R, Elias-López D, Cruz-Bautista I, Roldán-Valadez E, Martagón AJ, Aguilar-Salinas CA. Metabolic Score for Visceral Fat (METS-VF), a novel estimator of intra-abdominal fat content and cardio-metabolic health. Clin Nutr. 2019 Jul 30. pii: S0261-5614(19)30294-8. doi: 10.1016/j.clnu.2019.07.012.

Evidencia reciente vincula la acumulación de grasa intra-abdominal como un factor de riesgo para modificaciones en la covarianza de las conexiones neuronales y cognición. Para explorar el fenómeno de acumulación de grasa visceral, se desarrolló un indicador para estimar la acumulación de grasa intra-abdominal utilizando el indicador METS-IR y la relación cintura-estatura, además de edad y sexo. El indicador metabólico de adiposidad visceral (METS-VF, por sus siglas en inglés), es predictor de diabetes mellitus e hipertensión incidente y sus manifestaciones correlacionan con perfiles metabólicos adversos. Le evidencia que vincula adiposidad visceral y demencia en pacientes con DM2 no ha sido previamente explorada.

3. Predicción de demencia y deterioro cognitivo asociado a DM2 en población Mexicana

3.1. Validación fenotípica del score de Exalto et al.

Bello-Chavolla OY, Aguilar-Salinas CA, Avila-Funes JA. The type 2 diabetes-specific dementia risk score (DSDRS) is associated with decreased cognitive performance, disability and frailty amongst Mexican community-dwelling elderly. Arch Gerontol Geriat. 2019. [In press].

Una de las áreas de oportunidad en el estudio del cálculo de riesgo en demencia consiste en la identificación de factores que pudieran servir como medios para reducir la carga de riesgo asociada. En el caso de la demencia, las intervenciones no han sido muy exitosas y pocos factores han sido identificado como protectores en estudios epidemiológicos (7). Para abordar éste problema en población Mexicana, estudiamos a pacientes con DM2 de la Cohorte de Coyoacán en quienes se hizo el cálculo de riesgo de demencia utilizando el *Diabetes Specific Dementia Risk Score* (DSDRS), validado por Exalto y coaboradores. Al evaluar el fenotipo con el más alto riesgo identificado por DSDRS se identificó que se trataba de sujetos con fragilidad, dependencia funcional y un grado leve de deterioro cognitivo. Estos sujetos tenían además disminuciones importantes en su calidad de vida y estaban en riesgo de desnutrición. Dado que DSDRS sólo identifica a los sujetos basado en factores asociados a diabetes, nuestros resultados demuestran el papel de la predicción de riesgo en la identificación de sujetos quienes se beneficiarían de abordaje multidisciplinario para reducir no sólo la carga asociada a diabetes sino también la de factores relacionados a funcionalidad que podrían aminorar la comorbilidad en ésta población.

3.2. Asociación de deterioro cognitivo con síndromes geriátricos

15. Bello-Chavolla OY, Aguilar-Salinas CA, Avila-Funes JA. Geriatric Syndromes and Not Cardiovascular Risk Factors are Associated with Cognitive Impairment among Mexican Community-Dwelling Elderly with Type 2 Diabetes. Rev Invest Clin. 2017;69(3):166-172.

Una de las preguntas iniciales dentro del proyecto es si el deterioro cognitivo asociado a DM2 podría atribuirse a factores relacionados a la salud cardiovascular y la diabetes o en mayor medida a la comorbilidad de síndromes geriátricos en ésta población. Diversos estudios han confirmado que los pacientes con DM2 tienen un mayor riesgo de desarrollar síndromes geriátricos y que, en turno, los síndrome geriátricos contribuyen al desarrollo de deterioro cognitivo. En éste estudio, demostramos en pacientes con DM2 de la Cohorte de Coyoacán, que los síndromes geriátricos se asociaban a deterioro cognitivo en DM2, con menos contribución de factores de riesgo cardiovascular. El tamaño de muestra fue modesto puesto que sólo se consideraron casos con diagnóstico previo de DM2 por lo cual no pudo descartarse el papel de estos factores en la función cognitiva en DM2.

Supplement Article

Epidemiology of diabetes mellitus in Mexico

Omar Y. Bello-Chavolla, Rosalba Rojas-Martinez, Carlos A. Aguilar-Salinas, and Mauricio Hernández-Avila

Type 2 diabetes is the main health problem in Mexico. The large and growing number of cases and the remarkable economic impact of the disease support this statement. The condition is expressed at an earlier age and at a lower body mass index in Mexican mestizos compared with the age and body mass index reported in Caucasians. In addition, Mexican mestizos have an increased susceptibility to developing diabetic nephropathy. The Mexican health system needs major adjustments in order to prevent and treat type 2 diabetes. Treatment is not currently based on the needs and expectations of the patient. As a result, it is insufficient, belated, and costly. Close to 20% of the preventable deaths in Mexico are caused by diabetes and related metabolic diseases. Even a small decrease in this rate could result in substantial savings for the Mexican healthcare system.

INTRODUCTION

Close to 80% of the 415 million people with type 2 diabetes mellitus (T2DM) worldwide live in middle- and low-income countries. A significant proportion of them (41.1 million) reside in Latin America.[1] The age-adjusted prevalence for the region is 9.2% for adults (aged 20–79 years). Two of the 10 leading countries for the number of cases are located in the Americas (Brazil, 14.3 million, and Mexico, 11.5 million). T2D is a prominent public health problem in Mexico. An alarming, rapidly growing trend in prevalence has been observed in this country during the past few decades. Not only is this prevalence associated with an increase in cardiovascular risk, it also confers an increased risk for diabetic retinopathy, limb amputations, and kidney failure. Population growth, aging, and major changes in lifestyle have all contributed to an increased prevalence of T2D. Medical care for T2D and its complications entails an elevated cost for the national health system, as well as significant expenses for patients and their families.[2]

As with the majority of chronic nontransmissible diseases, T2D occurs as a result of both environmental and genetic factors; lifestyle plays a decisive role in determining whether genetic predisposition will lead to disease. Within the past 30 years, the Mexican population has become concentrated in urban centers, which has contributed to a change in dietary patterns, with a significant increase in the consumption of total calories, processed food, simple carbohydrates, soft drinks, and some sources of saturated fat. Using the 2006 National Health Survey, Barquera et al.[3] estimated that the average diet composition was 61% carbohydrates, 12% protein, and 26% fat (7.5% saturated fat). Nearly 36% of adults had an excessive carbohydrate intake; the corresponding percentage for fat was close to 13%. A large proportion of the population had a lower-than-expected intake of vegetables, vitamin A, and folic acid. The mean fiber intake was 20.7 g/day. In addition, the urban environment favors the use of cars and limits physical activity. The result of these changes is chronic exposure of the population to a positive caloric balance and a rapid rise in the prevalence of obesity,[3] which is a major

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Table 1 **Mortality attributable to diabetes mellitus**

Year	No. of deaths	Mortality rate (per 100 000 population)	Percentage
2000	46 525	46.26	10.7
2001	49 855	48.96	11.3
2002	54 828	53.21	12.0
2003	59 119	56.73	12.6
2004	62 201	59.0	13.2
2005	67 090	64.5	13.6
2006	68 353	65.2	13.9
2007	70 451	66.6	13.7
2008	75 572	70.8	14.0
2009	77 699	72.2	14.2
2010	82 964	74.0	14.5

Data from Health Secretariat/Dirección General de Información en Salud. Extrapolated from a database of deaths between 1979 and 2008 (Instituto Nacional de Geografia y Estadistica/ Secretaria de Salud) and from population projections in Mexico for 2005–2050 and retrospective projections for 1990–2004 (Consejo Nacional de Población 2006).

determinant of the incidence of T2D. Obesity prevalence among adults in Mexico increased from 20.9% to 32.4% between 1994 and 2012; in contrast, the prevalence of people who are overweight (defined as a body mass index [BMI] between 25 and 29.9 kg/m^2) remained unaltered (close to 38%).

The impact of T2D on mortality has progressively increased in Mexico. In 1970, T2D was the 15th most common cause of death. However, it moved up to the 9th and then the 4th main cause of general mortality by 1980 and 1990, respectively.[4] Since 1998, T2D has been among the leading causes of death in Mexico.[5] In 2000, T2D became the foremost cause of general mortality in Mexico, being responsible for 10.7% of the deaths registered that year.[6] As shown in Table 1, the T2D mortality rate has increased since then, reaching 14.5% in 2010. Since the year 2000, T2D has been the most common cause of death in women and the second most common cause in men, after coronary heart disease, a condition that can frequently result from T2D.[6] Mortality rates have increased more for men (42.2–51.6/1 000 000 population; 22.2% increase) than women (51.2–61.8/ 100 000 inhabitants; 17.1% increase). For the entire diabetic population in Mexico, the average age at death is 66.7 years.

Diabetes-related mortality is higher in central and northern Mexico. Mexico City is nearly 30 points above the national average, followed by the State of Coahuila. The Mexican states with lower mortality rates attributable to T2D are Quintana Roo (37.14/100 000), Chiapas (46.68/100 000), and Baja California Sur (50.76/100 000).[7] In contrast with trends in other countries, the mortality rates for T2D, coronary heart disease, and stroke in Mexico have maintained a steady rise between 2000 and 2013.[8]

PREVALENCE

Mexico is one of the few countries in the Americas in which 4 population-based health surveys have been conducted in the past 3 decades. Prevalence data from National Health Surveys performed in 1993,[9] 2000,[10] and 2006[11] are derived from the numbers of previously diagnosed (PD) subjects and cases found during the surveys (FP). The most recent data were obtained in 2012, but the prevalence of the previously undiagnosed cases has not been reported.[12] The data shows that T2D prevalence has increased from 6.7% in 1993 (PD, 4.6%; FP, 2.1%) to 7.5% in 2000 (PD, 5.8%; FP, 1.7%),[10] and 14.4% in 2006 (PD, 7.3%; FP, 7.1%).[11] The increases were similar for both sexes and for rural and urban areas. Results from the 2012 National Health and Nutrition Survey (ENSANUT 2012) show that the prevalence of T2D based on PD was 9.2% in adults aged 20 years and older, meaning that 6.4 million Mexican adults have been diagnosed with T2D.[13] The highest prevalence was found in adults aged 60–69 years (26.3%), with men having the highest prevalence between the ages of 50 and 59 years and women between the ages of 60 and 69 years (Figure 1).

Early-onset T2D (defined as onset before 40 years of age) has increased in recent years, from 1.8% in 1993 (PD, 1.0% and FP, 0.8%)[14] to 2.3% in 2000 (PD, 1.5% and FP, 0.8%)[15] and 5.7% in 2006 (PD, 1.5% and FP, 4.21%).[16] The prevalence of undiagnosed T2D is almost 3 times greater than the PD cases, which might indicate less frequent use of medical services by younger people and a lack of awareness of the disease.

T2D screening and diagnoses are below international standards.[11] The proportion of the population with undiagnosed T2D found by the survey in 2006 was practically the same as the proportion of cases with a previous medical diagnosis: 7.1% and 7.3%, respectively. This proportion contrasts with the low rates (5%–10%) reported for European countries.

The prevalence of T2D is higher in the urban areas of central-western Mexico, among people with 6 or fewer years of education and a medium or high socioeconomic level and among people enrolled at the Institute for Security and Social Services for State Workers (in Spanish) (Table 2). Prevalence also increases along with body mass index scores (Figure 2). Overall in Mexico, the prevalence of T2D is significantly higher in populations with a family history of T2D, obesity (Figure 3), and the presence of concurrent chronic diseases such as hypertension, hypercholesterolemia, kidney disease, and microalbuminuria.[10]

SCREENING

The Mexican adult population who were screened for T2D in the year preceding the survey increased from

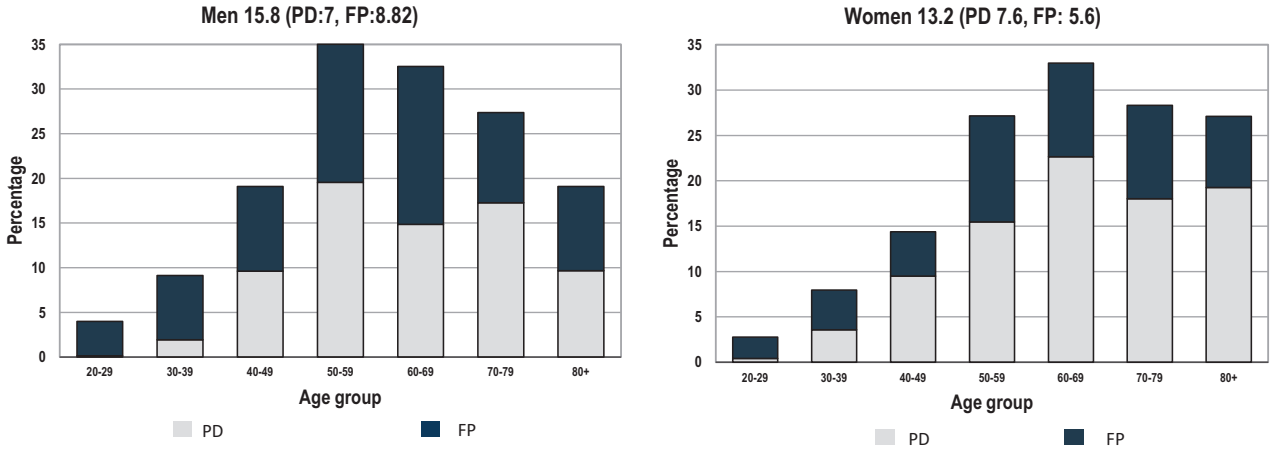

Figure 1 **Prevalence of diabetes mellitus according to age group, type of diagnosis, and sex.** Data from the National Health and Nutrition Survey 2006 (ENSANUT 2006).[16] *Abbreviations:* FP, cases found during the surveys; PD, previously diagnosed subjects.

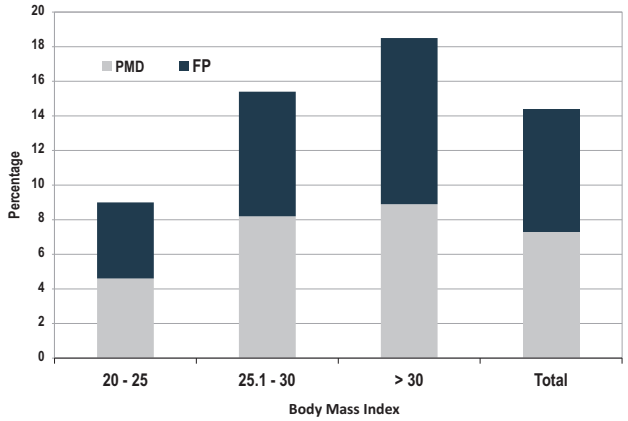

Figure 2 **Prevalence of type 2 diabetes according to type of diagnosis and body mass index.** Data from the National Health and Nutrition Survey 2006 (ENSANUT 2006).[16] *Abbreviations:* FP, cases found during the surveys; PD, previously diagnosed subjects.

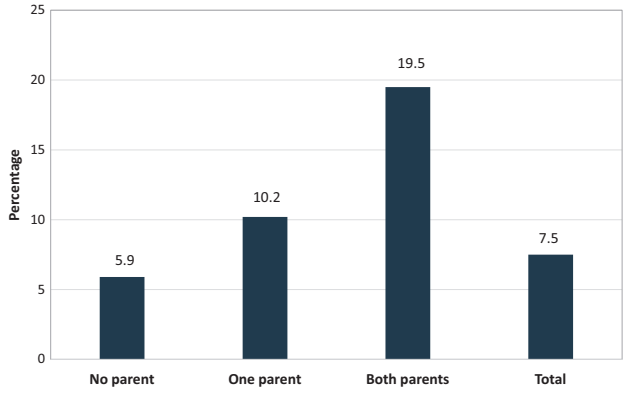

Figure 3 **Prevalence of type 2 diabetes in population over 20 years of age according to family history in parents.** Data from the National Health Survey 2000 (ENSA 2000).[15]

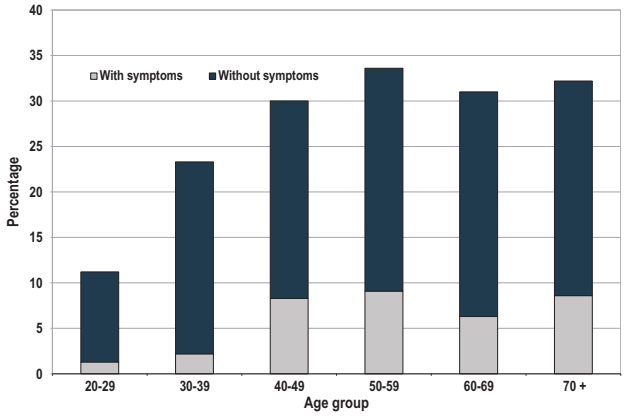

Figure 4 **Prevalence of diabetes symptoms among individuals attending prevention programs for type 2 diabetes screening.** Data from the National Health and Nutrition Survey 2006 (ENSANUT 2006).[16]

10.5% in 2000 to 22.7% in 2006. Of these, 12% in 2000 and 6.2% in 2006 did not receive their screening results.[17] One-fifth of the adults who attended a preventive medical service for T2D screening during the year before ENSANUT 2006 presented with symptoms related to the disease. This proportion was higher in people aged 40–59 years and those aged 70 years and older (Figure 4).

CLINICAL EXPRESSION OF T2D AND ITS COMORBIDITIES IN MEXICO

Based on the National Health Survey (ENSA) 2000[15] and ENSANUT 2006,[16] the most common characteristics of patients with T2D are an average age of 55.8 years in males and 56.4 years in females, overweight (27.9 kg/m^2 for males and 28.9 kg/m^2 for females), waist circumference of 99.9 cm in males and 99.3 cm in females, and a time period since the diagnosis of

Table 2 **Prevalence of type 2 diabetes according to sociodemographic characteristic, type of diagnosis, and sex**

Sociodemographic characteristic	Male			Female			Total		
	PD	FP	Total	PD	FP	Total	PD	FP	Total
Locality type									
Rural	5.2	4.3	9.5	5.8	5.3	11.1	5.5	4.8	10.3
Urban	7.5	10.0	17.5	8.1	5.6	13.7	7.8	7.7	15.5
Region									
North	6.1	6.9	13.0	6.3	6.2	12.5	6.2	6.5	12.7
Center-West	9.8	9.9	19.7	10.5	6.4	16.9	10.2	8.1	18.3
Center	6.6.	11.6	18.2	7.6	4.4	12.0	7.1	7.7	14.8
South-Southwest	5.0	5.1	10.1	6.3	5.8	12.1	5.7	5.5	11.2
No. of school, years									
$\leq$6	9.7	8.5	18.2	11.9	7.0	18.9	11.0	7.6	18.6
>6	4.8	9.1	13.9	2.9	4.0	6.9	3.8	6.6	10.4
SEL									
1–2 decils	6.1	4.6	10.7	7.3	5.6	12.9	6.8	5.1	11.9
$\geq$3	7.5	11.1	18.6	7.9	5.6	13.5	7.7	8.2	15.9
Enrollment									
IMSS	9.5	12.1	21.6	7.3	5.4	12.7	8.3	8.5	16.8
ISSSTE	17.3	7.6	24.9	7.7	7.7	15.4	11.8	7.7	19.5
SPSS	5.5	7.0	12.5	9.0	4.1	13.1	7.5	5.3	12.8
Private	0	0	0	25.3	6.0	31.3	10.4	2.5	12.9
Other	4.7	9.3	14.0	9.7	4.5	14.2	8.0	6.1	14.1
Neither	5.1	7.5	12.6	7.2	5.8	13.0	6.2	6.6	12.8

Data from the National Health and Nutrition Survey 2006 (ENSANUT 2006).[16] *Abbreviations:* FP, cases found during the surveys; IMSS, Instituto Mexicano del Seguro Social; ISSSTE, Instituto de Seguridad Social y Servicios a los Trabajadores del Estado; PD, previously diagnosed subjects; SEL, Socioeconomic level; SPSS, Seguro Popular de la Secretaria de Salud.

9.3 years in males and 8.4 years in females. The average age at diagnosis was 48 years, with women being diagnosed at a younger age than men. A high percentage of the population with T2D in the study had at least one cardiovascular risk factor (86.7%; including hypercholesterolemia, arterial hypertension, and smoking); if only modifiable risk factors are considered, the percentage is 65%. Nearly half the patients had hypertension (35.5% of males and 46.6% of females). One-third of the patients with hypertension were diagnosed during the study; the most common blood pressure abnormality was the coexistence of both systolic and diastolic hypertension (50.3%). Among PD patients with hypertension, only 80% had received hypertensive treatment and only 30.6% of those patients had blood pressure levels below 140/90 mmHg. Smoking was registered for 14.5% of patients, and 28.7% of patients reported having a family history of coronary artery disease. Dyslipidemia is one of the most common comorbidities in T2D, with associated triglycerides and non-HDL cholesterol levels being higher than in the general population. LDL cholesterol levels above 100 mg/dL were observed in 74.8% (95% confidence interval [CI]: 72.5%–76.9%) of T2D patients who were PD; however, only 7.6% (95% CI: 6.3%–9.1%) of those individuals knew they had hypercholesterolemia.

A high percentage of women with T2D had at least one pregnancy during their lifetime (n = 2373, 94.7%); this proportion was similar to that found in patients without T2D. However, the number of women who had suffered at least one abortion was higher in the group with diabetes (odds ratio [OR] = 1.62, 95% CI: 1.53–1.83) and a similar trend was found for stillbirth (OR = 1.99, 95% CI: 1.75–2.3); these differences were confirmed to be significant when adjusted by age. Fertility control is part of T2D management in order to reduce the obstetric morbidities that can result from unplanned pregnancies. Nevertheless, a high percentage of women with T2D did not use contraceptive methods during their reproductive years (42.5%) and this rate was not significantly different in women without T2D (38.8%). The lack of difference in the use of contraceptive methods by women with childbearing potential, with or without T2D, is a concern. However, the observation provides a window of opportunity for public health officials to devise policies aimed at reducing diabetes-related obstetric complications.

TYPE 2 DIABETES EXPRESSION IN DIFFERENT AGE GROUPS

Age is one of the most important determinants of T2D incidence, which varies from 3.2% in the population aged 20–29 years to 32.75% in people aged 60–69 years, and decreasing to 26.21% in the population over 70 years of age. The age of onset determines clinical characteristics and the burden of disease, with early onset increasing the social and economic burden because of

chronic complications and premature disability during productive years. In 2006, the prevalence of early-onset T2D was 5.8%; most of these individuals ignored their condition. However, because of the age distribution of the population, 22.7% of people with T2D are under the age of 40 years.

A substudy of the 1994 survey reported a T2D prevalence of 1.8% in the population under 40 years of age,[18] representing 14.8% of all T2D cases. Later, the 2000 survey reported a T2D prevalence of 2.3% for the 20- to 40-year age group; early presentation of T2D occurred in 13.2% of the T2D population. ENSANUT 2006 showed a substantial increase in the prevalence of disease both in the general population and in the 20- to 40-year age group (14.4% in the general population and 5.8% in the 20- to 40-year age group), reflecting a nearly 2-fold increase in the prevalence of T2D from the year 1994. The growing trend in T2D prevalence in Mexico is stronger for early-onset T2D, with the number of patients increasing from 318 400 in 1994 to 1 662 870 in 2006.

Early-onset T2D affects a heterogeneous population. Two-thirds of these individuals have a BMI $>25\ kg/m^2$, with hypertension and hypoalphalipoproteinemia being common comorbidities (32.5% for arterial hypertension and 79.3% for hypoalphalipoproteinemia). The conditions are usually treated with oral glucose-lowering agents. In contrast, insulin is more often used as a part of T2D treatment in nonoverweight patients. Within this subgroup, a study identified cases of MODY (with mutations in *HNF1α* or *HNF14α*) or positive anti-GAD antibodies (6% of cases).[19]

When compared with the overall population with T2D, the young T2D population had a higher prevalence of underdiagnosis (70%) and more school years, but a lower socioeconomic level. In terms of cardiovascular risk factors, the young population had higher alcohol and tobacco consumption, an average BMI of $27\ kg/m^2$, and a lower prevalence of hypercholesterolemia. In contrast, hypoalphalipoproteinemia (HDL cholesterol <40 mg/dL) was more common among them. Diabetic retinopathy was present in 7.6% of patients younger than 40 years of age, and 6.3% of these patients were referred after having suffered a previous myocardial infarction. Despite these complications and outcomes, few young patients undertake preventive measures, with very few receiving statins or acetyl-salicylic acid or follow-up with an ophthalmologist.

Elderly patients with T2D also comprise a heterogeneous population with two extremes.[20,21] The first is composed of T2D patients who have a longer exposure to the disease and who are, thus, prone to chronic complications, increasing third-party dependence, and requiring more complex disease management. The second group is composed of patients diagnosed with T2D after the age of 70 years; these patients have a low prevalence of microvascular complications, and their glucose levels can be kept stable with one or two oral hypoglycemic agents. Both groups are represented in similar proportions. Among older individuals with T2D, the mean age at diagnosis is 57 ± 10 years and the time of exposure to the disease is 11 ± 8 years. Almost half of them had been diagnosed 10 or more years ago, and the mean BMI was $28.4 \pm 5\ kg/m^2$; 82.2% were treated with only one hypoglycemic agent, and 7.6% received insulin treatment. Cardiovascular risk factors were common in this age group; 37.6% had smoked at least one cigarette within the previous month before the survey, 60% had hypertension, and 88.7% were taking one or more antihypertensive agents. Microalbuminuria was detected in 48.4% of cases. Geriatric problems were also common; 8.8% of patients within this age group had suffered a fall within the last year, motor limitations were reported by 30% of patients, and 17.8% reported regular use of sedative agents.

TREATMENT AND CONTROL

Correction and control of hyperglycemia is the basis for the prevention of microvascular complications (kidney disease, neuropathy, and retinopathy). A large percentage (93.3%) of T2D patients in ENSANUT 2012 reported receiving pharmacologic treatment; 84.8% were receiving oral agents for hypoglycemia, 6.8% were receiving insulin, and 2.5% were receiving a combination of both.[22] The mean HbA1c level, a marker of glycemia within the previous 6–8 weeks, was 9.3% (2.2% below the value reported in 2006). Only 25% of T2D patients had a HbA1c level $<7\%$; severe hyperglycemia (HbA1c $> 9\%$) was found in 50.3% of cases. These percentages are not satisfactory. In comparison, data from the Diabetes in Canada Evaluation, showed that 51% of patients had a HbA1c level $<7.0\%$, and in the United States the National Health and Nutrition Examination Surveys indicated that 57% of patients had HbA1c concentrations $<7.0\%$.

The inadequacy of glycemic control in Mexico cannot be attributed only to lack of access to medical care; 94.1% of patients had at least 1 medical evaluation in the previous year. Only 24.17% and 1.86% of patients considered diet and physical activity to be part of their treatment, respectively. On the other hand, alternative medicine was the treatment option for 6.1% of the T2D population. Only 21.7% of patients in ENSANUT 2012 had glycemic levels determined 4 or more times per year, and 7.7% of ENSANUT 2012 patients had at least 2 HbA1c determinations every year. Factors associated

Table 3 **Characteristics of patients with type 2 diabetes in the 2012 National Health and Nutrition Survey**

Characteristic	Value (95% CI)[a]
Age (yrs)	56.9 (56.6–57.0)
Time since diagnoses (yrs)	9.2 (9.0–9.6)
With pharmacologic treatment (%)	85.6 (85.0–86.1)
HbA1c <7% (%)	25.6 (20–31.2)
HbA1c >9% (%)	50.3 (44.6–55.9)
Four or more visits to a medical unit per year (%)	65.4 (64.9–66)
Two or more HbA1c measurements per year (%)	7.7 (7.3–8.2)
Current statin use (%)	2.6 (1.6–3.6)
Annual foot exam	14.7 (14.1–15.2)
Annual eye exam	8.6 (8.1–9.0)

[a]Values are presented as means or percentages and 95% confidence intervals.

with unsatisfactory glycemic control included age, low BMI, longer duration of T2D, and insulin use. The lack of adherence to dietary advice is a multifactorial issue. For example, the participation of dietitians and diabetes educators is very limited in a large proportion of primary care units because the Mexican health system was designed to treat acute infectious diseases. Major qualitative changes are needed to ensure that access to medical care results in measures that help patients modify unhealthy behaviors.[21] Table 3 describes the characteristics of patients with T2D in the 2012 National Health Survey.[22]

Only 80% of individuals with T2D and hypertension received antihypertensive medication, and 76.6% of cases had blood pressure levels higher than the therapeutic goals. Nearly half the patients with both T2D and hypertension had no knowledge of having high blood pressure. Only 5% of PD and treated patients reached their therapeutic goals, and nearly one-fifth of patients with hypertension did not receive treatment, despite being aware of their diagnosis. Additionally, less than 10% of T2D patients were being treated with statins, even though this therapy was indicated in more than half of the cases. Interventions that have been proven to result in a reduction of chronic complications, such as the regular administration of low-dose acetyl-salicylic acid, are not well implemented (only in 10% of cases). As a result, the proportion of patients who fulfill the attention quality indicators for T2D is low. Gakidou et al.[23] compared the data from Mexico against results of surveys conducted in the United States, Asia, and Europe. Mexico's performance regarding the attention given to hyperglycemia and other comorbidities associated with T2D was poor; less than 5% of cases reached therapeutic goals as measured by HbA1c concentrations, blood pressure, and LDL cholesterol levels. Only 20% of cases received adequate treatment without reaching treatment goals; one or more of the therapeutic goals had not been diagnosed or treated in the rest of the cases. The country with the best performance was the United States, with 10% of patients receiving optimal treatment, 50% insufficient treatment, and 40% having at least one diagnosis omitted.

CHRONIC COMPLICATIONS

Screening for T2D chronic complications is an area of opportunity for improving diabetes care in Mexico. Only 14.7% of T2D patients had an annual foot exam, 8.6% underwent retinopathy detection, and 12.6% had an albuminuria measurement. In this population, 14.6% referred to having some degree of retinopathy, 13.4% had lost sensation in at least 1 part of their bodies, 9.4% reported having had ulcers in legs or feet, 4.9% were blind, 3% had some amputation, 2.3% had been diagnosed with a diabetic foot, and 1.2% had received dialysis.

T2D is one of the main causes of premature disability, blindness, terminal chronic kidney disease, and nontraumatic amputations, as well as 1 of the 10 most frequent causes of hospitalization in Mexican adults. In 2009, 2.8% of hospital discharges in Mexico were due to the management of T2D complications. The institution with the heaviest diabetes-related burden is the Instituto Mexicano del Seguro Social, which provides medical care to 44.9% of the country's T2D population. The Health Ministry delivers care to 36.2% of T2D patients; in its hospitals, there were 51 807 discharges following treatment for T2D in 2007,[22] and 36% of them were due to chronic complications.[24] A study conducted in the State of Mexico,[25] which included 44 458 subjects diagnosed with T2D, registered the presence of diabetic retinopathy in 10.9% of the studied population, diabetic nephropathy in 9.1%, peripheral neuropathy in 17.1%, ischemic cardiomyopathy in 4.2%, and stroke in 1.7%. In the Mexico City Study, the prevalence of proliferative and nonproliferative diabetic retinopathy was 8% and 40%, respectively; the incidence of retinopathy after a 4-year follow-up period was 22.5%.[26] Insufficient information exists regarding the impact of chronic complications on nutritional aspects (i.e., sarcopenia).

FUTURE ESTIMATES FOR THE INCIDENCE OF T2D COMPLICATIONS

Based on the data of patients with T2D from ENSANUT 2006, Reynoso-Noverón et al.[27] estimated that, in Mexico, 112 cases per 1000 persons with T2D will suffer at least 1 ischemic coronary event within the next 20 years. In the same period, there will be 889 433 new cases of heart failure, 2 048 996 events of myocardial infarction, 798 188 stroke events, and 491 236 nontraumatic amputations attributable to T2D. The

expected mortality rate due to T2D is reported to be 539 per 1000 persons with the disease, and average life expectancy is 10.9 years.

COST OF DIABETES

The direct and indirect costs of T2D treatment are remarkable. In 2010, researchers from the National Institute of Public Health in Mexico calculated that the more substantial direct costs are associated with medications ($133 143 734), followed by complications ($110 410 928), medical consultations/diagnosis ($59 734 448), and hospitalization ($39 937 331). Indirect costs are mainly due to permanent disability ($409 205 846), followed by costs due to premature mortality ($19 623 029) and temporal disability ($6 372 059). Both the direct and indirect costs are paid mainly by patients and social security institutions.[28]

During 2012, 168 406 hospital admissions were associated with diabetes-related complications, accumulating over 685 208 days of hospital stay. With an average cost per bed-day of $2150–$5500 Mexican pesos, this amounted to a per-stay cost burden of $1473 million to $3768 million pesos. These figures do not include costs for emergency care, nutritional care, or rehabilitation.

AREAS LACKING SUFFICIENT INFORMATION

Despite the information obtained by the National Health Surveys, there are still aspects of T2D epidemiology in Mexico that have not been fully explored; 2 examples of information gaps are the prevalence of T2D in special groups and the incidence of diabetes. The first is attributed to the lack of representative studies that explore diabetes prevalence in children, adolescents, pregnant women, type 1 diabetes, indigenous groups, and groups with higher risk for secondary diabetes. Studies within the pediatric population are usually limited to cohort studies of cases in reference hospitals; such studies report that the percentage of T2D cases has increased 2-fold in recent years. Only one study used a population-based approach.[29] The situation is similar for gestational diabetes mellitus; a recent study of 905 female patients of the National Institute of Perinatology founded a prevalence of 10.3% using the American Diabetes Association criteria and 30.1% when using the criteria of the International Association of Diabetes and Pregnancy Study Groups.[30] While there are reports on the prevalence of T2D in some indigenous Mexican groups (Mazatecas,[31] Otomíes,[32] Pimas,[33] Yaquis, Tepehuanas, Purépechas, Huicholes, and Mexicaneros[34,35]), these studies have small sample sizes and do not represent the indigenous Mexican population. Despite these limitations, most recent reports show a trend towards increasing prevalence, similar to what has been observed in rural populations. Studies focused on indigenous groups that live in urban areas are required because these individuals have gone through rapid lifestyle modifications and have a greater incidence of T2D.[36,37] Additionally, there are no national records or interinstitutional databases that allow evaluation of the Mexican national health system's effectiveness in T2D treatment.

The Mexican government has implemented several initiatives to decrease the exposure of the population to calorically dense products (i.e., sodas and junk food) during the past 6 years. These included taxes and the regulation of mass media. Assessment of the impact of these interventions is ongoing.

CONCLUSION

T2D and other chronic diseases must be confronted with appropriate actions. This has been the proposal of national prevention plans[38,39] and it follows the recommendations of the World Health Organization[40] and the Pan American Health Organization.[41] The goals are preventing new cases, decreasing the incidence of complications, and reducing mortality and disability.

The natural history of T2D can be modified. In particular, actions that halt the growing trend of obesity may have a remarkable impact on T2D incidence in Mexico. In addition, taking action against obesity may decrease the proportion of T2D cases with comorbidities, which are associated with higher morbidity and mortality (i.e., lipid disorders or arterial hypertension). Campaigns to stimulate the adoption of a healthy lifestyle should be established with the goal of facilitating permanent change and messages should be adapted to the needs of various subsets of the population. For the assessment of such interventions, the development of prognostic tools and the creation of pharmacoeconomic models should be built in the next few years.

The national health system needs major adjustments to confront the challenges caused by T2D. Primary care units should be organized to make diagnoses on time and provide low-cost, structured prevention programs that include the provision of patient-centered nutritional support. In addition, a renewed focus on the effectiveness of the interventions should be reinforced. The lack of effectiveness is explained by factors attributable to the health system, physicians, and patients. Diabetes management is based on principles that differ from those of communicable diseases, requiring a complex educational process to understand the disease, behavioral changes by the patient, the long-term use of multiple drugs, frequent clinical evaluations, and a unified effort by the patient, specialists,

family members, and the community.[42] The structure and procedures currently implemented by most healthcare institutions in Mexico are not equipped for such a treatment approach. The length of medical consultations should be sufficient to implement a treatment plan and to detect chronic complications. The prominent role of highly specialized physicians should be replaced with greater participation of a wide range of health professionals (i.e., nutritionists, physical education specialists, psychologists, educators, and physical therapists, among others). Involvement of the family, in order to support lifestyle changes and other elements that are critical for treatment adherence, must be taken into consideration in a systematic manner.[43,44] Empowerment of the individual to make wise decisions regarding his or her lifestyle and T2D treatment is feasible. Treatment should be adapted to the patient's needs and expectations.[45] Surveillance programs (using internationally accepted indicators) may have a significant impact, in less than a decade, on decreasing the cost and burden imposed by T2D on the healthcare system. Specifically, close to 20% of the preventable deaths in Mexico are caused by T2D. Even a small decrease in this rate could result in substantial savings for the healthcare system in Mexico.

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THE JOURNAL OF LATIN AMERICAN GERIATRIC MEDICINE REVIEW ARTICLE

Management of type 2 diabetes in the elderly patient

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Abstract

Type 2 diabetes is a rising global problem; elderly patients have the highest prevalence and their management is complicated by the presence of comorbidities and age-related changes. When establishing a treatment regime for elderly individuals, concerns in terms of functional status, living arrangements, the presence of frailty, cognitive impairment, and risk of hypoglycemia must be considered before selecting specific treatments. Geriatric assessment must be sought to maximize the potential benefit of treatment. Glycemic targets must take into consideration the presence of comorbidities, life expectancy, and the risks associated with tight glycemic control. In general, HbA1c goals between 7.5-8.0% are regarded as appropriate for elderly individuals. Regardless, goals must be adjusted in relation to treatment response and expected complications. Diet therapy and physical activity are the cornerstone of treatments to improve glycemic control and maintain an adequate functional status; pharmacological first-line therapy includes the use of metformin, which carries a low risk of hypoglycemia and has been associated with improved outcomes. Consideration of combined therapy must be weighed against hypoglycemia and cardiovascular risk, expected adverse reactions, and potential benefits from more intensive treatment regimes. Cardiovascular risk management must be focused on hypertension management and lifestyle changes such as cessation of smoking and moderate weight loss; statin use must be individualized considering life expectancy, cognitive status, and the presence of frailty to improve benefits. (J Lat Am Geriat Med. 2017;3:26-36)

Key words: *Type 2 diabetes. Management. Metformin. Frailty. Antidiabetic medication.*

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CHALLENGES OF TYPE 2 DIABETES MELLITUS MANAGEMENT IN THE ELDERLY PATIENT

Type 2 diabetes mellitus (T2D) is a chronic, degenerative disease that represents a significant health burden worldwide, being especially relevant in elderly patients and affecting up to 20% of the population. The elderly patient with T2D belongs to a rather heterogeneous spectrum of disease presentation. This age group may include cases with early onset T2D patients that have long disease exposure and high susceptibility for the development of chronic complications, which increases the chance of dependence and complex management[1]. But also, T2D patients diagnosed at an older age, usually ≥ 70 years, have a low prevalence of microvascular complications and can reach glycemic targets with one or two antidiabetic agents. The complexity of management is increased by the interaction of T2D with comorbidities and geriatric syndromes, which increases the likelihood of poor management, additional diabetes-related complications, and preventable mortality[2]. Recent

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evidence suggests that the combination of geriatric syndromes and cardio metabolic conditions significantly impact functional and cognitive capacity, especially in elderly women[3]. The demonstration of the interaction of geriatric syndromes and T2D for the development of comorbidity, frailty, functional and cognitive dysfunction has not been extensively studied.

Even though the prevalence of frailty in elderly patients might be up to 25%, the inclusion of this population in randomized clinical trials has been inconsistent, making the availability of reliable clinical data rather scarce[4] on the development of geriatric complications such as urinary incontinence, falls, frailty, cognitive impairment, dementia, and functional dependence[5]. Furthermore, diabetes has been associated to an increased risk of disability in mobility, activities of daily living (ADL) and instrumented activities of daily living (IADL)[6]. The International Diabetes Federation (IDF) recommends initiating T2D management in elderly individuals considering functional status in three categories: functionally independent, functionally dependent, and end-of-life care. They further categorize functional dependence per the presence of frailty and/or dementia[7]. The recommended assessment requires a multidisciplinary approach to assess functional capabilities as well as medical and psychosocial comorbidities for the designation of treatment and rehabilitation plans, management of comorbidities, and requirements for long-term and end-of-life care[8]. Table 1 summarizes the primary evaluations and procedures required for a simplified assessment before initiating treatment.

Living arrangements and psychosocial support are important determinants of success in T2D management[4]; this is especially true for patients living in long-term care facilities. Functional dependence modifies self-care responsibilities and requirements for disease management in this population. Recommendations by the American Diabetes Association (ADA) suggest that this population receive more simplified treatment regimes, liberal diet plans, implementation of physical activity and exercise, and avoidance of sliding scale insulin regimes. Specific comments on these strategies will be discussed in later sections.

Elderly patients with T2D and hypoglycemic medications are at a higher risk of developing complications that are implicit to this treatment[9]. Older patients with frailty are at an increased risk of falls and disability, and this is especially true for individuals with comorbid T2D[9] in whom sarcopenia or muscle mass loss occurs at higher rates because of increased catabolism. A higher rate of falls in T2D patients has been associated to the occurrence of frailty, cognitive impairment and, most importantly, to the rate of hypoglycemia in elderly individuals[10]. Further, the occurrence of chronic diseases associated with protein malnutrition, muscle wasting, and frailty have directed to resolution of hyperglycemia and normalization of HbA1c levels, leading to the coining of the term "burnt-out diabetes"; frailty has also been associated to increased insulin resistance in obese frail individuals[11]. The effect of frailty must be considered when establishing a plan for T2D management in elderly populations[8,11].

Hypoglycemia risk is an important challenge that must be addressed in elderly individuals with T2D for management implementation[7,8]. Hypoglycemia increases the risk of morbidity, mortality, frailty, and disability, leading to impaired quality of life in elderly individuals with T2D[9]. In the elderly, autonomic dysfunction may lead to decreased recognition of hypoglycemic events, thus increasing the risk of severe hypoglycemic episodes that require hospitalization, which might lead to increased cognitive and physical dysfunction[11,12]. The interplay between hypoglycemia, polypharmacy, frailty, and dementia in elderly individuals further complicates management; frail patients with tight glycemic control as indicated by decreased HbA1c levels or with medications that increase hypoglycemia risk (long-acting sulfonylureas and complex insulin regimes) tend to have an increased risk of hypoglycemic events, especially at the onset of consistent weight loss[13]. Furthermore, the rate of occurrence of hypoglycemic events has been linked to an increased risk of developing dementia, falls, and frailty[10-14]. The consideration of treatment goals and minimizing exposure to hypoglycemia-inducing medications is an important consideration for control and prognosis of T2D individuals and must be individualized for every patient.

TREATMENT GOALS

Elderly patients with T2D have a higher rate of vascular complications, including heart failure and coronary artery disease[15]. In patients with long disease exposure (> 10 years) the rate of microvascular complications exceeds the rate of cerebrovascular disease, especially in the case of diabetic eye disorders[9]. The role of glycemic control has been extensively studied for young adults, and large trials have been conducted comparing standard versus intensive glycemic targets. Nevertheless, evidence from the largest trials, including the UK Prospective Diabetes Study (UKPDS), the Action to Control Cardiovascular Risk in Diabetes

Table 1. Interventions and procedures for geriatric assessment before treatment initiation

Assessment	Tools and procedures	Relevance
Physical performance	SPPB and IDOP 3-step package	Assessment of balance and gait speed (both), as well as gait power (SPPB) which is impaired in frail patients
IADL	Lawton index, Barthel IADL index	Diabetes increases risk of disability and IADL impairment
ADL	Katz index, Barthel ADL index	Screening methods for assessment of complex regime implementation
Cognition	Mini-Mental State Examination, Montreal Cognitive Assessment, MiniCog	MiniCog is more specific for elderly with T2D, all are screening and non-diagnostic
Depressive symptoms	Geriatric Depression Scale - GDS	Depression is a common comorbidity in T2D patients and increases the risk of cognitive impairment
Frailty	Fried Frailty Phenotype	Diabetes increases the risk of frailty and the development of complications and premature mortality
Nutrition	Mini-Nutritional Assessment - MNA	Designing nutritional interventions and identifying patients at risk of malnourishment
Quality of Life (QoL)	Audit of Diabetes Dependent Quality of Life Senior - ADDQoL Senior, SF-36 questionnaire	Validated for older people with diabetes Validated in nursing homes; SF-36 evaluates quality of life in 8 domains
Cardiovascular risk assessment	Globorisk score[1]	Cardiovascular risk assessment might be relevant for prevention of complications and further functional impairment

ADL: activities of daily living; IADL: instrumented activities of daily living; SPPB: Short Physical Performance Battery.
Adapted from: Sinclair A, Dunning T, Rodriguez-Mañas L. Diabetes in older people: new insights and remaining challenges. Lancet Diabetes Endocrinol. 2015;3:275-85, and IDF Global Guideline for Managing Older People with Type 2 Diabetes, International Diabetes Federation, 2013. [1]Hajifathalian K, Ueda P, Lu Y. A novel risk score to predict cardiovascular disease risk in national populations (Globorisk): a pooled analysis of prospective cohorts and health examination surveys. Lancet Diabetes Endocrinol. 2015;3:339-55.

(ACCORD) trial, the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trial, and the Veterans Affairs Diabetes Trial (VADT) have included a low proportion of elderly patients with ages ranging from 53 to 66 years, with most including less than 2% of adults aged ≥ 80 years[16]. Most trials have excluded elderly populations due to the high rate of hypoglycemia with intensive glycemic targets (HbA1c < 7%), which makes the application of major trial results a challenge.

In setting a target goal for T2D management in elderly patients, an estimation of benefits in terms of hyperglycemia management and prevention of micro and macrovascular complications must be contrasted to the risk of treatment complications[8]. In terms of available data, there is no evidence for increased protection against major cardiovascular events for intensive glycemic control in the first 10 years of treatment[17,18]; additionally, the ACCORD trial showed increased mortality in the group of intensive glycemic control[19]. Reduction of microvascular complications have mostly been reported for the UKPDS trial, which included a younger population and showed benefits mostly after 8-15 years of intensive glycemic control[20]. However, harms of intensive glycemic control have been reported in all four major studies, showing an increased risk of hypoglycemia and an associated increase of decline in cognitive function[21]; age, longer disease exposure, polypharmacy, and cognitive impairment put elderly individuals at a higher risk of hypoglycemic episodes.

Glycemic goals for elderly individuals have not shown benefits for HbA1c levels ≤ 7.5%. Nevertheless, consensus data[22] show that HbA1c levels > 9% lead

to increasing rates of polyuria, fatigue, and cognitive impairment. Therefore, HbA1c range levels for optimal treatment are between 7.5-8.0% and must be adjusted based on perceived patient preferences[10], including burden of treatment (especially insulin injections), continuous glucose monitoring, life expectancy, and associated complications associated to disease exposure. When exploring factors that influence glycemic target decisions, one study showed that more intensive control targets were associated with higher baseline HbA1c levels, weight, and male physicians; glycemic target goals had an average HbA1c of 7.0%[23]. In general, disease duration, age, and polypharmacy did not affect glycemic target decisions; once the HbA1c targets have been reached, treatment de-intensification must be considered per patient's preferences and clinical assessment[24].

TREATMENT CHOICE

Diet and exercise

Elderly patients with T2D are at an increased risk of developing malnutrition[25]. In older patients, both community-dwelling and those living in long-term care facilities, a body mass index (BMI) level in the underweight category has been associated with increased mortality[26]. However, changes in body composition with aging modify the predictor capacity of frailty in elderly patients[27]. In addition, malnutrition has been associated to adverse outcomes, including pressure ulcers, delirium, depression, decreased bone mineral density, and frailty[28]. Therefore, evaluation of patients at higher risk using screening tools and biochemical assessment might be necessary before establishing a dietary lifestyle intervention[8]. Weight reduction must be gradual, especially because weight loss in overweight and obese patients can result in nutritional deficits and decreased mineral bone density. A combined approach of physical activity adjusted for functional status along with nutritional therapy with consistent carbohydrate amounts to prevent hypoglycemia and protein intake adapted to frailty status must be individualized per patient's needs[8,28]. This intervention improves functional status, psychological and cognitive function, and glycemic control[8].

SPECIFIC ANTIDIABETIC AGENTS

Pharmacological therapy in the elderly patient with diabetes must be managed in accordance with the presence of comorbidity; pharmacokinetic modifications associated with ageing and the presence of polypharmacy must be considered when prescribing for this age group. In general, high quality evidence studies that evaluate glycemic treatment in older adults, especially those over 80 years of age, are lacking. Therefore, most data are based on small-scale sub-analyses of patients included in larger studies within the required age range.

Metformin is regarded as the first-line therapy for the management of elderly patients with T2D[8,29]. However, when HbA1c levels are not achieved, the second-line agent is not well-established. Table 2 outlines the main pharmacological options for the management of T2D in older adults.

Metformin

Most guidelines recommend metformin as first-line therapy for the treatment of T2D in elderly patients[4,7,29]. When compared to other oral glucose-lowering agents, metformin has a low risk for hypoglycemia and generally has a favorable safety profile; the concomitant use of sulfonylureas and metformin[30] might lead to an increased risk of hypoglycemia compared to monotherapy, and this has also been shown for other medications. The use of metformin as monotherapy leads to a decrease in 0.5-1.0% of HbA1c levels and increases insulin sensitivity whilst promoting weight loss[10].

Two randomized clinical trials (RCT), the ADOPT[31] (A Diabetes Outcome Progression Trial) and the SPREAD-DIMCAD[32] (Study on the Prognosis and Effect of Antidiabetic Drugs on Type 2 Diabetes Mellitus with Coronary Artery Disease) trials showed a reduction in cardiovascular mortality associated with metformin use in comparison to sulfonylureas, which has also been observed in a few observational studies[29]. However, follow-up in these studies has been short and the reduction in cardiovascular outcomes has been modest; furthermore, meta-analyses have shown inconsistent results[33]. Thus, results on the effect of metformin on cardiovascular mortality must be interpreted with caution.

A relevant safety concern for the use of metformin in elderly patients is its use in patients with impaired kidney function. The safety concern was based on early pharmacokinetic studies, which showed that patients with severely impaired kidney function had an increased risk of lactic acidosis. In elderly patients, estimation of renal function based on serum creatinine measurements might be incorrect and result in overestimation of kidney

Table 2. Pharmacological options for management of type-2 diabetes in the elderly

Medication group	Glycemic control	Adverse effects and safety concerns	Potential benefits
Biguanide (metformin)	1-2% reduction in HbA1c	Risk of lactic acidosis eGFR must be measured for all patients taking this medication Consider dose adjustment for patients < 45 ml/min/1.73 m^2; do not use for eGFR < 30 ml/min/1.73 m^2 or in decompensated heart failure Functional and frailty status must be considered because of unintentional weight loss Gastrointestinal adverse effects	Reduced cardiovascular events and mortality Not associated with weight gain or hypoglycemia First-line therapy for patients without impaired renal function
Sulfonylureas (glipizide, gliclazide)	1-2% reduction in HbA1c	Risk of hypoglycemia and weight gain; combination with metformin increased hypoglycemia risk Avoid long-acting sulfonylureas due to increased risk of hypoglycemia (glyburide)	Cardiovascular benefit has not been consistently shown
Glinides (repaglinide, nateglinide)	0.4-0.9% reduction in HbA1c	Risk of hypoglycemia and associated weight gain Nateglinide must be avoided in patients with renal failure	Shorter half-life when compared to sulfonylureas Might be useful in patients with bad eating habits with frailty or dementia
Thiazolidinediones (pioglitazone)	1-2% reduction in HbA1c	Fluid retention, weight gain, increased risk of heart failure Increased fracture risk	Increased risk of heart failure and myocardial infarction (the latter for rosiglitazone)
A-glucosidase inhibitors (acarbose)	0.4-0.9% reduction in HbA1c	Gastrointestinal adverse events	Reduction of cardiovascular events in patients with carbohydrate intolerance Reduction of postprandial hyperglycemia
GLP-1 agonists (exenatide, liraglutide)	1% reduction in HbA1c	Gastrointestinal adverse events (can be minimized with gradual dose increase), unintentional weight loss (should be avoided in frail patients)	Low risk of hypoglycemia; reduces fasting and postprandial hypoglycemia Uncertain risk of acute pancreatitis
DPP-4 inhibitors (sitagliptin, saxagliptin, linagliptin)	0.5-0.8% reduction in HbA1c	Uncertain risk of acute pancreatitis and joint pain	Neutral effects on major cardiovascular events, risk of heart failure still not clear
SGLT2 inhibitors	0.5-0.7%	Weight loss, blood pressure lowering, vulvovaginal candidiasis and urinary tract infection Avoid for eGFR < 60 ml/min/1.73 m^2 Risk of euglycemic diabetic ketoacidosis	Reduction in rates or cardiovascular events and mortality Ameliorates progression of kidney disease
Insulin	Variable	Risk of hypoglycemia and weight gain Requires self-monitoring, especially prandial insulin regimes Might not be the best choice for patients with frailty or dementia	Long-acting insulin can be a safer choice in combination with oral glucose-lowering agents

DPP-4: dipeptidyl peptidase-4; eGFR: estimated glomerular filtration rate; GLP-1: glucagon-like peptide-1; SGLT2: sodium-glucose co-transporter type 2.

Adapted from: Sinclair A, Dunning T, Colagiuri S. Managing older people with type 2 diabetes: global guideline. International Diabetes Federation 2013. Lipska KJ, Krumholz H, Soones T, Lee SJ. Polypharmacy in the Aging Patient: A Review of Glycemic Control in Older Adults With Type 2 Diabetes. JAMA. 2016;315:1034-45. Maruthur NM, Tseng E, Hutfless S, et al. Diabetes Medications as Monotherapy or Metformin-Based Combination Therapy for Type 2 Diabetes: A Systematic Review and Meta-analysis. Ann Intern Med. 2016;164:740-51.

dysfunction. Instead, the use of estimated glomerular filtration rate (eGFR) must be encouraged for decision making[34]. Current guidelines recommend caution and frequent monitoring when implementing metformin treatment in patients with eGFR < 60 ml/min/1.73 m^2 and it is contraindicated for eGFR < 30 ml/min/1.73 m^2. Nevertheless, evaluation of kidney function must be sought in every elderly patient prior to metformin initiation and must be evaluated in every consult, given the possibility of decreased kidney function in this population[7]. Furthermore, recent observational data has suggested that historical contraindications of metformin use, such as chronic kidney disease (CKD), congestive heart failure (CHF), and chronic liver disease (CLD) might benefit with the use of metformin[35] and has had changes approved by the Food and Drug Administration (FDA)[36] for CHF and CKD. Given that elderly patients usually have additional comorbidities associated with T2D, metformin use in individuals with CKD, CHF, and CLD must be individualized to maximize the potential clinical benefit.

Adverse effects of metformin use include gastrointestinal effects and unintended weight loss (usually associated with side effects). This latter effect might be significant for patients at higher risk of complications such as individuals with frailty syndrome[7,29]. Nevertheless, there has been some data regarding a possible protective effect of metformin on the development of frailty and frailty-associated complications. However, in a cohort study of 2,415 elderly individuals with T2D, metformin compared with sulfonylurea was associated with a 30% decreased risk of mortality among those without any frailty-related diagnoses, but was not significantly associated with decreased risk of mortality among those with frailty-related markers. Clinical trials evaluating the effect of metformin on pre-frail individuals and on the progression and prevention of frailty are currently ongoing and pending preliminary results[11,37,38]. Metformin has also been linked to vitamin B12 deficiency in several studies; a recent meta-analysis demonstrated that metformin use decreased vitamin B12 levels by 57 pmol/l, which might lead to a deficiency status in patients with T2D[39]. The decrease in vitamin B12 levels has been shown to be more important for at-risk populations including elderly individuals; susceptibility for vitamin B12 testing included comorbidities and chronic microvascular complications, but was not consistently done in elderly patients[40].

Sulfonylureas and glinides

Sulfonylureas and glinides are a reasonable first-line therapy when metformin use is contraindicated or if the patient cannot tolerate the adverse events from metformin use[7,29]. The risk of hypoglycemia and increased weight gain associated with the use of both pharmacological classes limits the use of these medications in elderly populations. Initial monotherapy with sulfonylureas is not supported by current evidence[41]. The American Geriatrics Society recommends against the use of long-acting sulfonylureas (glyburide) in elderly patients because of an increased risk of hypoglycemia[42]. The World Health Organization (WHO) recommended that gliclazide should be considered as the preferred sulfonylurea in elderly patients, with glimepiride and glipizide as acceptable alternatives; these recommendations were supported by the ADVANCE study, which showed no increase in weight gain and low rates of hypoglycemia for gliclazide[43].

Glinides have a shorter half-life (60-90 minutes) when compared to sulfonylureas. Both repaglinide and nateglinide should be taken before meals and can be skipped in patients with frailty and dementia and irregular eating habits; they usually have a lower rate of hypoglycemia[7]. Nateglinide should be avoided in patients with severe kidney failure[10].

Dipeptidyl peptidase-4 inhibitors

The safety profile of dipeptidyl peptidase-4 (DPP-4) inhibitors makes them a feasible and tolerable option for use in the elderly[44]. Because there is decreased incretin inactivation and its action is glucose-dependent, risk of hypoglycemia is minimized in the elderly[45]. The evidence of DPP-4 inhibitors in the elderly has mostly been shown in subgroup analyses of large clinical trials; of the approved molecules, vildagliptin and linagliptin have shown greater evidence of safety and efficacy in patients > 75 years of age[46,47]. However, comparisons have mainly been assessed against placebo and not against another approved monotherapy[48].

Safety concerns on DPP-4 inhibitors included conflicting reports on an increased fracture risk and increased risk of heart failure or hospitalization due to hear failure. Two recent meta-analyses evaluated the available evidence on the incidence of fracture risk and determined that the use of DPP-A inhibitors does not modify bone fracture risk in comparison to placebo or other antidiabetic medications[49,50]. The risk of heart

failure progression or hospitalization due to heart failure is a concern that has limited the use of these agents in high-risk patients. However, meta-analyses of this safety issue have shown mixed results with mostly marginal, non-significant increases in heart failure risk, especially with saxagliptin[51,52]. The use of DPP-4 inhibitors is attractive in the elderly, remaining an alternative treatment to metformin or as an add-on therapy to reach glycemic goals; potential neuroprotective benefits are being evaluated for its effect on cognition[53].

Alpha-glucosidase inhibitors

Acarbose is an alpha-glucosidase inhibitor that reduces intestinal absorption of glucose; it has mainly been used to treat postprandial hyperglycemia and carries a low risk of hypoglycemia in elderly populations[54]. Acarbose has recently been studied for its effect on postprandial hypotension, which is a phenomenon that increases the risk of falls, mortality, and cardiovascular adverse outcomes in elderly patients[55]. Acarbose has been shown to attenuate the decrease in postprandial systolic pressure, syncope, falls, dizziness, and weakness by reducing splanchnic gastrointestinal circulation[56]. Its safety profile has made it an adequate alternative first-line treatment for patients who do not tolerate metformin treatment or who have failed glycemic goals with metformin alone; gastrointestinal side effects might contribute to discontinuation, but this has not been consistent across trials and they are usually present at higher dosages[57]. Some studies have suggested a potential cardiovascular benefit, but mostly on combinations with other protective measures[58].

Glucagon-like peptide-1 agonists

Data on the use of glucagon-like peptide-1 (GLP-1) receptor agonists has been scarce in elderly patients, but generally showed an efficacy and safety profile similar compared to younger populations[7]. A recent study showed that lixisenatide has a pharmacokinetic, efficacy, and safety profile that suggests it is useful in elderly patients[59]. However, attention must be paid in differentiating effects of short-acting (exenatide) and long-acting GLP-1 agonists. Glycemic targets are more easily reached with long-acting GLP-1 agonists, and vomiting and nausea are less compared to short-acting agents[60,61].

Gastrointestinal side effects are significant with GLP-1 agonists[62], but are mostly seen in early use and have been shown to decrease with gradually increasing dosages[63]. The GLP-1 agonists, especially liraglutide, have been associated with moderate weight reduction and low-risk hypoglycemia[64]. However, evidence of its efficacy and safety in elderly obese individuals has not been studied; weight loss might be a cause of concern in frail individuals[7,8].

SODIUM-GLUCOSE CO-TRANSPORTER-2 INHIBITORS

Inhibition of sodium-glucose co-transporter-2 (SGLT2) causes glycosuria dependent on blood glucose levels and glomerular filtration rates. It is therefore contraindicated in patients with impaired glomerular function, which may limit its use in elderly populations[65]. Data on canagliflozin suggests that there is a significant but non-sustained decrease in body weight and systolic blood pressure, which has been consistent with data found in younger populations[66]. Side effects of SGLT2 inhibitors limits their applicability in elderly patients, given reports of increased urinary frequency, vulvovaginal mycotic infections, urinary tract infections, postural hypotension, dehydration, and falls, which might discourage their prescription in this population[58].

Recent data from the EMPA-REG study suggested that empagliflozin might have cardiovascular benefits, especially in the setting of heart failure, signaling a role for its use in high-risk elderly patients[67]. In addition, a follow-up report on this study reported a decrease in the rate of progression of kidney disease in patients at high cardiovascular risk[68]. However, these studies did not include an older population and its efficacy in cardiovascular risk reduction was marginal. Outcome data for elderly populations at high risk must be evaluated in longitudinal studies to investigate the potential cardiovascular benefit of SGLT2 inhibitors.

Thiazolidinediones

Thiazolidinediones carry a low risk of hypoglycemia. However, their side effect profile makes them a poor candidate for treating T2D in elderly patients[7]. Studies showing an increased risk of fractures in women above and below 50 years and in males above 50 years have been consistently reported for both rosiglitazone and pioglitazone[69,70]. Furthermore, there is an increased risk of heart failure and risk of worsening in patients with established heart failure

reported for thiazolidinediones as well as increased cardiovascular mortality for rosiglitazone, which limits their prescription for high-risk patients[71,72]. Besides additional side effects, including weight gain, fluid retention and edema, the ACCORD-MIND trial suggested a potential effect of thiazolidinediones on cognitive decline, which might be detrimental given the increased risk of cognitive impairment in elderly individuals with diabetes[73]. Therefore, prescription of these drugs must be individualized and considered only in selected cases.

Insulin treatment

In elderly patients, management of hyperglycemia has differential benefits in terms of adequately controlled basal and postprandial glucose levels[74]. The various presentations of insulin must therefore be individualized per the patient's context. Simple regimen insulin levels have shown benefit in both glycemic control and reducing the rate of hypoglycemia; the use of rapid insulin analogs has also been a matter of concern, and they are generally less prescribed in this population in comparison to long-acting insulin, which has been related to lower hypoglycemic nighttime events[75,76]. The device used for administration has been scrutinized in some studies, suggesting that vial and syringe methods yield lower treatment persistence and decreased adherence as well as lower hypoglycemic episodes compared to pen initiators, with no difference between insulin-naive and non-naive patients[77]. Nevertheless, most trials have small sample sizes and have inadequate methodological quality, which impairs the ability to make specific recommendations.

Another issue to take into consideration when initiating insulin management in elderly patients is their functional level and dependence on IADL and ADL. For individuals in long-term care facilities, the use of oral agents or basal insulin was evaluated in one RTC, showing that there were no differences in glycemic control, rate of hypoglycemia, and number of complications, emergency room visits, and mortality[78]. For community-dwelling individuals, insulin glargine or determir as a basal insulin regime has been shown in prospective RCTs to achieve adequate glycemic control and reduced daytime hypoglycemia rates compared to thiazolidinediones, insulin lispro and normal pressure hydrocephalus and lifestyle/dietary measures[79] and in addition to concomitant oral antidiabetic drugs[80]. The comparison of adjuvant oral antidiabetic agents and insulin treatment as monotherapy has shown significant clinical improvement in glycemic control, whilst reducing insulin requirements. Combinations with sulfonylureas should be avoided, given the increase in hypoglycemic events, and combinations with metformin diminish weight gain with no increase in adverse events[81]. Therefore, insulin treatment should be considered in elderly patients as a second- or third-line treatment to achieve glycemic goals, especially in undernourished subjects. Regimes should mostly consist of basal insulin combined with metformin unless it is not well tolerated; prandial insulin results in a higher rate of hypoglycemia and prescription errors compared to long-acting basal insulin regimes[7].

CARDIOVASCULAR RISK MANAGEMENT

Cardiovascular disease is the most prevalent cause of mortality in elderly T2D patients. Smoking discontinuation and treatment with low-dose aspirin should be considered in elderly individuals according to life expectancy. These interventions have a greater benefit/risk ratio in this age group and should thus be considered for prevention of cardiovascular disease[28]. Hypertension plays a significant role in this association with cardiovascular mortality, contributing to 75% of specific complications[82]. Antihypertensive medications have been associated with a reduced cardiovascular morbidity and reduced incidence of stroke and heart failure, without a significant impact on mortality. Consensus has been reached on a target blood pressure (BP) goal of 140/90 mmHg; no significant benefit has been seen with lower blood pressure targets and there have even been reports of increased mortality for BP < 115/65 mmHg[83]. Lifestyle intervention is based on decreased sodium impact and it generally has a minimal impact on BP control. The drug of choice for elderly patients with T2D with hypertension and/or albuminuria is either an angiotensin-converting enzyme inhibitor (ACEI) or an angiotensin-II receptor blocker (ARB)[84]; both have shown benefit on decreasing the risk of major cardiovascular events and reduction in the progression of kidney disease[7,85]. Add-on therapies include combinations with thiazide diuretics, beta-blockers, and calcium channel blockers; however, benefit is inferior with those therapies compared to ACEI/ARB.

The use of statins for secondary prevention of cardiovascular disease in elderly patients remains an

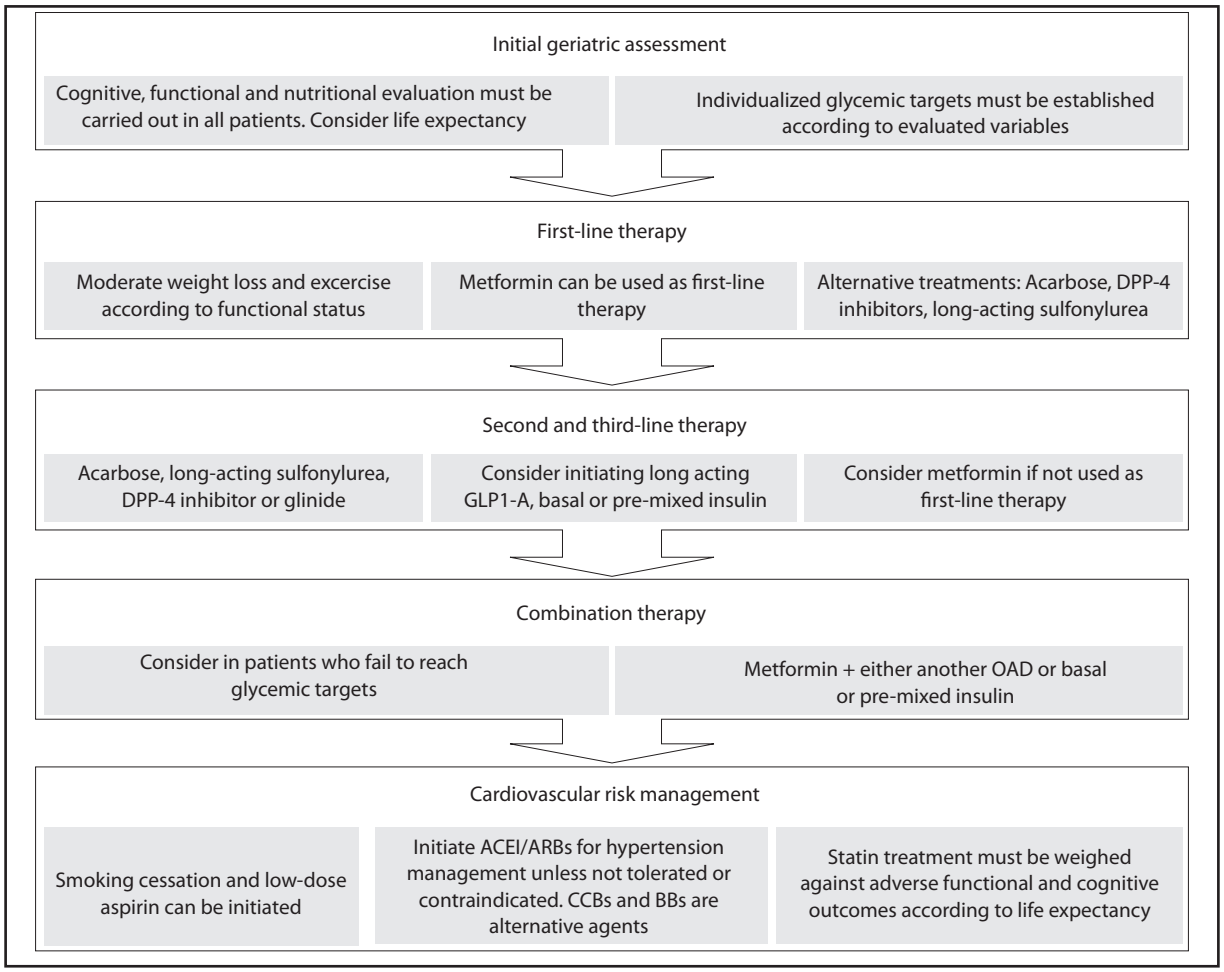

Figure 1. *Proposed algorithm for type-2 diabetes management in elderly individuals.*
ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin-II receptor blocker; BB: beta blocker; CCB: calcium channel blocker; DPP-4: dipeptidyl peptidase-4; GLP-1: glucagon-like peptide-1; OAD: oral antidiabetic drug.

issue of controversy; some epidemiological data suggest that the relative risk of coronary heart disease associated with high cholesterol decreases with age[86] and that there is an inverse relationship between stroke incidence and cholesterol levels[87]. Therefore, benefits of statin treatment in individuals aged 75 and over requires clinical judgment.

High-intensity statins in the elderly carry an increased risk of adverse events, especially in individuals with frailty and sarcopenia in whom myalgia and myositis are more frequent[88]. Data on the effect of statins and cognitive outcomes has been inconsistent, with one study reporting increased cognitive improvement in patients with established dementia after statin discontinuation[89] and pooled analyses reporting no association[90]. Statin prescription should therefore weigh potential benefits and harms of therapy, and consider life expectancy, low-density lipoprotein cholesterol levels, functional and cognitive status, as well as cardiovascular risk to make informed decisions and maximize treatment benefits[7].

CONCLUSIONS AND PERSPECTIVES

Management of T2D in elderly patients is complex and requires a full evaluation of comorbidities, geriatric syndromes, and socioeconomic background to improve prescription and minimize the effect of adverse events. Glycemic goals should not be stringent and must be based on life expectancy and functional and cognitive status and must adjust to the living arrangements of elderly individuals. Intensive glucose control has been associated with adverse outcomes and should not be used routinely. Randomized controlled trials comparing oral antidiabetic medications are scarce and generally have low methodological

quality; thus, clinical judgment is required for adequate prescription.

In an elderly patient with newly diagnosed T2D it is reasonable to start with lifestyle intervention strategies along with metformin treatment as first-line therapy to reach glycemic goals. Alternatives include DPP-4 inhibitors, acarbose, and long-acting sulfonylureas of glinides, though the latter two must be evaluated in terms of independence and cognitive function. In general, most oral antidiabetic medications are well tolerated in elderly patients; however, thiazolidinediones, short-acting sulfonylureas, and SGLT2 inhibitors should not be routinely prescribed and only be used in very specific settings. Combined therapy should be considered in patients who cannot reach glycemic goals with metformin or in whom the first-line therapy was not well tolerated; insulin regimes must be simple and mostly based on basal insulin. Figure 1 resumes a proposed algorithm for T2D management in elderly individuals.

High-quality RCTs are required to analyze the efficacy and safety of oral antidiabetic medications against metformin, and combinations should be further evaluated for hypoglycemia risk and adverse event rates. Specific evaluations in patients with frailty, cognitive impairment, and comorbidities must be carried out, and long-term follow-up is especially required to evaluate the risks and benefits of cardiovascular risk management in this population. This creates an area of opportunity for future research and calls for evaluation of current practices in the management of T2D in elderly individuals.

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REVIEW ARTICLE

Pathophysiological Mechanisms Linking Type 2 Diabetes and Dementia: Review of Evidence from Clinical, Translational and Epidemiological Research

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Abstract: ***Background*:** Type 2 diabetes represents an increasing health burden world-wide and its prevalence in particularly higher in elderly population. Consistent epidemiological evidence suggests an increased risk of dementia associated to type 2 diabetes; the mechanisms underlying these associations, however, remain unclear.

***Objective*:** The study aims to review epidemiological, clinical and pre-clinical data that weigh on pathophysiological links, mechanisms of disease and associations between type 2 diabetes and dementia to identify areas of opportunity for future research.

***Method*:** We searched the following electronic bibliographic databases: PUBMED, EMBASE, SCIELO, MEDLINE and OVID for clinical, translational and epidemiological research literature that summarize diabetes-related risk factors for dementia, metabolic and neurological changes associated to T2D, evidence of therapeutic approaches in type 2 diabetes and its pathophysiological implications for dementia.

***Result*:** Type 2 diabetes mellitus increases risk for all-cause dementia, vascular dementia and Alzheimer's disease. The most evaluated mechanisms linking both disorders in pre-clinical studies include an increase in neuronal insulin resistance, impaired insulin signaling, pro-inflammatory state, mitochondrial dysfunction and vascular damage which increase deposition of β-amyloid, tau proteins and GSK3β, leading to an earlier onset of dementia in individuals with impairment in the glucose metabolism. Neuroimaging and neuropathology evidence linking cerebrovascular lesions, neurodegeneration and particularly small-vessel disease in the onset of dementia is consistent with the increased risk of incident dementia in type 2 diabetes, but consistent evidence of AD-related pathology is scarce. Epidemiological data shows increased risk of dementia related to hypoglycemic episodes, glycemic control, metabolic syndrome, insulin resistance and genetic predisposition, but the evidence is not consistent and statistical analysis might be affected by inconsistent covariate controlling. Therapeutic approaches for T2D have shown inconsistent result in relation to dementia prevention and delay of cognitive decline; lifestyle intervention, particularly physical activity, is a promising alternative to ameliorate the impact of disability and frailty on T2D-related dementia.

***Conclusion*:** Vascular disease, inflammation and impaired brain insulin signaling might occur in T2D and contribute to dementia risk. Evidence from epidemiological studies has not consistently reported associations that could integrate a unified mechanism of disease in humans. Evaluation of the effect of antidiabetic medications and non-pharmacological interventions in dementia prevention in type 2 diabetes is promising but has thus far offered inconsistent results.

Keywords: Type 2 diabetes, diabetes-related dementia, Alzheimer's disease, glycemic control, diabetes complications, dementia.

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1. INTRODUCTION

Type 2 diabetes (T2D) represents an increasing health burden world-wide and its prevalence is particularly high in elderly population. The global trend of ageing is increasing world-wide, especially in the group of elderly people and it is estimated that in 2030, there will be approximately 690 million elderly persons [1]. A review that evaluated the incidence and prevalence of dementia in the recent years concluded that dementia-risk trends are decreasing in some countries over the years, mainly due to improved elderly care [2]. The observed association between dementia and T2D has gathered special attention due to an increasing prevalence and incidence of both dementia and T2D world-wide. Based on epidemiological projections, there is expected to be 552 million of persons with T2D in 2030, most of them in low to middle income countries [3, 4]. Furthermore, there will be an increasing incidence of T2D in developing countries, which is estimated to increase up to 34.0% based on estimations from the Future Elderly Model [5].

Dementia attributable to T2D is relevant due to the increasing prevalence and incidence of both diseases and lack of information about specific pathogenesis of this association [6]. Despite consistent epidemiological evidence, the mechanisms underlying the association of dementia and T2D remain unclear. In this review, we revisit the association of dementia attributable to T2D, with focus on evaluating known risk factors and potential pathophysiological mechanisms in favor and against common disease pathways in both disorders that have been shown in recent studies. We also analyze areas lacking sufficient information in epidemiological, clinical and translational research, which could be helpful for researchers to identify areas of opportunity related to specific pathophysiological mechanisms and risk factors attributable to T2D. Furthermore, we discuss the available evidence on targeted interventions and treatments which could be useful in developing studies focused on preventive schemes for dementia in patients with T2D.

2. METHODS

We searched PUBMED, EMBASE, SCOPUS, MEDLINE and OVID for clinical, translational and epidemiological research literature that summarize diabetes-related risk factors for dementia, metabolic and neurological changes during T2D and the evidence of therapeutic approaches in T2D and its pathophysiological implications for dementia. We reviewed articles which studied diabetes-related risk factors for dementia including risk factors for dementia attributable to T2D, dementia attributable to tight glycemic control, depression in T2D and dementia, and genetic risk factors for dementia in T2D. The review of the metabolic and neurologic changes in T2D and its role in cognitive dysfunction and dementia included: dementia in pre-diabetes and metabolic syndrome, the role for insulin secretion and sensitivity in dementia and T2D, neuroimaging and neuropathology in patients with T2D and its correlation with dementia-related brain changes. Finally, we reviewed the evidence of therapeutic approaches in T2D and its pathophysiological implications for dementia research including the effect of T2D-related medication in dementia risk, medications for cardiovascular risk in T2D and dementia risk and evidence for potential non-pharmacological prevention strategies for dementia in T2D.

3. DIABETES-RELATED RISK FACTORS FOR DEMENTIA

Consistent epidemiological evidence has demonstrated a role of T2D as a risk factor for the development of all-cause dementia. A recent meta-analysis of longitudinal cohort studies concluded that T2D increases the risk of AD in up to 50% (RR 1.5 95%CI 1.2-1.8) and for vascular dementia more than two-fold (RR 2.5 95%CI 2.1-3.0) [7]. Similar results were observed in another meta-analysis, in which the risk for overall dementia associated to T2D increased up to 70% (RR:1.7 95% CI 1.5-1.8), for AD up to 60% (RR 1.6 95%CI 1.4-1.8) and two-fold for vascular dementia (RR 2.2 95%CI 1.7-2.8) [8]. This association also holds true for cognitive function in T2D; indeed, patients with long-standing T2D without diagnosed dementia have lower cognitive function, particularly in attention, working memory and executive functions [3,4,9].

Common risk factors for all-cause dementia in T2D have been studied as secondary outcomes in most studies. Risk factors associated to dementia in T2D include older age, family history of dementia, smoking and comorbidities including hypertension, obesity, dyslipidemia and stroke [11]. Nevertheless, mid-life obesity and hypertension have a paradoxical risk association, given that in later-life, there is evidence suggesting a lower risk of dementia in T2D [12]. Additional risk factors which have not been associated to dementia-risk in T2D, but which have been shown in general population include: sleep disturbances [13], hyperlipidemia and history of depression [14]. Protective factors for dementia shown in general population have also been studied for diabetes, including years of formal education, measured by the grade level attained and/or colleague attendance, occupation and physical activity [15,16]. A systematic review linking variables that contribute to incident all-cause dementia including occupation, lower level of education and socioeconomic status found inconclusive results in patients with T2D [17].

3.1. Risk Factors for Dementia Attributable to T2D

As discussed previously, patients with T2D present an increased risk for all-cause dementia with traditional risk factors for all-cause dementia compared to general population. Indeed, in a retrospective cohort including patients with T2D and dementia, the authors reported that patients with T2D and dementia are shown to be older, more likely female, had higher rates of smoking, longer duration of diabetes and more frequent use of T2D medication [18]. Elderly T2D patients often present with comorbidity, which increases the risk of functional and cognitive impairment; furthermore, a positive correlation between cumulative comorbidities in patients with T2D in relation to dementia risk has been reported. A large prospective cohort conducted in Taiwan reported a higher risk of incident all-cause dementia with increasing number of common comorbidities in elderly individuals with T2D, including hypertension, dyslipidemia, cerebral artery disease, stroke, and kidney disease, adjusted for age [19]. An increase in comorbidities, particularly meta-

bolic disturbances and dyslipidemia have also been linked to increased pro-inflammatory state; T2D has also been linked to chronically increased pro-inflammatory state, with overexpression of cytokines, chemokines and complement proteins, similar to what is seen in post-mortem brains with AD [20]. In mice, chronic inflammatory states have been linked to increased microglial activation [21] and overexpression of tau proteins [22]. This suggests that a pro-inflammatory state as seen in T2D and increased comorbidities may contribute to dementia risk and might mediate these epidemiological observations. Increases in oxidative stress have also been described in AD and has been linked to endothelial dysfunction, which increases the production of reactive oxygen species [23, 24]; a highly oxidative environment, such as what is observed after major cardiovascular events, might interact with amyloid-β (Aβ) plaques and mitochondrial dysfunction to impair tight junction proteins, thus impacting vascular permeability of relevant brain substrates, including insulin [25]. These alterations might interact to increase dementia risk in individuals with high cardiovascular risk, including T2D.

There have been efforts to identify risk factors solely attributable to T2D, including glycemic level and control (26,27,28], years of diabetes exposure [26,29], use of exogenous insulin [30], endogenous hyperinsulinemia [26,31], insulin resistance [32] and hypoglycemia [33] yielding inconclusive results. Research conducted in a large cohort in Ontario, Canada concluded that elderly individuals with recently diagnosed T2D had 12% higher risk in men and 14% higher risk in women for incident all-cause dementia compared to age-matched controls, suggesting that T2D may be considered a risk factor regardless of years of T2D exposure [34]. As will be discussed later, these results lead to the hypothesis that metabolic syndrome and insulin resistance might mediate early stages of dementia and cognitive decline, especially in AD [35,36].

Observational studies have reported an association between insulin secretion and dementia in non-diabetic patients, suggesting that patients with low levels of fasting insulin had higher risk of developing incident dementia, likely attributable to decreased β-cell function [37], nevertheless, a recent meta-analysis showed that rather hyperinsulinemia was correlated with both decreased cognitive function and incident dementia [38]. As commented before, endogenous hyperisulinemia is a predictor of MCI and studies have suggested that insulin variations throughout life may modulate progression of dementia in T2D. A recent hypothesis suggests that alterations in glucose metabolism due to impairment in insulin signaling, inflammation, accumulation of glycation end-products and oxidative stress in the neurons might mediate the progression of dementia [39]. Certainly, the crescent interest in the use of intranasal insulin treatment is a novel area of interest, reporting a good response in functional status and daily activity, but not significantly in cognitive functions [40]. The role of endogenous hyperinsulinemia in subjects with and without T2D and the use of intranasal insulin as a potential dementia treatment are still in progress and may be an area of opportunity for further research.

Whether dementia can currently be considered a T2D complication remains a controversial subject. Pathophysiological correlations between both disorders have been sought out and a potential microvascular component for dementia in T2D has been evaluated in both epidemiological and imaging studies. This was suggested in a longitudinal study performed by Exalto *et al*. where they followed a group of T2D patients and reported that patients with severe diabetic retinal disease are at an increased risk for dementia, reflecting an association with cerebral microvascular disease and incident dementia, even when this is an unknown etiology [41]. Furthermore, impaired renal function also has been shown to be a an independent risk factor for incident dementia, as reported in the TABASCO trial, a prospective cohort that described the association with renal function and brain function using MRI and reported that both conditions increased risk for cognitive decline four-fold as shown by reduced cerebral and hippocampal volume [42]. The Diabetes-Specific Dementia Risk Score (DSDRS), a recent predictive score for evaluation and prediction of incident dementia in T2D developed by Exalto *et al*, considered microvascular complications, including diabetic retinopathy and diabetic kidney disease, and macrovascular complications, including stroke, myocardial infarction and diabetic foot disease, as significant predictors for all-cause incident dementia in elderly patients, suggesting that all risk factors attributable to T2D for incident dementia are accumulative and could be potential targets for intervention [43]. The causal role of microvascular disease has been questioned, especially since neurological changes have been observed in individuals with T2D without evidence of end-organ microvascular damage [44,45]. Disability and functional impairment related to microvascular complications might also be a likely link between T2D-related microvascular complications and dementia, especially since diabetic retinopathy leads to visual impairment, neuropathy to decreased mobility and depression and T2D also leads to sensorineural hearing-loss as a result of microvascular damage [46]. The role of rehabilitation and multidisciplinary interventions to reduce cognitive changes in individuals with disability due to microvascular complications remains to be evaluated as a preventive measure for dementia in these patients with longstanding T2D.

3.2. Hypoglycemia Due to Tight Glycemic Control in T2D and Dementia Risk

Management of glucose levels in the elderly patient with T2D is complex and must consider evaluation of functional and cognitive status to improve glycemic control, long-term functional outcomes and quality of life [47]. Intensive glycemic control in elderly individuals is controversial and has not shown clear cognitive benefits [48], furthermore, regimes based on strict HbA1c goals increased the risk of hypoglycemia, hospitalization and falls, with increased risk of functional impairment [49]. This has led to the suggestion that T2D treatment is often not adequately suited to elderly patients, with many studies suggesting that a large proportion of patients might be overly treated, increasing the risk of hypoglycemia, frailty and dementia [50,51]. Furthermore, a cross-sectional study reported that elderly patients with T2D-related comorbidities including renal insufficiency and cognitive impairments are potentially over treated according to different guidelines [52]. Another retrospective study analyzed risk factors for T2D overtreatment and reported that

individuals with recent cardiovascular events had an increased risk of developing hypoglycemic events [53]. Furthermore, hypoglycemia risk is exacerbated with the use of exogenous insulin and sulfonylureas, especially in patients with chronic kidney disease [54].

There is evidence that supports the hypothesis that hypoglycemic events may contribute to a worsening of the clinical course of dementia and that the risk for incident dementia is exacerbated with each additional episode of hypoglycemia. This was established in a large 27-year follow-up cohort, where authors concluded that in elderly patients with long-standing T2D, cumulative number hypoglycemia increases proportionally the risk for incident dementia [55]. Additionally, an MRI-based analysis of the Atherosclerosis Risk in Communities (ARIC) cohort study showed that patients with hypoglycemia had smaller total brain volume and cognitive decline over 15 years; however, this evidence was not replicated in the ACCORD-MIND MRI trial, where hypoglycemia was not linked to decreased brain volume and abnormal white matter volume [56,57]. Nevertheless, both studies showed poorer cognitive outcomes and a higher rate of cognitive decline in patients with symptomatic hypoglycemia requiring medical assistance. This had led to question whether there is a need of de-intensifying treatment goals in patients with dementia and T2D or those with T2D at increased risk of dementia with specific use of medication that does not contribute to increased risk of hypoglycemic events. Furthermore, a study suggested that a good maintenance of glycemic control may reduce the risk cognitive decline, especially in those with long-standing diabetes, more than a specific goal of treatment [58], reinforcing the idea that specific treatment guidelines must be developed for the treatment of T2D in elderly patients with dementia, MCI or at risk of cognitive decline and dementia.

3.3. Depression in T2D and Dementia

Another condition related to an increased risk for incident dementia is depression. The relation between depression and T2D appears to be bidirectional. Evidence suggests that in patients with depression, there is 60% higher risk of developing incident T2D. This association is attributable to unhealthy behaviors and physiological changes that induce high body stress [59]. This results in dysfunction of the hypothalamic-pituitary-adrenal axis, sleep disturbances and a pro-inflammatory state which leads to impairments in the glucose metabolism [60]. On the other hand, a meta-analysis report that patients with T2D are up to 25% higher risk of developing depression [59]; this risk is even higher in patients using exogenous insulin and in those with T2D complications, particularly nephropathy, neuropathy and sexual dysfunction [61]. This bidirectional association has been linked to worsening course of T2D, and aggravated severity across the range of complications in T2D. The link between T2D, depression and dementia has been explored. Evidence suggest that in patients with depression and T2D there is two-fold higher risk of incident dementia compared with T2D patients without depression [40,41]. In a nation-wide prospective cohort study including subjects with depression and T2D, subjects with both conditions had two-fold higher risk for incident dementia, suggesting a role for depression as an independent risk factor [62, 63]. Conversely, cognitive dysfunction and depressive symptoms could interact and impact adherence of T2D treatment and thus affect glycemic control in T2D patient [64] which can deteriorate glycemic control and lead to more intensive treatment strategies which could impair functional and cognitive status, thus increasing dementia risk in T2D [52,65].

3.4. Genetic Risk Factors for Dementia in T2D

Genetic risk for dementia has become a relevant area of research with potential implications in dementia prevention. Risk variants in the APOE4 gene, specifically with the APOEε4 allele, have been associated to an increased risk of all-cause dementia in most populations [9,66,67]. The APOEε4 gene causes morphologic changes in neuron architecture in patients with dementia, a trait that may be exacerbated in patients with T2D. The association between the APOEε4 gene and T2D was first reported in the Honolulu Heart Program, where subjects with T2D and carriers of the APOEε4 gene had a 3-fold higher risk of developing hippocampal neuritic plaques (95%CI 1.2-7.3), a 3.5 fold-risk of accumulating neurofibrillary tangles in the cerebral cortex (95%CI: 1.2-7.3) and 2.5 fold-risk to develop the same structures in the hippocampus, specifically in AD (95%CI 1.5-3.7) [68,69]. The APOEe4 has been reported to be a frequent variant in some populations [70–72]. Although there is no clear mechanism that completely explains why this allele increases the risk of AD, there are hypothesis proposing a reduction in Aβ-plaque clearance, which increases inflammation and leads to intensification of neurodegeneration. A recent review suggested that currently it is unknown whether the presence of this allele confers an accumulation of misfolded AD-related proteins or if there is a loss of protective mechanisms attributable to this gene [73,74]. Furthermore, APOEε4 allele has been detected in amyloid plaques [75], and may cause mitochondrial disruption and neuronal damage *via* its receptors [76]; the risk allele has also been implicated to promote tau phosphorylation in animal models [77].

Another recently discovered variant which increases risk of incident dementia in T2D is the HHEX_23 AA genotype, which represents the first novel association observed for T2D patients. The HHEX_23 gene codifies for the insulin degrading enzyme (IDE), which can contribute to the pathogenesis of AD and whose alterations have been linked to neurological and cognitive changes in both human and animal models [78]. One study performed in Scandinavian population reported an increased risk of incident dementia in carrier patients of the AA variant in HHEX_23 and observed a significant interaction with the presence of T2D, increasing substantially the risk of incident dementia and AD. In the same study, carriers of the variant presented significant reductions in hippocampal, and gray and white matter observed in MRI imaging. Further studies need to establish the complete role of IDE in AD tissues, and the role of the HHEX_23 genotype in different populations. Genome-wide association studies that evaluate association of genetic variants with dementia risk in individuals with T2D are required to evaluate contribution of ethnic-specific variants in relation to cognitive and pathologic changes linked to dementia, with attention to specific etiologies subtypes of dementia. Evaluation of genetic risk factors for T2D and its implications in

future dementia risk must also be explored in future studies, with attention to preventive strategies in at-risk groups to reduce T2D incidence or the influence of lifestyle changes in the modification of future dementia risk. Functional evaluations in *in vivo* animal models must also be conducted to identify the pathogenic role of such variants and its potential clinical implications.

The identification of specific risk factors for incident dementia in T2D are complex, mainly due to the intrinsic methodological limitations of most studies. First, many of the studies have different main outcomes, with most primarily evaluating the association between dementia and diabetes and risk factor evaluation mostly relegated to secondary analyses which can present biases due to limited power, variable selection and inconsistent definitions and reporting. Second, many of these studies have different methods to define dementia, which might impact patient selection, affect epidemiological estimations of dementia and affect observed associations with potential risk and protective factors. Third, there is a need of longitudinal cohort studies to assess the interaction of traditional risk factors and T2D attributable risk factors, which is an area, lacking sufficient information. Development of future studies to evaluate risk factors for dementia in T2D should focus on follow-up of T2D subjects with known baseline characteristics, consistent definitions and sufficient time to determine disease onset and etiology. Fig. (**1**) resumes the role of risk factors, both traditional and T2D-specific, in modifying dementia risk and leading to clinical and pathologic features of dementia.

4. METABOLIC AND NEUROLOGIC CHANGES IN T2D AND ITS ROLE IN COGNITIVE DYSFUNCTION AND DEMENTIA

4.1. Dementia in Pre-diabetes and Metabolic Syndrome

Evidence from animal and cell models have unveiled common underlying mechanisms linking dementia and T2D, including impairments in insulin signaling and transport, pro-inflammatory state, oxidative stress, mitochondrial dysfunction, advanced glycation end-products, total cholesterol and the APOEe4 allele. As discussed before, there is evidence which suggests that dementia risk attributable to T2D starts to develop as early as pre-diabetic stages and even in the metabolic syndrome, a relation which has been consistently proven. Compared with matched controls, patients with metabolic syndrome and impaired glucose tolerance have reduced cognitive function [79]. Additionally, certain types of clinical and pathological features of dementia appear earlier in individuals with impaired glucose metabolism, which indicates that there may be a prodromal stage linked to insulin resistant or hyperglycemic stages that is present before the onset of dementia. This was suggested in a prospective evaluation performed in the Framingham Heart Study third generation, which assessed dementia-free subjects and demonstrated impaired memory, visual perception and attention performance in individuals with impaired fasting glucose. MRI evaluation showed decreased total brain and occipital lobar gray matter volumes, suggesting structural brain changes which had previously been related to dementia in patients with impaired glucose metabolism [80]. Another retrospective study conducted in Singapore reported that the presence of metabolic syndrome increases risk MCI

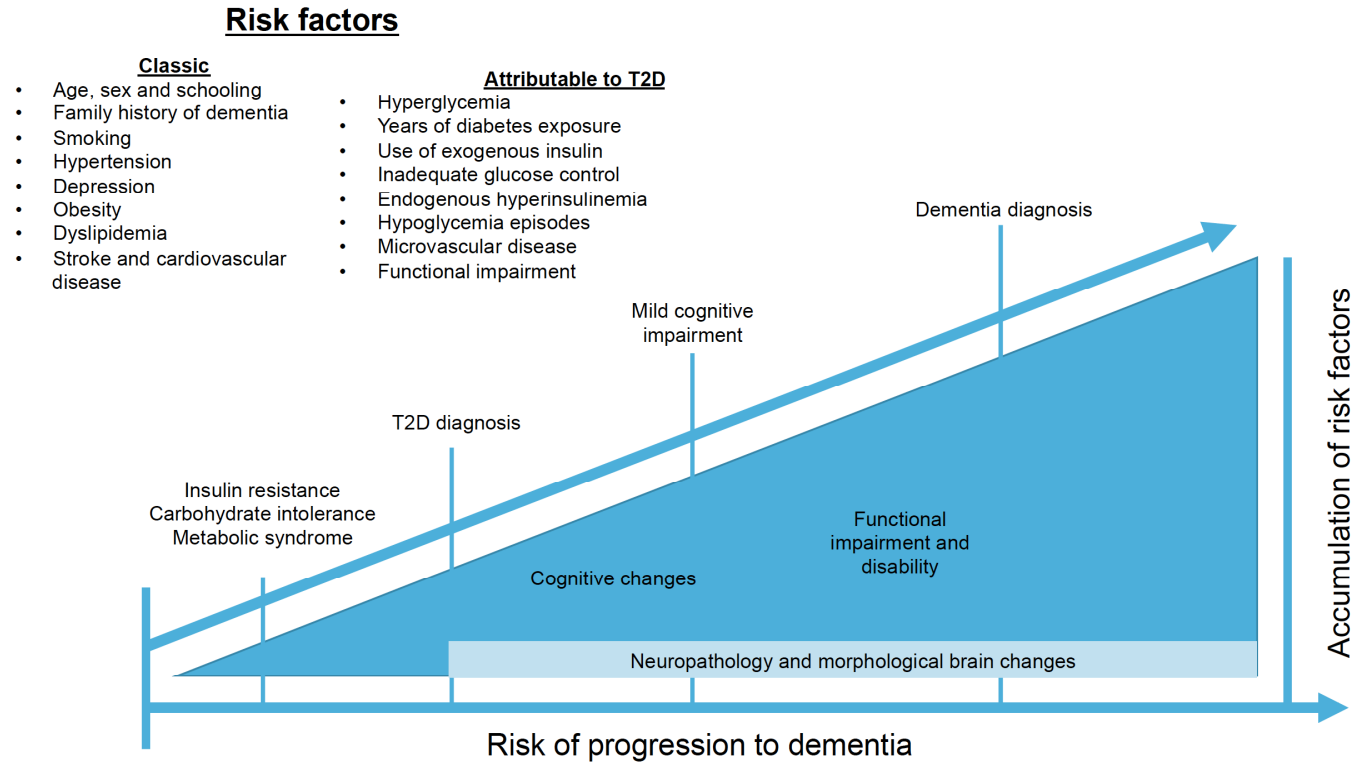

Fig. (1). Cumulative effect of risk factors for dementia in type 2 diabetes (T2D). Evidence suggests that risk factors for dementia in T2D might be classic or attributable to T2D and are cumulative across different metabolic states in interaction with dynamic cognitive and functional changes which increases the risk of cognitive impairment and progression to dementia.

four-fold with a higher risk of progression to dementia compared with controls [81]. This may suggest that cognitive changes may begin prior to T2D onset and are exacerbated with the presence of T2D [82].

Accumulated evidence suggests that the interaction of hyperglycemia and hyperinsulinemia is accumulative across different metabolic stages and is sharply exacerbated in patients with T2D in an age-dependent manner. However, longitudinal studies including elderly populations have suggested that poor glycemic control in patients with T2D contributes to accelerate cognitive dysfunction independently of age [83, 84]. Similarly, young patients with metabolic syndrome have lower cognitive performance and impaired brain structural integrity compared with matched controls, suggesting that metabolic alterations have neurological impacts regardless of age [85]. The interaction of metabolic alterations and age regarding its impact on cognition and brain architecture remain as an attractive area of opportunity for research. Longitudinal studies which evaluate cognitive changes, modifications in brain structure and incident dementia from impaired glucose tolerance through the development of T2D may clarify the clinical course of cognitive dysfunction related to impaired glucose metabolism and tolerance and determine its impact in future dementia risk.

4.2. A Role for Insulin Secretion and Sensitivity in Dementia and T2D

As mentioned earlier, insulin treatment and endogenous hyperinsulinemia could play a major role in the pathogenesis of cognitive dysfunction and dementia in T2D. It is known that insulin acts as a competitive inhibitor of the enzyme that degrades the Aβ plaque, therefore reducing its clearance and potentially leading to accumulation and deposition of Aβ plaques [86]. There is also a hypothesis linking altered brain insulin and insulin-like growth factor-1 (IGF-1) signaling with dementia, especially AD [87, 88]. This may question that the fluctuation in glucose levels may not be the key factor in incident dementia, but instead a fluctuation in insulin levels. This was evaluated in a prospective study conducted in Sweden, in which the authors reported that patients with lower insulin sensitivity, as evaluated using euglycemic hyperinsulinemic clamp, were shown to have 55% higher risk of vascular dementia and those with lower first-phase insulin response assessed using an oral glucose tolerance test had 32% higher risk of AD, suggesting that impaired β-cell function and decreased peripheral insulin sensitivity increase the risk of dementia independent of T2D status [89].

Insulin also has displayed a significant role in modulating cortical responses and cognitive function. In humans, this was demonstrated in a study that assessed cerebro-cortical activity using magnetoencephalography with two-step euglycemic-hyperinsulinemic clamping in obese patients, which concluded that insulin plays a major role in regulating cerebral metabolism by modulating cerebro-cortical activity. This same effect was observed in obese individuals with insulin resistance and carriers of the insulin receptor substrate (IRS)-1 Gly972Arg polymorphism [90]. Another study which evaluated insulin resistant patients using combined FDG-PET and euglycemic-hyperinsulinaemic clamping showed an increased brain glucose metabolism in insulin-sensitive patients, compared with insulin-resistant patients, especially in those brain areas dedicated to subverting appetite and reward [91]; these observations strengthen the link between insulin signaling in the brain, behavioral and cognitive changes related to whole-body glucose metabolism and insulin sensitivity. Despite the strong experimental evidence, these studies do not clarify the precise mechanisms link insulin resistance to the brain, neither if generalized insulin resistance or organ-specific insulin resistance affect neuronal insulin sensitivity. Hypotheses that may explain this relation suggest that long-standing T2D leads to decreased insulin transport mediated by the blood-brain barrier, which leads to decreased brain insulin signaling [92,93]. Studies performed in rodent models have proposed that impairments in insulin receptor signalin, promotes synthesis of glycogen synthase kinase 3β (GSK3β), increases in production of enzymes involved in Aβ processing (β and γ-secretase) and hyperphosphorylation of tau protein [94]. The impact of GSK3β on cognitive function was demonstrated in a study which evaluated that inhibition of GSK3β with lithium chloride in mice leads to decreased cognitive dysfunction compared to controls [95]. Furthermore, mice which are morbidly obese and glucose intolerant at young ages have shown profound cognitive impairment by 12 months, despite not showing significant increases in Aβ formation, proposing a role for peripheral insulin resistance in modulating dementia risk in T2D. [96].

Along with this evidence, recent studies of intranasal insulin use in patients with cognitive impairment have reported improvement in memory and other cognitive functions and tests, offering empirical evidence for a role of insulin in improving cognition [97–99]. This evidence is paradoxical with epidemiological findings, which have shown that exogenous insulin administration increases dementia risk; arguably, the use of exogenous insulin might increase hypoglycemia risk, which might function as a confounder in these associations [100]. In murine models, acute but not chronic intranasal insulin has been show to improve cognitive function, an effect which is halted in diabetic mice [101,102]. Future studies should evaluate the impact of insulin use in cognition and dementia risk, considering confounders such as treatment adherence, hypoglycemia episodes and glycemic targets adjusted for functional status. The role of intranasal insulin to prevent or delay cognitive dysfunction in T2D calls for the development of longitudinal studies with sufficient follow-up to evaluate relevant cognitive outcomes related to functional status and its effect in quality of life in elderly patients with T2D.

4.3. Neuroimaging and Neuropathology in Patients T2D and Correlation with Dementia-related Brain Changes

The correlation of clinical and epidemiological observations linking cognitive dysfunction, dementia and T2D have not shown consistent results in imaging studies. As mentioned earlier, neuroimaging studies have focused efforts in linking changes in brain architecture, brain atrophy, white matter integrity and vascular pathology in patients with T2D; however, few studies have focused on the impact of such changes in cognitive function, progression of cognitive decline and dementia [103]. As commented before, MRI studies have revealed a correlation between brain atrophy and

cognitive dysfunction in patients with T2D, who show reduced brain volume up to 0.5-2.0% compared with controls, particularly in gray and white matter integrity [104]. There is also an increased number of micro-infarctions in lacunar regions caused by small-vessel disease in patients with T2D, compared with controls; nevertheless, the evidence for micro or macrovascular disease as the sole cause for brain vascular pathology in patients with T2D remains controversial. Considering these observations, most conclusions drawn in relation to the etiology of cognitive dysfunction in imaging studies are limited. Imaging studies evaluating trajectories of T2D patients compared to age-matched healthy controls should help unravel structural differences underlying T2D compared to normal aging and studies correlating these changes to cognitive dysfunctions are required to establish a causal and pathophysiological role for such observations in MCI and dementia. Furthermore, most imaging studies are limited by their cross-sectional design and do not include patients with recently-diagnosed T2D [103,104]. Imaging evidence that support most longitudinal observations in dementia research in T2D remains an area of opportunity for further research.

In AD, the characteristic neuropathological feature is the inclusion of extracellular Aβ plaques in neurons, which consist mainly in aggregated Aβ, which is a 4-kDA peptide derived from a sequential cleavage of the Aβ precursor protein (APP) [105]. In T2D, there is a deposit of Aβ plaques in the β-pancreatic cell, also known as human islet amyloid polypeptide (hIAPP) [106-108]. In mice, hIAPP seems to induce apoptosis in the β-pancreatic cell [108] promoting an impaired glucose tolerance; hyperamylasemia in murine causes deposition of hIAPP deposition in cerebral blood vessel walls, leading to endothelial dysfunction and modulated by the APOEε4 gene [109]. Evidence regarding Aβ-pathology in humans with T2D has not been consistent. Autopsy analyses of the Rush Longitudinal Cohort of Aging reported a negative association between T2D and HbA1c on global AD-associated pathology but reported higher odds of cerebral and subcortical infarction, likely supporting the hypothesis of vascular-mediated pathology in T2D-related dementia risk [110]. Furthermore, autopsy, cerebrospinal fluid and PET studies including T2D subjects have not shown increased extracellular deposits of Aβ or increased intraneuronal aggregates of hyper-phosphorylated tau protein or increased biomarkers of Aβ compared with individuals without T2D [111,112]. Nevertheless, imaging and cerebrospinal fluid evaluations of individuals with T2D from the Alzheimer's Disease Neuroimaging Initiative showed decreased lower bilateral frontal and parietal cortical thickness and increased cerebrospinal fluid total and phosphorylated tau, suggesting an impact of T2D in neurodegeneration independent of AD-related pathology [113]. Overall, no overarching, unifying mechanism has been proposed relating T2D, cognitive dysfunction and dementia based on neuropathological or neuroimaging studies, most likely due to the heterogeneity of evaluated populations, scarcity of long-term evaluations which correlate these findings and inconsistent definitions regarding the differences between diabetes-related cognitive dysfunction, MCI and dementia, which have led to inconclusive observations [100]. Studies focused on precise identifications of T2D-related pathological changes in the brain in individuals with and without MCI and dementia must be conducted to reduce the heterogeneity of available evidence and relate these findings with clinical and imaging observations to propose a unified mechanism for the pathways involved in dementia risk in T2D. Fig. (**2**) resumes available evidence regarding metabolic and neurological changes linking T2D and dementia.

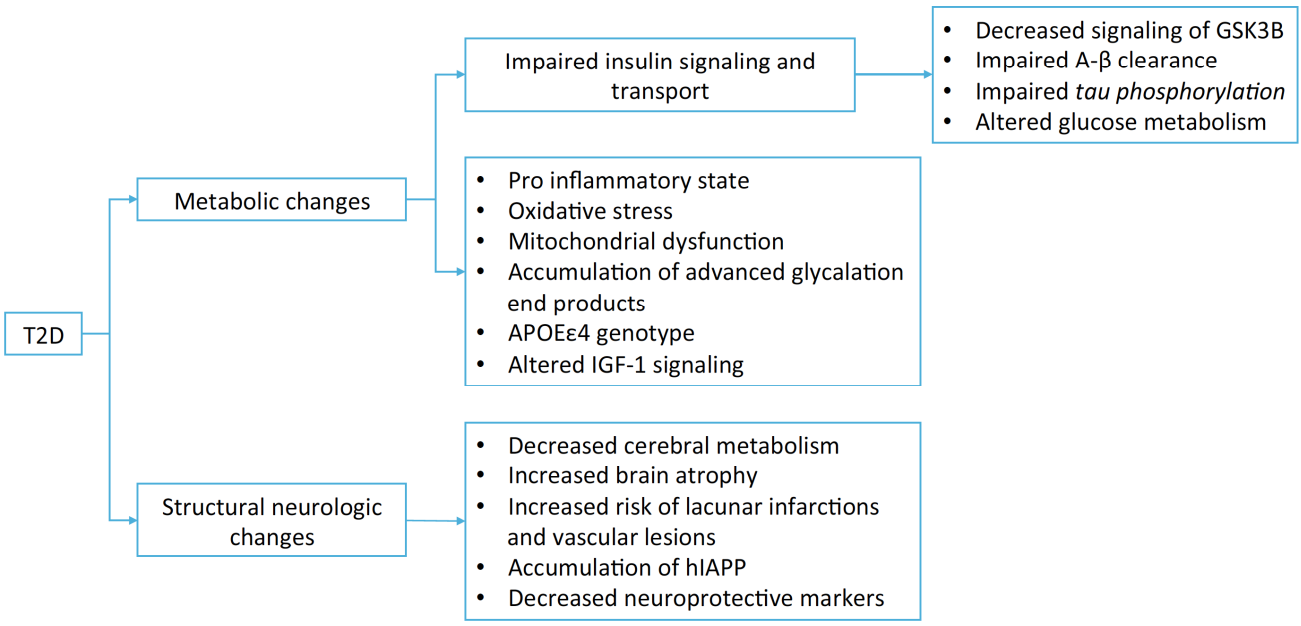

Fig. (2). Changes attributable to T2D in dementia. T2D causes metabolic and morphological changes which are common in dementia. The most recent hypothesis suggest that changes are divided in those that are related to the impaired insulin action, those related to T2D pathophysiology and neurological changes that can be seen in imaging studies.
Abbreviations: T2D: Type 2 diabetes; hIAPP: human islet amyloid polypeptide; GSK3B: glycogen synthase kinase 3β, IGF-1: Insulin-like growth factor 1.

5. EVIDENCE OF THERAPEUTIC APPROACHES IN T2D AND ITS PATHOPHYSIOLOGICAL IMPLICATIONS FOR DEMENTIA RESEARCH

5.1. Effect of T2D-related Medication in Dementia Risk

The impact of T2D treatment in dementia risk remains controversial. In prospective cohorts, including the ACCORD-MIND study, patients with T2D under intensive glycemic control do not experience improved cognition but have significantly lower decreases in gray matter decline [114]. A systematic review by the Cochrane collaboration suggested that evidence regarding the cognitive effect of antidiabetic medication is inconsistent, with the clearest evidence suggesting no benefit of intensive versus standard glycemic control in elderly subjects [115]. Nevertheless, studies which evaluate the effect of T2D treatment on dementia risk have offered a different picture. The SALSA study, a large prospective cohort conducted in Mexican-American subjects, reported a greater risk of incident dementia and cognitive impairment in patients who were untreated for T2D [116]. Another study performed by the 10/66 Dementia Research Group (DRG) in Mexican population reported that the risk of incident dementia was higher in undiagnosed patients with diabetes, who were untreated at that point [117]. A meta-analysis pooling report related to epidemiological studies suggested a decreased incidence of dementia with the use of insulin sensitizers, particularly metformin and thiazolidinediones (TZDs) [118]. These studies have shown that glycemic control at least in part modifies dementia risk, an observation that has been evaluated regarding different specific medications for T2D.

Metformin, the first-line therapy for T2D management, has been evaluated compared to other hypoglycemic medications in relation to its cognitive effect and impact on dementia risk. Compared to sulfonylureas, metformin leads to decreased dementia risk in elderly patients under 75 years, particularly in those with preserved renal function [119]. Nevertheless, one nested longitudinal case-control study suggested that long-term metformin use was associated with higher risk of AD [120]. Metformin has also been linked to reduced dementia risk in comparison to TZDs [121]. Combination therapy with metformin has been assessed in some studies, in one observational study in Taiwan, investigators reported that the combined use of metformin and sulfonylureas had a decreased risk of incident dementia compared with controls [122]; in contrast sulfonylurea monotherapy has not been reported to have significant reduction of incident dementia, compared with metformin [119]. Pilot data from a randomized placebo-controlled clinical trial showed that metformin was associated with improvements in executive functioning, learning, memory and attention with increased orbitofrontal cerebral blood flow [123]. The positive effects of metformin on dementia-related pathophysiology have been evaluated in pre-clinical studies. Metformin and saxagliptin therapy in D-galactose models of AD have shown reversal of memory impairment, oxidative stress, inflammation and tau hyperphosphorylation secondary to impaired insulin signaling [124]. Metformin activates AMPK-dependent pathways, which decreased Aβ-related mitochondrial dysfunction, upregulated genes linked to neuroprotection and decreased activity of caspase 3/9 activity and cytosolic cytochrome c in human neural stem cells [125]; furthermore, metformin was shown to decrease Aβ-induced apoptosis in a MAPK-JNK-dependent way in hippocampal neurons and reduce effects of amyloid deposition in long-term potentiation in murine models fed with high-fat diet [126,127].

The most consistent observations for reductions of dementia incidence in epidemiological settings related to monotherapy for diabetes have been shown for pioglitazone. An observational study reported 47% lower incidence of dementia in T2D patients using pioglitazone for a 6-year period follow-up [128]. Similar results were obtained in a retrospective cohort in Taiwan, where there was a 50% reduction of incident dementia for high-cumulative dose users of pioglitazone [129]. In pre-clinical studies, TZDs have been shown to inhibit neuroinflammation, reduce Aβ-accumulation and plaque formation by promoting Aβ-clearance, and reducing mitochondrial dysfunction and tau-hyperphosphorylation in mice [130,131]. The use of TZDs in reducing dementia-risk in patients with T2D is still an area of opportunity for research, but numerous potential beneficial results in cognition and dementia-related pathophysiology have been reported in animal experimental models and pilot studies of human subjects, which has led to planning and development of future clinical trials (NCT0193156).

Dipeptidyl peptidase-4 inhibitor (DPP-4) inhibitors and long-acting sulfonylureas are attractive medications which have only begun to be explored in dementia research. DPP-4 inhibitors have shown modest but beneficial effects on cognition in individuals with and without cognitive impairment and AD [132]; in pre-clinical studies, DPP-4 inhibitors have been shown to ameliorate cognitive deficits, decrease Aβ formation and deposition and cytotoxicity through activation of AMP-K dependent pathways, which decreased activation of GSK3β and tau hyperphosphorylation by improving insulin action [133,134]. As discussed previously, research evaluating the role of sulfonylureas in dementia prevention have been limited by the increased risk of hypoglycemia in elderly individuals, especially those with functional impairment; the long-acting sulfonylurea glimepiride has been proposed as an attractive alternative, given its use in elderly individuals and its comparably lower hypoglycemia risk. Evidence form pre-clinical studies have shown a role for glimepiride as an antagonist of acetylcholine esterase, a common pharmacological target for dementia, and that glimepiride treatment downregulates binding of Aβ plaques, thus reducing intracellular cholesterol accumulation and activation of phospholipase A2, leading to decreased neuronal synaptic damage [135,136]. Overall, available evidence regarding the use of antidiabetic drugs to reduce T2D-related dementia risk, dementia prevention or cognitive benefits in human subjects are limited by the scarcity of randomized clinical trials, inconsistent follow-up time and lack of statistical power in observational reports. Ongoing clinical trials must assess the benefits of antidiabetic medications longitudinally and correlate their usefulness against functional status.

5.2. Medications for Cardiovascular Risk in T2D and Dementia Risk

The role of medication routinely administered to T2D patients to modify cardiovascular risk and dementia risk is mostly observational. In Taiwan, a large prospective cohort in patients with T2D who were using angiotensin-converting-enzyme inhibitor (ACEI) and/or angiotensin II receptor blockers (ARBs) found that both drugs decrease the incidence of vascular dementia, but not AD [137]. This is explained partly because higher blood pressure levels lead to progression to atherosclerosis and hypoxia, which induces brain damage [138]. Another hypothesis is an increased activation in the renin-angiotensin-aldosterone pathway in T2D, which leads to accelerated progression to dementia [137]; therefore, blockade of this pathway using ACEI/ARB may result in decreasing dementia incidence. Additionally, T2D is associated with impaired autonomic nervous system response, leading to endothelial damage through sustained vasoconstriction, increasing atherosclerosis and formation of atherosclerotic plaque risk, thus decreasing the cerebral blood flow and damaging synaptic connections and neuronal activity in regions involved in cognitive functions including limbic regions, association areas and white matter that links association areas [139]. This effect could be reduced through adequate management of blood pressure and a reduction of sympathetic blood flow, interventions which should be addressed both pharmacologically and increasing physical activity in at-risk individuals. The pathophysiological impact of such interventions remains to be elucidated in future studies.

Although studies have reported that statins are also a protective factor for incident dementia, especially in AD [140], there is evidence suggesting that in general population, statins given at late life do not prevent cognitive decline or dementia [141]. One meta-analysis that included prospective studies which assessed the potential benefit of statins concluded that there is a significant reduction of incident dementia [HR 0.71, 95%CI 0.61-0.82) [142]. The effects of statins in the central nervous system might be explained by the lipid reduction alone; however, a role for the pleiotropic effects of statins, including anti-inflammatory, anti-oxidant, pro-fibrinolytic and anti-proliferative have been proposed as mechanisms to reduce progression to dementia but have not been evaluated in models of diabetes. Furthermore, modifications of cholesterol content in the brain seems to promote a non-amyloidogenic processing pathway at the level of the cell surface, which reduced amyloid accumulation and plaque formation [142]. Another hypothesis is that statins regulate the synthesis of cholesterol end-products like isoprenoids (farnesyl pyrophosphate and geranyl pyrophosphate) which modify the activity of neuronal signaling proteins like RAS and RHO. However, data is still inconclusive and most proposed pathophysiological mechanisms remain a hypothesis [143]. The role of statins in the modification of T2D-related dementia risk is relevant, considering the role of statins in cardiovascular protection in T2D; however, these mechanisms and clinical associations have not been extensively studied and remain as areas of opportunity for future research.

One study performed in Taiwanese subjects reported that daily low mean doses of 40mg of acetylsalicylic acid (Aspirin) reduces de risk of AD-dementia, but not non-AD-dementia, in patients with T2D. Nevertheless, the same report suggested increases in risk of all-cause incident dementia for doses >40mg compared to non-aspirin users [144]. The proposed mechanisms underlying their observations suggest a role for low-dose aspirin in modulating endothelial function and reducing the pro-inflammatory state that has been linked to AD and T2D pathophysiology but an increased risk for vascular dementia and functional impairment with higher doses. These findings are supported by a recent meta-analysis, which suggests that use of non-steroidal anti-inflammatory drugs (NSAIDs), reduces risk of AD in the general population [145]. Further longitudinal data from observational studies including individuals with T2D and the development of randomized, controlled clinical trials are required to assess the role of aspirin and NSAIDs and its adequate dosages in modifying future dementia risk.

5.3. Evidence for Potential Non-pharmacological Prevention Strategies for Dementia in Type 2 Diabetes

Given the recognition of risk and protective factors for dementia that are potentially modifiable, interest has grown in the development of screening methods to identify at-risk patients for the development of studies focused on dementia prevention in T2D. Recently, a novel diabetes-specific risk score for 10-year prediction of incident dementia in 10 years was developed and validated in American population by Exalto *et al*. The score considers variables including age, schooling, depression and T2D-related comorbidities and complications including microvascular disease, diabetic foot, cerebrovascular disease, cardiovascular disease and acute metabolic events including symptomatic hypoglycemia and hyperglycemic crises. The Diabetes-Specific Dementia Risk (DSDRS) was developed aiming at introducing dementia prediction into daily life clinical practice to detect subjects at high risk of incident dementia [43]. Most of the evaluated factors, as discussed previously, have been linked to increased disability and impaired functional status, both of which increase dementia risk. Future studies should evaluate the role of multidisciplinary interventions in reducing dementia risk by targeting disability, impaired quality of life and functional status related to micro and macrovascular complications of T2D. Evaluating the role of DSDRS in assessing dynamic changes in risk overtime or the development of a dynamic risk score for follow-up would aid in monitoring changes in dementia risk and developing tailored interventions for at-risk individuals.

Since intensive glycemic control has been linked to poorer outcomes in elderly patients with T2D with limited cognitive benefit, a growing interest has emerged in evaluating non-pharmacological interventions, particularly cognitive and metabolic screening, diet and physical activity. One way of detecting patients at high risk of dementia is by detecting MCI [146], this subset of patients have shown a higher risk of developing dementia compared to patients without T2D and MCI [66]. Routine cognitive evaluations should be conducted for T2D patients and studies must be assessed to evaluate the optimum point to intensify screening by focusing on maximizing risk reduction. Views proposed

in other reports also support that preventive measures in the general population, including healthy lifestyle and a favorable cardiovascular risk factor profile, maybe in accordance of reducing the incidence of T2D, which is the better preventive measure to impact its associated dementia risk [147]. The Alzheimer Disease Association concluded in a recent review that there is strong evidence to suggest that regular physical activity, management of cardiovascular risk factors including obesity, smoking and hypertension and healthy lifestyle changes including dietary and cognitive training can delay onset of dementia [15]. Indeed, recent evidence has suggested that physical activity modulates the increases in dementia risk attributable to both of APOEε4 allele and T2D in population that has an increased incidence of T2D, mostly by reducing cardiovascular risk, modulating peripheral insulin sensitivity and improving functional status [148]. Non-pharmacological interventions are promising in modifying long-term dementia risk and are currently an area of opportunity for future research. Table **1** resumes the observed mechanisms for both pharmacological and non-pharmacological interventions in modifying T2D-related dementia risk.

Table 1. Clinical benefits and proposed mechanisms of medications commonly used in T2D on dementia risk.

Medication	Clinical Evidence	Proposed Mechanisms
Metformin	•Decreased dementia risk in patients <75 years, with preserved renal function •Decreased dementia risk in combination with sulfonylureas or pioglitazone •Improves in executive functioning, learning, memory and attention with increased orbitofrontal cerebral blood flow from randomized, controlled, short-term clinical trials	•Activation of AMPK-dependent pathways, decreasing Aβ-related mitochondrial dysfunction, activity of caspase 3/9 activity and cytosolic cytochrome c in human neural stem cells. •MAPK-JNK-dependent decreases in Aβ-induced apoptosis in hippocampal neurons. •Reduction on the effects amyloid deposition in long-term potentiation in mice. •Reversal of memory impairment, oxidative stress, inflammation and tau hyper-phosphorylation in mice.
Pioglitazone	•47% lower incidence of dementia in diabetic patients using pioglitazone for a 6-year period •50% reduction of incident dementia for high dose users.	•Inhibition of neuroinflammation in mice. •Reduction in Aβ-accumulation and plaque formation by promoting Aβ-clearance •Reduction ofmitochondrial dysfunction and tau-hyperphosphorylation
DPP-4 inhibitors	•Modest but beneficial effects on cognition in individuals with and without cognitive impairment and AD.	•Amelioration of cognitive déficits, Aβ formation and deposition and cytotoxicity through activation of AMP-K dependent pathways. •Decreases in activation of glycogen synthase kinase 3β (GSK3β) and tau hyperphosphorylation by improving insulin action.
Sulfonylureas (glimerpiride)	•Not available	•Antagonist of acetylcholine esterase •Downregulates binding of Aβ-plaques, reducing intracellular cholesterol accumulation and activation of phospholipase A2.
ARB/ACEI inhibitors	•ACEI and/or ARB decrease incidence of vascular dementia, but not AD	•Blood pressure control reduces progression of aterosclerosis and hipoxia. •Inactivation of the renin-angiotensin-aldosterone pathway, which has been linked to accelerated progression of cognitive decline. •Alleviation of endotelial dysfunction, leading to improved cerebral blood flow and neuroprotection.
Statins	•Reduction in the incidende of all-cause and AD-dementia.	•Activation of non-amyloidogenic processing pathways at the level of the cell surface, thus reducing plaque formation. •Reduction of cholesterol end-products, improving neuronal signaling. •Anti-inflammatory, anti-oxidant, pro-fibrinolytic and anti-proliferative effects.
Aspirin	•40mg of acetylsalicylic acid (Aspirin) reduces de risk of AD-dementia, but not non-AD-dementia •Increases in risk of all-cause incident dementia for doses >40mg compared to non-aspirin users	•Modulating endothelial function and reducing pro-inflammatory state linked to AD and T2D pathophysiology •Increased risk of bleeding, leading to vascular dementia and functional impairment with higher doses.

Abbreviations: T2D: Type 2 diabetes; AD: Alzheimer's disease, Aβ: Amyloid β, ACEI: Angiotensin converting enzyme inhibitors, ARB: Angiotensin receptor blockers, DPP4: Dipeptidyl peptidase 4 inhibitors.

Table 2. Areas of opportunity for research in T2D-related dementia risk. Conclusions in gaps in knowledge and required studies in diverse areas of opportunity for research in the evaluation of the effect of T2D in cognition and dementia risk. Abbreviations: T2D: Type 2 diabetes; AD: Alzheimer's disease, GWAS: Genome-wide association studies, EWAS: Epigenome-wide association studies.

Research Area	Areas of Opportunity for Research
Pre-clinical	•Experimental designs with increased correlation with observational and clinical findings. •Confirmation of findings from previous studies evaluating impact of biological factors in cognition in models of dementia, AD and T2D. •Effects of antidiabetic effects of medication in *in vivo* models of dementia, AD and T2D. •Systems biology approach to correlate findings with metabolomics, proteomics and gene expression studies.
Observational	•Development of cohort studies aimed at evaluating risk factors for dementia focused in T2D patients, with strong and consistent cognitive outcomes. •Standardization of follow-up time and risk factor evaluation, to ensure statistical power in population-based samples. •Consistent covariate controlling, including traditional risk factors for dementia, use of medication and functional status, along with standardization of analytic techniques for comparison across-studies. •Evaluating the effect of ethnicity, gender and socioeconomic factors in T2D-related cognitive impairment and dementia risk. •Development of GWAS, EWAS and whole-exome sequencing studies aimed at evaluating ethnic-specific variants that increase risk of dementia in T2D, with particular focus on the effetc of known and novel variants attributable to increased T2D risk in susceptible populations
Clinical	•Development of experimental models to confirm findings from pre-clinical studies. •Development of long-term clinical trials evaluating the effect of T2D-related medications in cognitive outcomes in individuals with T2D. •Longitudinal imaging studies paired with metabolic evaluations to assess observations obtained through observational and pre-clinical studies. •Evaluate the direct effect of functional impairment and metacolic changes in cognition in patients with T2D. •Development of specific treatment reccomendations and studies on the effect of deintensification of clycemic control on cognition an dementia risk

CONCLUSION

Here, we resume an overview of pathophysiological links between T2D and dementia reviewing evidence from epidemiological and clinical studies in correlation with data obtained through biological an experimental medicine. A consistent mechanism linking T2D to dementia risk has not been reported in human research and evidence regarding animal models is skewed towards shared mechanisms between AD-dementia and T2D. Current hypothesis have overviewed the role of metabolic disturbances in T2D, including cerebral insulin resistance, accumulation of glycation end products and inflammation, correlated with the effects of T2D and cardiovascular medication on cognition and dementia risk. Epidemiological and clinical studies have also suggested that the clinical course of T2D, including inadequately intensive glycemic control, increasing number or comorbid conditions or T2D-related complications might lead to impaired functional and cognitive status which increase dementia risk. Even though T2D patients are also affected by so-called traditional dementia risk factors, the identification of T2D-specific risk factors in samples powered to evaluate differences with rigorous dementia definitions and consistent confounding control are required for the development of screening methods for future research.

The heterogeneity of the available evidence of T2D-related factors and its role in dementia risk calls for targeted studies aiming at optimization of T2D treatment using a multidisciplinary approach that combines pharmacological and non-pharmacological interventions evaluating its cognitive affect and impact on dementia risk modification, some of these gaps are resumed in Table **2**. Likewise, possible interventions that can decelerate the progression to dementia and cognitive decline must be explored in both observational and experimental settings of individuals with T2D. Despite strong and consistent evidence in biological and experimental medicine, translational studies that evaluate findings from observational studies are required in the pre-clinical setting to study mechanisms of disease and alleviate the intrinsic confounding of most observational settings. Given the increase in T2D prevalence in an aging population, future studies should shed light on these gaps in knowledge and further out understanding in what is becoming an important emerging complication in elderly patients with T2D.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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METS-IR, a novel score to evaluate insulin sensitivity, is predictive of visceral adiposity and incident type 2 diabetes

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Abstract

Objective: We developed a novel non-insulin-based fasting score to evaluate insulin sensitivity validated against the euglycemic–hyperinsulinemic clamp (EHC). We also evaluated its correlation with ectopic fact accumulation and its capacity to predict incident type 2 diabetes mellitus (T2D).

Design and methods: The discovery sample was composed by 125 subjects (57 without and 68 with T2D) that underwent an EHC. We defined METS-IR as $\mathrm{Ln}((2*G_0)+TG_0)*BMI)/(\mathrm{Ln}(HDL\text{-}c))$ (G_0: fasting glucose, TG_0: fasting triglycerides, BMI: body mass index, HDL-c: high-density lipoprotein cholesterol), and compared its diagnostic performance against the M-value adjusted by fat-free mass (MFFM) obtained by an EHC. METS-IR was validated in a sample with EHC data, a sample with modified frequently sampled intravenous glucose tolerance test (FSIVGTT) data and a large cohort against HOMA-IR. We evaluated the correlation of the score with intrahepatic and intrapancreatic fat measured using magnetic resonance spectroscopy. Subsequently, we evaluated its ability to predict incident T2D cases in a prospective validation cohort of 6144 subjects.

Results: METS-IR demonstrated the better correlation with the MFFM ($\rho = -0.622$, $P < 0.001$) and diagnostic performance to detect impaired insulin sensitivity compared to both EHC (AUC: 0.84, 95% CI: 0.78–0.90) and the SI index obtained from the FSIVGTT (AUC: 0.67, 95% CI: 0.53–0.81). METS-IR significantly correlated with intravisceral, intrahepatic and intrapancreatic fat and fasting insulin levels ($P < 0.001$). After a two-year follow-up, subjects with METS-IR in the highest quartile (>50.39) had the highest adjusted risk to develop T2D (HR: 3.91, 95% CI: 2.25–6.81). Furthermore, subjects with incident T2D had higher baseline METS-IR compared to healthy controls (50.2 ± 10.2 vs 44.7 ± 9.2, $P < 0.001$).

Conclusion: METS-IR is a novel score to evaluate cardiometabolic risk in healthy and at-risk subjects and a promising tool for screening of insulin sensitivity.

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Introduction

Decreased insulin sensitivity, better known as peripheral insulin resistance (IR), is a state of ineffective insulin action in peripheral tissues, which leads to hyperinsulinemia and impaired lipid and glucose homeostasis (1). IR is a risk factor for the development of type 2 diabetes mellitus (T2D), which causes significant health burden world-wide (2). Evaluations of IR often require invasive methods, which has underscored the search for accurate indirect measures of IR (3). Development of accurate and sensitive screening measures to detect IR in early stages to prevent cardiometabolic complications has gained interest (2, 3), leading to the development of fasting insulin and non-insulin-based surrogate indexes of IR (4).

Fasting insulin-based indexes, which include the homeostatic model assessment for IR (HOMA-IR) and the quantitative insulin sensitivity check index (QUICKI), have long been employed as the basic evaluations for IR (5). Nevertheless, non-insulin-based fasting IR indexes have been developed to account for the low practicality and variability of insulin-based indexes, substituting insulin measurements for fasting triglycerides, glucose and lipoprotein measures. These include the product of glucose and triglycerides (TyG index), the product of glucose, triglycerides and BMI (TyG-BMI index), and the ratio of triglycerides divided by HDL-c (TG/HDL-c ratio) (6, 7, 8). However, evaluations of non-insulin-based indices against the gold standard, the euglycemic–hyperinsulinemic clamp (EHC), offer contrasting results in terms of diagnostic performance and correlation with direct measures of IR (9). In this work, we aimed to generate a non-insulin-based surrogate of insulin action with higher accuracy compared to other insulin and non-insulin-based methods against the EHC and estimated its correlation with pathophysiological components of IR and the metabolic syndrome (MS). Furthermore, we evaluated the capacity of the score to predict incident T2D cases.

Subjects and methods

Participants and study setting

In the discovery sample, we included men and women 20–79 years old, with a body mass index (BMI) between 18 and 34.9 kg/m^2 recruited from the outpatient Diabetes Clinic of a university hospital in Mexico City. Subjects with T2D participated if they had a glycated hemoglobin (A1c) concentration <8% were not receiving insulin and were treated only with metformin. No subject smoked tobacco, had cardiovascular disease, T2D complications or an acute infection. All biochemical and physiologic evaluations were completed within a month of initial recruitment. Subjects who voluntarily agreed to participate in the study signed an informed consent. The Human Research Ethics Committee of Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ) approved the study. All procedures were done in accordance with the Declaration of Helsinki.

Biochemical, insulin sensitivity and body composition evaluations

Fasting biochemical and anthropometric evaluations

A complete medical and family history, including use of medications was obtained from all subjects. Subjects were weighed on calibrated scales and height was determined with a floor scale stadiometer; BMI was calculated as weight in kg divided by the squared product of height in meters.

Blood was obtained between 08:00 and 09:00 h after 8- to 12-h fast. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2), and A1c levels using high-performance liquid chromatography (HPLC) (Variant II Turbo, BIORAD). Lipid concentrations (cholesterol, triglycerides and HDL cholesterol), apo A, apo B, uric acid, creatinine and hepatic enzymes were measured using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). LDL cholesterol was calculated with the Friedewald equation when triglycerides were <250 mg/dL.

Euglycemic–hyperinsulinemic clamp and body composition analysis

We performed a one-stage EHC in subjects who underwent a 12-h fast; subjects with T2D were instructed to suspend treatment three days in advance. The study was not performed if fasting glucose concentrations were >250 mg/dL. A priming dose of 200 U/m^2/min of insulin was infused for 5 min, followed by 100 U/m^2/min for 5 min; subsequently, insulin was infused at a rate of 50 U/m^2 body surface area (BSA)/min. Euglycemia (~100 mg/dL) was maintained by a variable infusion of 20% dextrose; arterialized blood samples using a hot box were obtained every 10 min during the final 30 min of the EHC to determine

glucose and insulin concentrations. Insulin sensitivity was determined by the glucose infusion rate or M-value (10) during the final 30 min adjusted for fat-free mass (MFFM) obtained by dual X-ray energy absorptiometry (DXA) with a GE Lunar iDXA densitometer. Subcutaneous and intra-abdominal adipose tissue volume were quantified using magnetic resonance imaging (MRI); intrapancreatic and intrahepatic triglyceride (IHTG) content was determined using magnetic resonance spectroscopy (11).

Development and validation of the METS-IR index

Association of fasting biochemical measures with MFFM

We used linear regression analysis to develop an explanatory model for MFFM using fasting biochemical measures and anthropometric measurements obtained from the day of the EHC. Variables were removed from the model until the best fitting model with the maximum adjusted R^2 was achieved.

Mathematical modeling

Standardized beta coefficients for the associated variables in the linear regression model were considered to generate an equation for an IR index. Associated variables with negative beta coefficients were included in the numerator and variables with positive beta coefficient were included in the denominator; the magnitude of the coefficient was used to equilibrate variable contributions within the equation and variables with non-parametric distribution were log-transformed to approximate normality. Finally, we performed algebraic transformations in the model until achieving the equation that produced scores with the higher correlation with MFFM obtained from linear regression analyses, adjusted for age, sex and T2D, which we named metabolic score for IR (METS-IR).

Validation of the METS-IR index against the MFFM from EHC

For validation of the index, we included 59 additional subjects who also underwent an EHC and body composition analysis using DXA, aged 20–79 years old and BMI > 30.0 kg/m^2, without diagnosed cardiovascular disease or acute infection and who were evaluated using a 2-h oral glucose tolerance test (GTT) to rule-out T2D. The comparison between the validation and the discovery cohorts (Supplementary Table 1, see section on supplementary data given at the end of this article) showed significant differences between both cohorts; to account for those differences, evaluation was initially carried out in the validation cohort (n = 59), followed by evaluation of the overall EHC cohort, including the discovery sample (n = 184). We calculated HOMA-IR ((Glucose*Insulin)/405) (12), QUICKI (1/(log insulin + log glucose)) (13), TyG index (Ln((Glucose*Triglycerides)/2) (6), TG/HDL ratio (triglycerides/HDL-c) (14)) and the TyG*BMI index (TyG-BMI) (15) from fasting biochemical parameters and anthropometric measures in both cohorts. Validation of METS-IR against other surrogate measures of insulin action were evaluated using MFFM values <25th percentile as a cut-off point for IR in our validation cohort.

Validation of METS-IR against the SI index from the modified FSIVGTT and HOMA-IR

A second validation was carried out in a cohort of 61 subjects, aged 18–55 years old, with BMI between 18.5 and 24.9 kg/m^2, healthy and who were not taking medications that interfere with insulin sensitivity. These subjects were also evaluated with a 2-h oral GTT to rule-out T2D and subsequently insulin sensitivity was assessed with a 3-h modified frequently sampled intravenous GTT (FSIVGTT). Glucose was administered intravenously at a dose of 0.3 g/kg for 60 s beginning at time 0 and insulin at a dose of 0.03 IU/kg at minute 20 for 5 min; blood samples were obtained at −15, −10, −5, 0, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160 and 180 min, and data were analyzed using the MINMOD Millennium software to estimate insulin sensitivity (SI index). METS-IR, HOMA-IR, TG/HDL ratio, TyG-BMI and the TyG index were also calculated using fasting laboratory values obtained before the FSIVGTT. A cut-off value for the SI index <5 μU/min/mL was defined as IR for this evaluation.

Prediction of T2DM incidence using METS-IR and validation against HOMA-IR

We then evaluated the capacity of the METS-IR index to predict incident T2D, in our metabolic syndrome cohort, which was developed with the aim to evaluate the risk of MS components in incident T2D, hypertension, and cardiovascular mortality in an urban population living in 9 different cities in Mexico. The inclusion criteria included individuals aged 25–69 years, BMI ≥ 23 kg/m^2, without T2D, hypertension or other significant cardiovascular comorbidities and obese individuals (BMI ≥ 30 kg/m^2) with at least one of the following conditions: blood pressure ≥140/90 mmHg, fasting glucose >100 mg/dL, total cholesterol >200 mg/dL and triglyceride levels

>150 mg/dL. Individuals with diagnosed T2D, coronary artery disease, cerebral vascular disease, alcoholism, taking corticosteroids, with liver disease, kidney dysfunction or life-threatening diseases that would prevent the two-year follow-up were excluded.

Subjects were interviewed to obtain medical history, sociodemographic information, dietary and physical activity habits and anthropometric measurements, including weight, height, and waist circumference. Blood pressure measurement was also performed. We obtained a 20 mL blood sample after 9- to 12-h fast to measure of glucose, insulin, total and HDL cholesterol, triglyceride, apolipoprotein B and C-reactive protein concentrations using the same laboratory techniques as described earlier. These same evaluations were carried out after a minimum of two-year follow-up. Incident T2D was defined as a construct of previous medical diagnosis of T2D, taking oral hypoglycemic medication and/or fasting glucose levels as determined by current ADA guidelines. Time to follow-up was estimated from recruitment up to the last follow-up or T2D diagnosis, whichever occurred first. We also performed validation of METS-IR against HOMA-IR using the baseline evaluation of our MS cohort, using scores >75th percentile as the cut-off point to define IR.

Statistical analysis

Intergroup differences and paired data

To evaluate intergroup differences in sociodemographic, biochemical measures and IR indexes, we used Student's *t*-test and Mann–Whitney *U* where appropriate. Frequency distribution of categorical variables is reported as frequencies and percentages and was compared between groups using chi-squared tests. For measurements in follow-up studies, we used Student's paired *t*-test and Wilcoxon's rank-sign tests, where appropriate. Logarithmic transformations were applied to approximate normality in those variables showing a non-parametric distribution. Data are presented as mean ± S.D. or as median and interquartile range.

Validation of METS-IR

We used partial correlation analysis to evaluate correlation of individual IR indexes, including METS-IR, against MFFM for the EHC cohort, SI index for the FSIVGTT cohort and HOMA-IR for the MS cohort, adjusted for age, sex and the presence of T2D where appropriate; we generated 95% confidence intervals for the correlations using bootstrap sampling drawing 2000 random stratified samples. Diagnostic performance was evaluated using areas under the receiving-operating characteristic curve (AUC of ROC) and 95% confidence intervals were estimated using bootstrap sampling drawing 2000 stratified random samples in both cohorts. To estimate differences between AUC of ROC curves, we performed non-parametric ROC tests using a stratified bootstrap sampling method using the *pROC* package from R, version 3.4.3, as proposed by DeLong *et al*. (16). The cut-off point was determined using the Youden index; sensitivity, specificity, positive and negative predictive values and likelihood ratios (PPV, NPV, LR(+), LR(−), respectively) were calculated using the *OptimalCutpoints* package from R, version 3.4.3 (17).

Correlation of METS-IR with pathophysiological components of IR and MS

To evaluate dose–response correlation of physiologic parameters with METS-IR scores, trend analysis with linear regression was used against quartiles of intrahepatic, intrapancreatic and intravisceral fat adjusted for age, sex and T2D. Intrapancreatic and intrahepatic fat, fasting insulin and body composition measures using DXA were evaluated to develop an explanatory model for METS-IR using linear regression analyses. Variables were removed from until achieving the model with the highest adjusted R^2 value.

Prediction of incident T2D using METS-IR

To evaluate the association of the METS-IR score with incident T2D, we performed survival analysis comparing across METS-IR terciles and quartiles using Kaplan–Meier curves compared with log-rank tests. Cox proportional-risk regression analyses were used to evaluate risk of incident T2D across terciles and quartiles of METS-IR scores adjusted for age, sex, family history of T2D, hypertension, physical activity, waist circumference and smoking. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 21.0), R software (Version 3.4.4) and GraphPad Prism, version 6.0.

Results

Study population

An outline of the study subjects and phases is presented in Fig. 1. In the discovery sample, we included 67 and 58 subjects with and without T2D, respectively. Subjects without T2D were predominantly female, younger and with a significantly lower BMI, A1c, fasting glucose, insulin and liver enzyme levels compared to subjects

with T2D ($P<0.001$). No significant differences were observed in triglyceride, total cholesterol, LDL-c, HDL-c and serum creatinine concentrations. As expected, raw M-values, weight-adjusted M-values, MFFM and QUICKI values were significantly higher for subjects without T2D ($P<0.001$); conversely, HOMA-IR, TG/HDL, TyG and TyG-BMI indexes were lower ($P<0.001$, Table 1).

Mathematical modeling of METS-IR

In linear regression analyses (Table 2), we identified significant associations between the MFFM and BMI, triglyceride, HDL-c and glucose concentrations ($r^2=0.309$, $P<0.001$). Standardized beta coefficients were negative for triglycerides, BMI and glucose ($\beta=-0.170$, $\beta=-0.186$ and $\beta=-0.305$, respectively) but positive for HDL-c ($\beta=0.194$). Given the near twofold difference in the coefficient for glucose, a twofold multiplier was added to reflect regression coefficients in the equation. Non-parametric variables were log-transformed and algebraic transformations were performed yielding the resulting equation METS-IR = (Ln ((2*G_0) + TG_0)*BMI)/(Ln (HDL-c)), where G_0 and TG_0 represent fasting glucose and triglyceride concentrations, respectively.

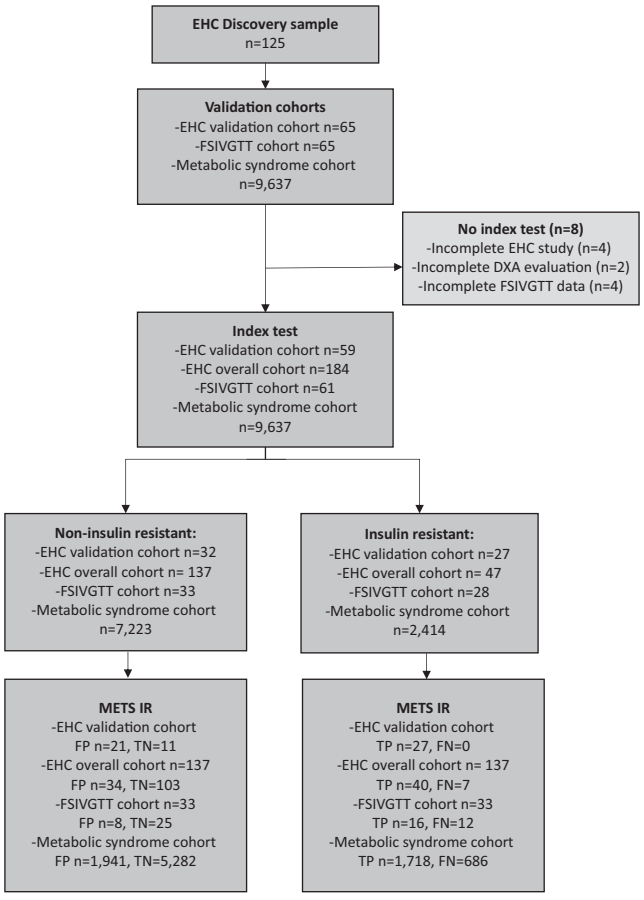

Figure 1
STARD diagram of study phases and populations used for the development, validation and prediction of incident type 2 diabetes with the METS-IR score. EHC, Euglycemic–hyperinsulinemic clamp; FP, False positive; FN, False negative; FSIVGTT, Frequently sampled intravenous glucose tolerance test; TN, True negative; TP, True positive.

Association of METS-IR and MFFM

The better correlation of fasting IR indexes and the MFFM was observed for METS-IR ($\rho=-0.569$, Supplementary Table 2), which was slightly higher than the TyG-BMI score ($\rho=-0.555$). For individuals without T2D, METS-IR also displayed the better correlation with MFFM ($\rho=-0.652$), followed by TyG-BMI ($\rho=-0.649$) and HOMA-IR ($\rho=0.646$). METS-IR had the highest correlation in subjects with T2D ($\rho=-0.504$), followed by the TyG-BMI index ($\rho=-0.442$). In linear regression analysis, METS-IR had a significant association with MFFM ($r^2=0.376$, $P<0.001$) adjusted for age, sex and T2D, which was higher than the explanatory model of the independent variables.

METS-IR, visceral adiposity and hyperinsulinemia

In our discovery sample, we observed a significant correlation between METS-IR and intravisceral ($\rho=0.660$, $P<0.001$), intrahepatic ($\rho=0.636$, $P<0.001$) and intrapancreatic fat ($\rho=0.408$, $P<0.001$). These associations were confirmed using linear regression analysis, adjusted by the presence of T2D, age and sex (Fig. 2). In addition, we observed a significant trend of higher METS-IR scores correlated with increasing percentiles of intravisceral, intrahepatic and intrapancreatic fat ($P<0.001$ for all). We found a significant correlation between fasting insulin concentration ($\rho=0.2608$, $P=0.001$) and METS-IR score, adjusted by age, sex and the presence of T2D. Using linear regression analysis (Table 3), we also observed that fasting insulin, intrahepatic, intravisceral and intrapancreatic fat explain 64.5% of the variability in METS-IR.

Diagnostic performance and validation of METS-IR against the MFFM

In the EHC cohort ($N=184$), we identified 47 subjects with IR using the MFFM value <25th percentile as a cut-off point (<6.39 mg/min/kg FFM), 20 subjects (16.0%) from the original cohort and 27 newly included subjects

Table 1 Sociodemographic variables, fasting biochemical measures, results of EHC and subrogate fasting insulin resistance indexes compared between diabetic and nondiabetic subjects in the discovery sample.

Parameter	Nondiabetic ($n=57$)	Diabetic ($n=67$)	*P*-value
Female sex (%)	39 (68.4%)	37 (54.4%)	0.110
Age (±S.D.)	32.19 ± 9.75	52.97 ± 12.08	<0.001
HbA1c (%)	5.20 ± 0.35	6.34 ± 0.69	<0.001
BMI (kg/m^2)	26.18 ± 3.79	28.78 ± 3.34	<0.001
Fasting glucose (mg/dL)	89.21 ± 7.67	118.07 ± 30.01	<0.001
Fasting insulin (µI/mL)	7.60 ± 4.72	10.50 ± 5.62	0.002
Triglycerides (mg/dL)	104.0 (80.0–158.8)	134.0 (106.5–179.5)	0.070
Total cholesterol (mg/dL)	174.68 ± 33.72	183.92 ± 35.67	0.133
LDL-c (mg/dL)	105.02 ± 12.15	108.47 ± 26.66	0.566
HDL-c (mg/dL)	43.91 ± 9.36	45.10 ± 12.75	0.506
Serum creatinin (mg/dL)	0.70 ± 0.18	0.73 ± 0.21	0.391
ALT (IU/L)	22.5 (17.0–32.3)	28.0 (21.0–39.5)	0.009
AST (IU/L)	22.5 (18.8–27.3)	27.0 (22.5–34.0)	<0.001
GGT (IU/L)	15.0 (11.0–25.3)	23.0 (16.0–30.5)	<0.001
M-value (mg/min)	567.71 ± 210.86	401.45 ± 154.71	<0.001
M-value adjusted by body mass (mg/min/kg)	8.22 ± 2.96	5.51 ± 2.29	<0.001
M-value adjusted by fat-free mass (mg/min/kg)	13.36 ± 4.76	9.01 ± 3.91	<0.001
HOMA-IR	1.39 (1.00–2.33)	2.70 (1.62–4.83)	<0.001
QUICKI	0.33 ± 0.04	0.36 ± 0.03	<0.001
TG/HDL	3.13 ± 2.32	4.30 ± 3.42	<0.001
TyG	8.52 ± 0.57	9.02 ± 0.58	<0.001
TyG-BMI	227.16 ± 43.03	258.23 ± 38.86	<0.001

ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BMI, Body mass index; EHC, Euglycemic-hyperinsulinemic clamp; HbA1c, Glycated hemoglobin; HDL-c, High-density lipoprotein cholsterol; HOMA-IR, homeostatic model assessment for IR; LDL-c, Low-density lipoprotein cholecterol; QUICKI, quantitative insulin sensitivity check index; TG/HDL, Ratio of triglycerides and high-density lipoprotein cholesterol; TyG, TyG index; TyG-BMI, TyG*BMI index.

(45.8%). METS-IR had an AUC of 0.845 (95% CI: 0.783–0.899) to identify IR in the combined validation cohort and 0.738 in the EHC validation cohort (95% CI: 0.601–0.866), which did not differ significantly ($P=0.18$) from the discovery sample (AUC: 0.853, 95% CI: 0.769–0.926, Fig. 3A and B). The AUC was lower for individuals with T2D (AUC=0.839, 95% CI 0.728–0.931) than for healthy individuals (AUC=0.852, 95% CI 0.778–0.920) but the difference was not statistically significant ($P=0.833$). A cut-off value for METS-IR of >51.13 had a sensitivity of 85.12% (95% CI 71.7–93.8%) and a specificity of 75.2% (95% CI 67.1–82.2%) to identify cases of IR diagnosed by MFFM. The NPV and PPV were 93.6% (95% CI 86.7–95.7%) and 54.0% (95% CI 44.2–75.7%), respectively; the positive and negative likelihood ratios were 3.43 (95% CI 2.50–4.70) and 0.20 (0.10–0.39).

Evaluation of other IR indexes using AUC of ROC curves (Table 4) against METS-IR showed the higher AUC for HOMA-IR, followed by METS-IR; among non-insulin fasting indexes, METS-IR had the higher performance, followed by the TyG-BMI index. We observed no significant differences in the AUCs of METS-IR, HOMA-IR and the TyG-BMI index; however, METS-IR had a significantly higher AUC than QUICKI, TyG index and TG/HDL ratio

Table 2 Linear regression analysis showing fasting laboratory values and anthropometric measurements associated to the M-value adjusted by fat-free mass, used for the development of the index.

	Beta	Standardized beta	*T*	*P*-value	95% CI
Variable					
Triglycerides	−0.012	−0.170	−1.983	0.050	−0.023 to 0.000
HDL-c	0.081	0.194	2.433	0.016	0.015–0.147
BMI	−0.236	−0.186	−2.235	0.027	−0.445 to −0.027
Glucose	−0.054	−0.305	−3.751	<0.001	−0.82 to −0.025

Adjusted for age, sex and the presence of T2D.
95% CI, 95% Confidence interval; BMI, Body mass index; HDL-c, High-density lipoprotein cholsterol; MFFM, M-value adjusted by fat-free mass; T2D, Type 2 diabetes mellitus.

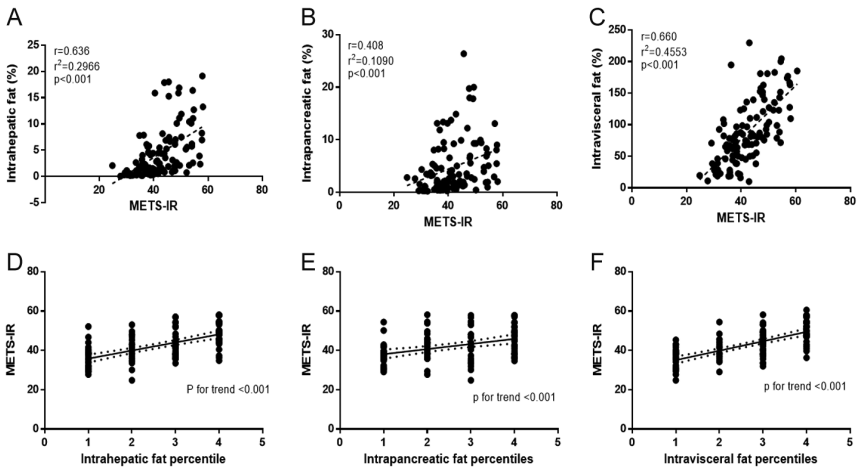

Figure 2
(A, B and C) show linear regressions and correlations between intrahepatic (A), intrapancreatic (B) and intravisceral (C) fat content, showing positive and significant associations with METS-IR values. Figures (D, E and F) show the comparison between METS-IR distributions according to intrahepatic (D), intrapancreatic (E) and intravisceral (F) fat content quartiles, showing a statistically significant trend ($P<0.001$) of increasing METS-IR values with higher intravisceral, intrapancreatic and intrahepatic fat content.

in both healthy subjects and the overall EHC cohort ($P<0.01$). In subjects with T2D, METS-IR and the TyG-BMI index had the best diagnostic performance; we observed no significant differences among AUCs of fasting indexes in subjects with T2D. Cut-off points along with its diagnostic performance indexes showed the higher sensitivity and NPV for METS-IR compared to other non-insulin-based fasting IR surrogates (Supplementary Table 3).

Validation against the modified FSIVGTT and HOMA-IR

In the second validation cohort against the SI index obtained from the modified FSIVGTT, we evaluated 61 healthy subjects, with an average age of 24.8±4.4 years and female predominance (73.8%). The average SI index was 6.23±3.58 and 28 subjects (45.9%) were classified with IR. Using partial correlation analysis adjusted by age and sex, all fasting indexes were significantly correlated to the SI index calculated by MINMOD (Supplementary Table 4). The highest AUC of fasting indexes (Fig. 3C) against IR (as defined by an SI index <5 µU/min/mL) was for QUICKI (AUC: 0.68, 95% CI: 0.54–0.82), followed by METS-IR (0.67; 95% CI: 0.53–0.81) and the TG/HDL ratio (AUC: 0.66, 95% CI: 0.53–0.80). We observed no statistically significant differences between the AUC of any of the fasting indexes (Table 4).

Finally, we validated the score using the baseline evaluation of our MS cohort ($n=9637$) against HOMA-IR, defining IR as HOMA-IR score >75th percentile (>3.78, Supplementary Table 5). Using partial correlation analyses, adjusted for age and sex, we found the highest correlation to HOMA-IR among non-insulin-based indexes for METS-IR ($\rho=-0.568$, 95% CI: −0.554 to −0.582), followed by the TyG-BMI index; as expected, the highest correlation was observed for the QUICKI index. Among non-insulin-based indexes, METS-IR had significantly higher AUCs compared to the TyG index and the TG/HDL ratio ($P<0.001$); we observed no significant differences between METS-IR and the TyG-BMI index (Fig. 3D and Table 4).

Prediction of incident T2D using baseline METS-IR

Our validation cohort included 9637 subjects for the baseline evaluation, from which 6144 completed the follow-up evaluation. We observed 331 cases of incident T2D over 14 850 accumulated person-years, yielding an incidence rate of 22.3 cases per 1000 person-years, or 5.4% in an average of 2.42 years of follow-up. Most subjects

Table 3 Linear regression analyses to evaluate the association of METS-IR and subrogates of insulin resistance, adjusted for age, sex and the presence of T2D.

	Beta	Standardized beta	*T*	*P*-value	95% CI
Parameter					
Fasting insulin	0.285	0.189	2.639	0.010	0.071–3.746
Intrahepatic fat	1.652	0.267	3.455	0.001	0.704–2.599
Intravisceral fat	3.217	0.274	3.092	0.003	1.154–5.281
Subcutaneous fat	5.892	0.88	4.309	<0.001	3.181–8.603

95% CI, 95% Confidence interval; METS-IR, Metabolic Score for Insulin Resistance.

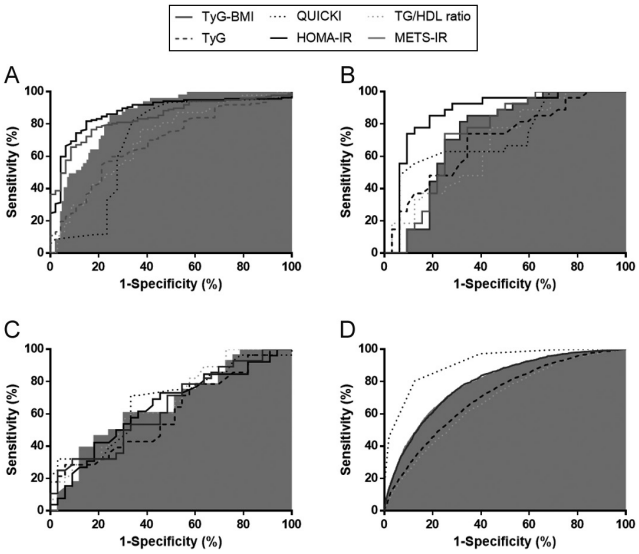

Figure 3

Receiver-operating characteristic (ROC) curves showing the weighed sensitivity and 1-specificity measures of fasting IR surrogates to identify cases of insulin resistance against (A). M-value adjusted by fat-free mass as the gold standard with a cut-off point <25th percentile in the combined EHC cohort ($n=184$). (B) M-value adjusted by fat-free mass as the gold standard with a cut-off point <25th percentile in the validation EHC cohort $n=59$), (C) SI index obtained using MINMOD analyses from modified FSIVGTT using a cut-off of <5 µU/min/mL ($n=61$) and (D) HOMA-IR values >75th percentile in the baseline evaluation of our MS cohort ($n=9637$). Shaded area represents area under the ROC curve of METS-IR. HOMA-IR, homeostatic model assessment for IR; METS-IR, Metabolic Score for Insulin Resistance; QUICKI, quantitative insulin sensitivity check index; TG/HDL, Ratio of triglycerides and high-density lipoprotein cholesterol; TyG, TyG index; TyG-BMI, TyG*BMI index.

were female (66.6%), with no significant differences in sex between those who developed T2D after follow-up in comparison to those who did not. The mean age of enrolled subjects was 42.63 ± 10.79 years, with a significantly higher age for subjects with incident T2D. Subjects who developed T2D had significantly higher BMI, glucose, insulin, triglyceride, total cholesterol along with lower HDL-c levels (Table 5). After follow-up, subjects with incident T2D had a significant increase in fasting glucose and a decrease in triglyceride, total cholesterol, HDL-c and without significant changes in BMI and fasting insulin levels.

Subjects who developed T2D had significantly higher METS-IR scores at baseline in comparison to those who did not (Fig. 4A, 50.2 ± 10.2 vs 44.7 ± 9.2, $P<0.001$). Both groups had a slight increase in METS-IR scores between visits, which remained significantly larger in subjects with incident T2D. Individuals in the highest METS-IR tercile had significantly higher T2D incidence over time, in comparison to the middle and lower terciles (log-rank test $P<0.001$; Fig. 4B). This observation was confirmed in Cox proportional risks regression analysis, which showed progressively higher risk of incident T2D for the highest (METS-IR >47.86; HR: 2.92, 95% CI: 1.87–4.55) and middle tercile (METS-IR: 40.16–47.86; HR: 2.38, 95% CI: 1.51–3.76) in comparison to the lowest tercile, adjusted by family history of T2D, age, sex, smoking, hypertension, physical activity and waist circumference. Using METS-IR quartiles, a score >50.39 was associated with the highest adjusted risk to develop T2D (HR: 3.91, 95% CI: 2.25–6.81; Supplementary Table 6).

Discussion

Here, we report a novel surrogate index to estimate insulin action validated against the EHC. Our model is calculated using fasting measures of glucose, triglycerides and HDL-c along with BMI, which are routinely obtained by primary care physicians, and do not rely on fasting insulin measurements, which are costly and have a high variability according to immunoassay technique utilized (18). METS-IR is a simple, indirect method for the detection of IR that correlates with pathophysiological components of the MS (i.e. intravisceral, intrahepatic and intrapancreatic fat) and is useful for prediction of incident T2D.

The performance of METS-IR was compared against other surrogate IR indexes. The higher correlations with the MFFM were observed for METS-IR, HOMA-IR, QUICKI and the TyG-BMI indexes and were similar to previous reports (19). METS-IR had a good diagnostic performance that was significantly higher than the TyG index and the TG/HDL ratio, but no different than the TyG-BMI index. Likewise, non-insulin-based indexes, particularly METS-IR and the TyG-BMI index, had a better correlation with MFFM than insulin-based indexes. Analyses of MS components have suggested a higher sensitivity for surrogates of obesity to identify adverse metabolic outcomes (20, 21). Therefore, the use of BMI in IR estimation might increase the spectrum of explained variability of the model and elucidates the increased correlation and diagnostic performance for both METS-IR and the TyG-BMI index, both of which include anthropometric measurements, against other non-insulin-based fasting surrogates of IR; this is relevant, since obesity is a known modifier of the reliability of insulin and non-insulin-based estimates of IR (22, 23, 24).

Table 4 Comparison of areas under the receiver operating characteristic curves for insulin resistance subrogate indexes as compared to the M-value adjusted by fat-free mass between the overall population, subjects with T2D and controls. Data are presented as AUC (95% CI)

Index	EHC cohort[+] ($n=184$)	EHC validation[+] cohort ($n=59$)	FSIVGTT cohort[^] ($n=61$)	Metabolic syndrome cohort[&] ($n=9637$)
METS-IR	0.845 (0.783–0.899)	0.738 (0.601–0.866)	0.669 (0.532–0.806)	0.800 (0.789–0.811)
HOMA-IR	0.875 (0.812–0.926)	0.868 (0.756–0.957)	0.645 (0.501–0.790)	–
QUICKI	0.702 (0.596–0.809)*	0.720 (0.518–0.846)	0.681 (0.544–0.818)	0.944 (0.940–0.949)*
TyG index	0.692 (0.609–0.771)*	0.692 (0.555–0.822)	0.632 (0.490–0.774)	0.715 (0.703–0.728)*
TyG-BMI index	0.841 (0.778–0.899)	0.733 (0.596–0.863)	0.640 (0.499–0.780)	0.800 (0.790–0.811)
TG/HDL	0.710 (0.626–0.790)*	0.672 (0.531–0.806)	0.663 (0.527–0.800)	0.690 (0.677–0.703)*

*$P<0.01$ against METS-IR in non-parametric ROC AUC comparison, [+]IR defined as MFFM <25th percentile, [^]IR defined as SI index <5 µU/min/mL, [&]IR defined ad HOMA-IR values >75th percentile.
95% CI, 95% Confidence interval; AUC, Area under the curve; HOMA-IR, homeostatic model assessment for IR; IR, Insulin resistance; METS-IR, Metabolic Score for Insulin Resistance; QUICKI, quantitative insulin sensitivity check index; TG/HDL, Ratio of triglycerides and high-density lipoprotein cholesterol; TyG, TyG index; TyG-BMI, TyG*BMI index; T2D, Type 2 diabetes mellitus.

METS-IR demonstrated significant correlations with visceral, intrahepatic and intrapancreatic fat content, known pathophysiological components of both IR and MS. Ectopic fat accumulation in muscle and liver tissue has been studied as a mechanism for the development of IR (25, 26); several studies have linked intrahepatic and intrapancreatic fat accumulation with IR (27, 28, 29, 30). Intrahepatic fat accumulation has also been linked to the development of hepatic IR, which significantly alters glucose and lipid homeostasis (31, 32). This translates into hyperglycemia, impaired lipemia and increases in body weight, all of which are mechanisms evaluated by our index (33). Evaluation of at-risk individuals using METS-IR would allow identification of pathophysiological alterations of IR, sparing the cost and variability of fasting insulin measurements.

Validation of the index against the MFFM was carried out combining the discovery sample with a set of obese individuals in which EHC data were available. In addition, we extended the validation to a second cohort of individuals with normal BMI, using as a gold standard a modified FSIVGTT with minimal model analysis to assess insulin sensitivity. This approach is reasonable, since minimal model approaches yield measures of insulin sensitivity that adequately correlate with estimations made by clamp techniques (34). Despite obtaining a lower correlation with SI than the observed with MFFM, METS-IR had a good diagnostic performance to identify cases of IR against other fasting surrogates of IR in this second cohort. Finally, we performed a third validation against HOMA-IR in a large cohort, in which the observations from validation against the other two measures of insulin sensitivity were replicated. Thus, METS-IR had good diagnostic performance in all three cohorts and was validated against three different methods to estimate insulin action.

IR is known to increase the risk for the development of dyslipidemia, hypertension, coronary artery disease and, particularly T2D (35, 36). Fasting IR indexes, including QUICKI, HOMA-IR, TyG index and the TG/HDL ratio have all been shown to predict incident T2D (37, 38, 39, 40). In our study, individuals in the highest percentile of METS-IR

Table 5 Anthropometric and laboratory measures for subjects with and without incident type 2 diabetes after 2-year follow-up in the validation cohort.

Parameter	Control		*P*-value*	Incident diabetes		*P*-value*
	Baseline	Follow-up		Baseline	Follow-up	
METS-IR	44.67 ± 9.22	45.71 ± 10.00	<0.001	50.23 ± 10.16	51.04 ± 10.90	0.021
Fasting glucose (mg/dL)	85.35 ± 10.45	85.53 ± 11.89	0.227	97.47 ± 13.42	112.69 ± 38.41	<0.001
BMI (kg/m^2)	28.65 ± 4.58	28.74 ± 4.66	0.001	31.01 ± 5.19	30.79 ± 5.18	0.061
Triglycerides (mg/dL)	187.90 ± 141.26	174.20 ± 112.42	<0.001	226.93 ± 154.87	207.59 ± 121.16	0.020
Fasting insulin (µl/mL)	11.75 ± 7.64	12.11 ± 10.14	0.007	15.82 ± 10.30	17.26 ± 15.37	0.083
Total cholesterol (mg/dL)	205.76 ± 40.94	197.78 ± 40.07	<0.001	211.17 ± 40.69	202.69 ± 39.33	<0.001
HDL-c (mg/dL)	44.81 ± 11.69	41.71 ± 12.11	<0.001	42.41 ± 10.97	40.20 ± 10.88	<0.001

**P*-value for paired comparison between baseline and follow-up.
BMI, Body mass index; HDL-c, High-density lipoprotein cholesterol; LDL-c, Low-density lipoprotein cholesterol; METS-IR, Metabolic Score for Insulin Resistance.

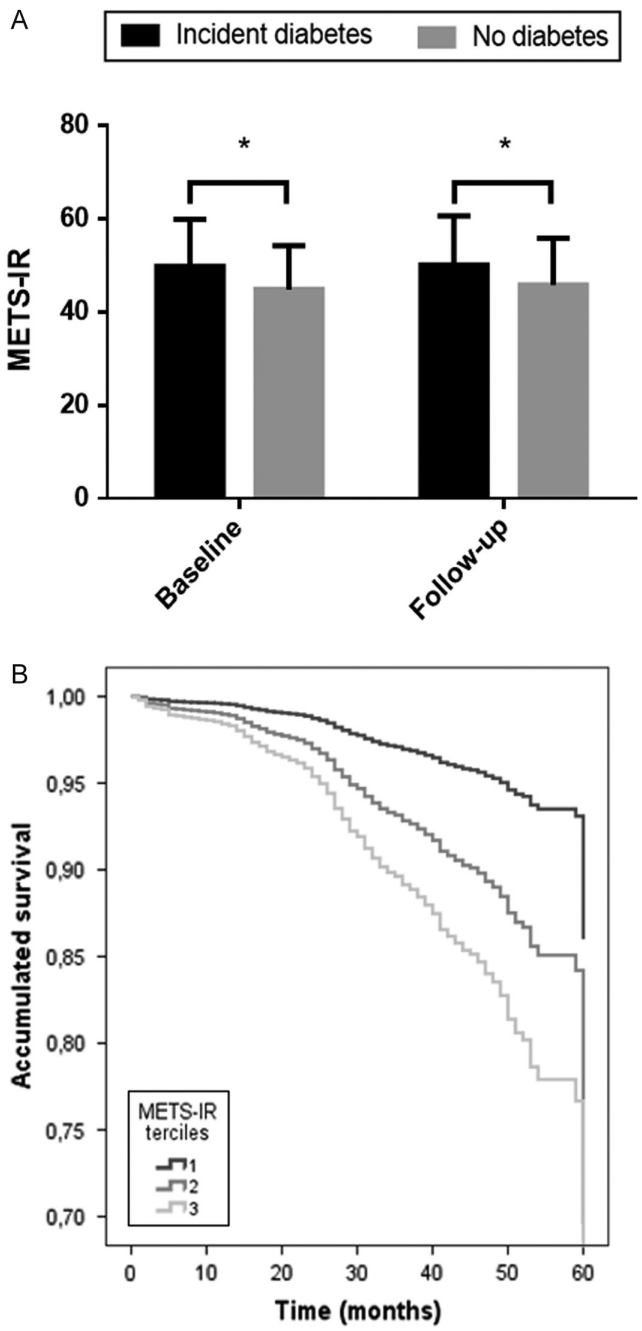

Figure 4

(A) Comparison of mean METS-IR values at baseline and follow-up between subjects with and without incident T2D. As seen in the figure both at baseline and follow-up mean METS-IR was significantly different between subjects with and without incident T2D ($P<0.001$). (B) Kaplan–Meier survival curves comparing T2D incidence between METS-IR terciles during a two-year follow-up period ($P<0.001$ for log-rank test).

score had a 3.9-fold increase in the risk of incident T2D after follow-up, compared to the lowest METS-IR quartile. In addition, individuals who developed T2D had higher baseline METS-IR scores and the risk of incident T2D was progressively higher for increasing METS-IR percentiles scores, an effect that was modulated by age. Application of METS-IR is feasible and reliable to identify subjects at-risk of developing T2D, which makes it a useful tool for application by primary care physicians, considering the practicality of its measurements and the pathophysiological correlations with components of MS and IR.

Our study had some strengths and limitations. METS-IR was validated against MFFM, the gold standard for assessing insulin action without the confounding effect of changes in body composition; normalization of M-values by FFM is a useful technique and reduces underestimation resulting from gender-related differences in fat mass compared to adjustment for body weight (41). METS-IR was validated in a group of patients with the clinical characteristics in which the estimation of insulin action is most likely to be clinically useful. Furthermore, it was validated also against the SI index, obtained with the minimal model approach. The evaluation of our index went beyond the assessment of the insulin action; it included also metabolic comorbidities and the ability to predict incident T2D. The limitations to be acknowledged include a relatively small sample size in the discovery sample and a small number of lean and healthy individuals in the validation sample. Further, we performed validation against the MFFM in an overlapping population with the discovery sample, which could lead to overestimation of diagnostic performance; this issue is common in the development of a surrogate index of insulin action due to the complexity and cost of the EHC. To account for these limitations, we performed validation against other estimators of insulin action and estimated confidence intervals using bootstrap methods; finally, we could replicate the results from the validation against MFFM. In addition, most studies comparing indirect fasting IR indexes use as the M-value adjusted by total body weight as the gold standard, while we used the MFFM, which makes comparisons with results from other studies less feasible. Lastly, because nearly half of the subjects evaluated in the discovery population had T2D, hyperinsulinemia during the one-stage EHC might not completely suppress hepatic glucose production, which might underestimate M-values for individuals with T2D (42); further, correlation with the MFFM and evaluation of ectopic fat accumulation across METS-IR percentiles had to be adjusted by T2D, age and sex, which leaves the possibility of residual confounding.

In conclusion, METS-IR is a novel score, which combines non-insulin fasting laboratory values and anthropometric measurements easily obtained during a primary care evaluation to evaluate insulin sensitivity and detect IR cases. Our index has a good correlation with the MFFM obtained from the EHC, ectopic fat accumulation and fasting insulin levels, which makes it a reliable indicator of overall IR. Furthermore, METS-IR displays a good predictive capacity to detect individuals at risk of developing T2D, which poses it as a complementary tool to conventional clinical predictors for the development of T2D. Therefore, METS-IR is a promising score to evaluate cardiometabolic risk, which makes it a useful tool for primary care physicians as a routine screening tool for metabolic health.

Supplementary data

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-17-0883.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Research idea and study design: O Y B C, I C B, P A V, C A A S; data acquisition: P A V, D G V, T V R, A R R, D S L, D M O, O A C, M R S G, A J M, L M H; data analysis/interpretation: O Y B C, P A V, C A A S; statistical analysis: O Y B C, A V V; manuscript drafting: O Y B C, P A V, C A A S, A V V; supervision or mentorship: C A A S, P A V. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Original article

Metabolic Score for Visceral Fat (METS-VF), a novel estimator of intra-abdominal fat content and cardio-metabolic health

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SUMMARY

Background & aims: Intra-abdominal and visceral fat (VAT) are risk factors for the development of cardio-metabolic comorbidities; however its clinical assessment is limited by technology and required expertise for its assessment. We aimed to develop a novel score (METS-VF) to estimate VAT by combining the non-insulin-based METS-IR index, waist-height ratio (WHtr), age and sex.

Methods: We developed METS-VF in a sample of 366 individuals with Dual X-ray absorptiometry (DXA). METS-VF was modeled using non-linear regression and validated in two replication cohorts with DXA (n = 184, with n = 118 who also had MRI) and bio-electrical impedance (n = 991). We also assessed METS-VF to predict incident type 2 diabetes (T2D) and arterial hypertension independent of body-mass index (BMI) in our Metabolic Syndrome Cohort (n = 6144).

Results: We defined METS-VF as: $4.466 + 0.011*(\text{Ln}(\text{METS-IR}))^3 + 3.239*(\text{Ln}(\text{WHtr}))^3 + 0.319*(\text{Sex}) + 0.594*(\text{Ln}(\text{Age}))$. METS-VF showed better performance compared to other VAT surrogates using either DXA (AUC 0.896 95% CI 0.847–0.945) or MRI (AUC 0.842 95% CI 0.771–0.913) as gold standards. We identified a METS-VF cut-off point >7.18 in healthy patients which has 100% sensitivity (95% CI 76.8–100) and 87.2% specificity (95% CI 79.1–93.0) to identify increased VAT (>100 cm^2). METS-VF also had adequate performance in subjects with metabolically-healthy obesity. Finally, in our metabolic syndrome cohort, subjects in the upper quintiles of METS-VF (>7.2) had 3.8 and 2.0-fold higher risk of incident T2D and hypertension, respectively ($p < 0.001$). This effect was independent of BMI for both outcomes.

Conclusion: METS-VF is a novel surrogate to estimate VAT, which has better performance compared to other surrogate VAT indexes and is predictive of incident T2D and hypertension. METS-VF could be a useful tool to assess cardio-metabolic risk in primary care practice and research settings.

1. Introduction

Accumulation of visceral adipose tissue (VAT) has been associated with insulin resistance (IR), the number of metabolic syndrome traits and an increased risk of developing type 2 diabetes (T2D), atherosclerosis, dyslipidemia, hypertension and coronary heart disease [1–5]. A gold standard to assess VAT is magnetic resonance imaging (MRI); nevertheless, MRI is expensive and needs to be performed and interpreted by a specialist. Other

Abbreviations: METS-VF, Metabolic Score for Visceral Fat; DXA, dual X-ray absorptiometry; MRI, magnetic resonance imaging; VAA, visceral adipose area; IR, insulin resistance; BIA, bioelectrical impedance; BMI, body-mass index; MS, metabolic syndrome; HR, hazard ratio; 95% CI, 95% confidence interval; AUC, area under the curve; ROC, receiver operating characteristic; VAT, visceral adipose tissue.

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imaging techniques include computerized tomography (CT), dual X-ray absorptiometry (DXA) and Bioelectrical Impedance Analysis (BIA), which are accessible, safe and highly correlated with MRI evaluations [6]. Despite increasing evidence regarding the clinical relevance of evaluating visceral adiposity, its application in everyday clinical practice is limited by equipment and technical difficulties.

Since visceral adiposity has significant metabolic burden and its assessment has been shown to be challenging, surrogate laboratory and anthropometry-based measures have been developed to estimate VAT. Routinely applicable anthropometrical indicators of VAT content include waist circumference (WC), body mass index (BMI), waist-to height index (WHtr) and waist to hip ratio (WHR, [7]); a limitation of these indicators is the challenge of distinguishing between subcutaneous adipose tissue and VAT, especially since these two compartments have opposed clinical and physiological implications. Both subcutaneous and visceral fat have been consistently associated with increased cardio-metabolic risk factors; however, the strongest adverse associations and the higher cardiovascular risk have been attributed to visceral fat depots [8]. This has brought VAT assessment to the spotlight of metabolic research, leading to the development of combined anthropometric and laboratory-based estimators, including the Visceral Adiposity Index (VAI), Lipid Accumulation Product (LAP), Estimated Visceral Area (EVA) and Deep Abdominal Adipose Tissue (DAAT) indexes, which have been validated in different ethnic groups as an effort to translate VAT estimation into epidemiological research and everyday clinical practice [9–12].

Since IR is associated with adipose tissue dysfunction and accumulation of VAT, we hypothesized that using an IR estimate along with anthropometric measures would increase the precision of VAT estimation. METS-IR, a novel non-insulin-based index to estimate IR, has proven to be more accurate to evaluate IR compared to other non-insulin-based indexes; METS-IR is also associated with pathophysiological components of metabolic syndrome and predicts T2D and hypertension [13]. Currently there is no VAT estimator that considers IR as a key component; furthermore, previous data showed a strong predictive capacity for VAT area using METS-IR. Known modifiers of VAT accumulation also include age and male sex; furthermore, body fat distribution could be a reliable marker of VAT accumulation and previous work has demonstrated that VAT accumulation could be accurately assessed using the WHtr [14,15]. In this work, we aimed to develop a novel estimator which combines METS-IR, WHtr, age and sex to estimate VAT using DXA-derived intra-abdominal fat mass estimation and validated against MRI measures of VAT. Furthermore, we evaluated a physiological correlation with adipokines for the index and its capacity to predict cardio-metabolic complications independently of increased BMI.

2. Subjects and methods

2.1. Participants and study setting

2.1.1. Discovery sample and physiological evaluations

In the discovery cohort, we included men and women aged 18–78, with BMI >18.5 kg/m^2 recruited from our clinical facilities. A complete medical and family history, including use of medication was obtained from all subjects from an interview by trained staff. Subjects with history of T2D, whom where not receiving insulin and were only treated with exclusively metformin were included, alongside with subjects with history of essential hypertension and dyslipidemia. No subject had cardiovascular disease, T2D complications, acute infection or any other lipodystrophic syndrome (e.g. HIV). All subjects were invited to participate in the study and signed and informed consent. The Human Research Ethics Committee of Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran (INCMNSZ) approved the study. All procedures were done in accordance with the Declaration of Helsinki.

2.2. Data collection

2.2.1. Biochemical evaluation

Blood samples were obtained after 8–12 h of fasting. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2). Lipid concentrations (cholesterol, triglycerides and HDL cholesterol) were measured using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). Plasma adiponectin, leptin, and fibroblast growth factor (FGF-21) concentrations were determined by performing ELISA assays (Merck Millipore). LDL cholesterol was calculated using Martin's equation [16]. METS-IR was calculated using the formula: (Ln ((2*G_0) + TG_0)*BMI)/(Ln (HDL-C)), where G_0 and TG_0 represent fasting glucose and triglyceride concentrations, respectively [13].

2.2.2. Anthropometry and body composition analysis

Anthropometry: Body weight was measured to the nearest 0.1 kg using a seca mBCA 514 medical body composition analyzer with 50 g gradation and height was measured to the nearest 0.1 cm using a seca 284 stadimeter with 1 mm gradation. WC was evaluated using an inelastic seca 201 tape with 0.1 cm precision directly over the skin at the mid-point between the ribcage and the iliac crest with the tape parallel to the floor and collocated after palpation. BMI was calculated as weight in kg divided by the squared product of height in meters. WHtr was calculated WC divided by height, both in centimeters. All anthropometric measures were performed after an 8–12 h fast by trained personnel the day of blood sample evaluations.

DXA evaluation: Whole-body fat, lean mass and visceral adipose tissue (VAT) mass were determined using dual energy X-ray absorptiometry (DXA) (GE Healthcare, CoreScan software version 16) by a certified densitometrist technician. All evaluations were carried out after at least a 4 h fast.

MRI evaluation: Abdominal MRI images were acquired using T1 and T2 sequences in the axial plane using a 3-T MRI scanner (Philips Achieva 3 T). Visceral adipose tissue area (VAA) was measured at the level of the L2–L3, L3–L4 vertebral superior endplate at the umbilicus level, with three slices obtained superior, and one slice inferior at 40 mm intervals (16 cm window); VAA was distinguished from subcutaneous adipose tissue based on the abdominal wall muscle layer. Images were analyzed by a single individual (EEV) using the Analyze software version 2.0.

BIA evaluation: Estimated visceral adipose tissue (EVAT) was measured using 8-point bioelectrical impedance analysis (BIA) with a SECA mBCA 515 medical body composition analyzer calibrated for Hispanic population. Measurements were obtained in subjects with a previous 8–12 h fast in a supine position by applying a low-intensity electric current between two pairs of electrodes placed in both feet of the subjects and in both hands by trained personnel dedicated to body composition analysis. Subjects were indicated to not wear metallic objects and were not consuming medication or had conditions (eg. lipodystrophy, edema) which interfered with the measurements. Body composition measurements were assessed the same day for each patient.

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2.3. Development and validation of the index

2.3.1. Mathematical modeling

To develop an estimator for intra-abdominal and visceral fat using DXA, we evaluated non-linear fits of individual crude and log-transformed variables aiming to maximize the explained variance of individual components. The working hypothesis of this work considered that the main predictors for intra-abdominal and visceral fat would include an insulin resistance component (METS-IR), an anthropometric measure of body-fat distribution (WHtr) as well as age, and sex, as suggested by previous research [8,14]. Based on these assumptions, we developed a linear combination of transformed variables which maximized the explained association for log-transformed intra-abdominal fat mass; estimated model coefficients ($\widehat{\beta}$) were calculated using the Levenberg–Marquardt algorithm. Model diagnostics were conducted using R^2 and Akaike's information criterion (AIC). The resulting model was termed Metabolic Score for Visceral Fat (METS-VF).

2.3.2. Validation of METS-VF against VAT from DXA, MRI and BIA

METS-VF was validated using three methods to assess VAT (Fig. 1). In the first cohort we included 184 additional subjects who also underwent a total body composition analysis using DXA, aged 20–79 years, without diagnosed cardiovascular disease, smoking history or T2D complications. Furthermore, in a subset of subjects from the validation cohort VAA was quantified in 118 patients using magnetic resonance imaging (MRI). Increased VAA-MRI >100 cm^2 according to previously-established cut-off values, validated for several ethnic groups [17]; in order to identify a cut-off for VAT-DXA mass in our population, we contrasted VAT-DXA and MRI values >100 cm^2 determined the cut-off using the Youden index (VAT-DXA >1389 g, 88.6% sensitivity, 88.9% specificity, AUROC = 0.919 95% CI 0.859–0.978).

A potential limitation of our approach is the assumption that increased in intra-abdominal fat or VAT would be proportional to metabolic disturbances. To mitigate this limitation, an independent sample of 91 metabolically healthy obese individuals (MHO) recruited from other ongoing studies was also assessed. We defined metabolically-healthy obesity as ≤1 NCEP ATPIII criteria for metabolic syndrome except for WC in individuals with BMI ≥30 kg/m^2, in whom VAT-DXA was assessed. This approach would reasonably test the ability of the index to detect VAT independent of significant metabolic abnormalities.

Finally, a third validation was carried out in a cohort of 991 subjects, aged 18–85 years; for validation of METS-VF in the BIA cohort, we defined increased EVAT as values >80th percentile. To contrast our novel score with currently validated models, we calculated the following adipose tissue subrogates: VAI, LAP, EVA and DAAT from fasting and anthropometrical measures in all cohorts [9–12].

2.3.3. Association of METS-VF with adipokine levels

In the subset of the validation cohort in whom VAA-MRI was estimated, plasma adiponectin, leptin, and fibroblast growth factor 21 (FGF-21) concentrations were also assessed. To evaluate the physiological dose–response correlation of adipokine profiles in the MRI sub-cohort, we compared adipokines across METS-VF terciles using trend analysis.

2.3.4. Prediction of incident T2D and hypertension using METS-VF in an open-population cohort

Finally, we evaluated the capacity of METS-VF to predict incident T2D and hypertension in our Metabolic Syndrome Cohort, an open population cohort with the objective to evaluate risk of MS components in the incidence of T2D, hypertension and cardiovascular mortality in an urban population living in 9 different cities in Mexico. The methodology, inclusion criteria and results are described elsewhere [13,15]. Incident T2D was defined as a construct of previous medical diagnosis of T2D, taking hypoglycemic medication and/or fasting glucose levels as determined by current ADA guidelines. Incident hypertension was defined as a construct of previous medical diagnosis of hypertension, taking anti-hypertensive drugs and/or blood pressure >140/90 mmHg as determined by current AHA guidelines. To better evaluate the independent role of METS-VF as a risk factor, analyses were stratified according to BMI category. Time to follow-up was estimated from recruitment up to the last follow-up or occurrence of the incident outcome, whichever occurred first.

2.4. Statistical analysis

2.4.1. Intergroup differences and paired data

To evaluate intergroup differences, we used Student's t-test and Mann–Whitney U where appropriate. Frequency distribution of categorical variables is reported as frequencies and percentages and was compared between groups using chi-squared tests. Logarithmic transformations were applied to approximate normality in variables showing a non-normal distribution. Data are presented as mean ± SD or median and interquartile range.

2.4.2. Validation of METS-VF

We used correlation analysis to evaluate association of VAT subrogates, including METS-VF, against VAT-DXA for the training and validation cohort, VAA-MRI and EVAT. Diagnostic performance was evaluated using areas under the receiving operating characteristic curve (AUROC). To estimate differences between AUROC we performed non-parametric ROC tests using stratified bootstrap sampling with the *pROC* R package. The cut-off point was determined using the Youden index; sensitivity, specificity, positive and negative predictive values and likelihood ratios (PPV, NPV, LR(+), LR(−), respectively) were calculated using the *OptimalCutpoints* R package. To evaluate concordance of VAT estimation using the index with VAT-DXA we performed Bland–Altman analyses.

2.4.3. Prediction of incident T2D, hypertension using METS-VF

To evaluate the association of METS-VF and incident T2D and hypertension we performed survival analysis comparing across METS-VF quartiles, quintiles and cut-off values using Kaplan–Meier curves compared with log-rank tests. Cox proportional-risk regression analyses were used to evaluate risk of incident outcomes adjusting for physical activity, family history of T2D and/or hypertension, prevalent hypertension and smoking. All statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 21.0), R software (Version 3.5.1) and GraphPad Prism (Version 6.0).

3. Results

3.1. Study populations

In the discovery cohort we included 366 individuals with mean age 46.14 ± 14.07 and female predominance (79.1%). Amongst the discovery cohort, 43 subjects were within normal weight (11.7%), 125 were overweight (34.2%) and 198 were obese (54.1%). T2D was present in 62 individuals (17.1%) and hypertension in 54 (14.9%) (Table 1). General assessment of DXA-derived body composition analyses stratified by sex is presented in Supplementary material. In the validation cohort, we included 184 individuals with mean age 46.37 ± 13.98, female predominance (65.6%) and higher T2D

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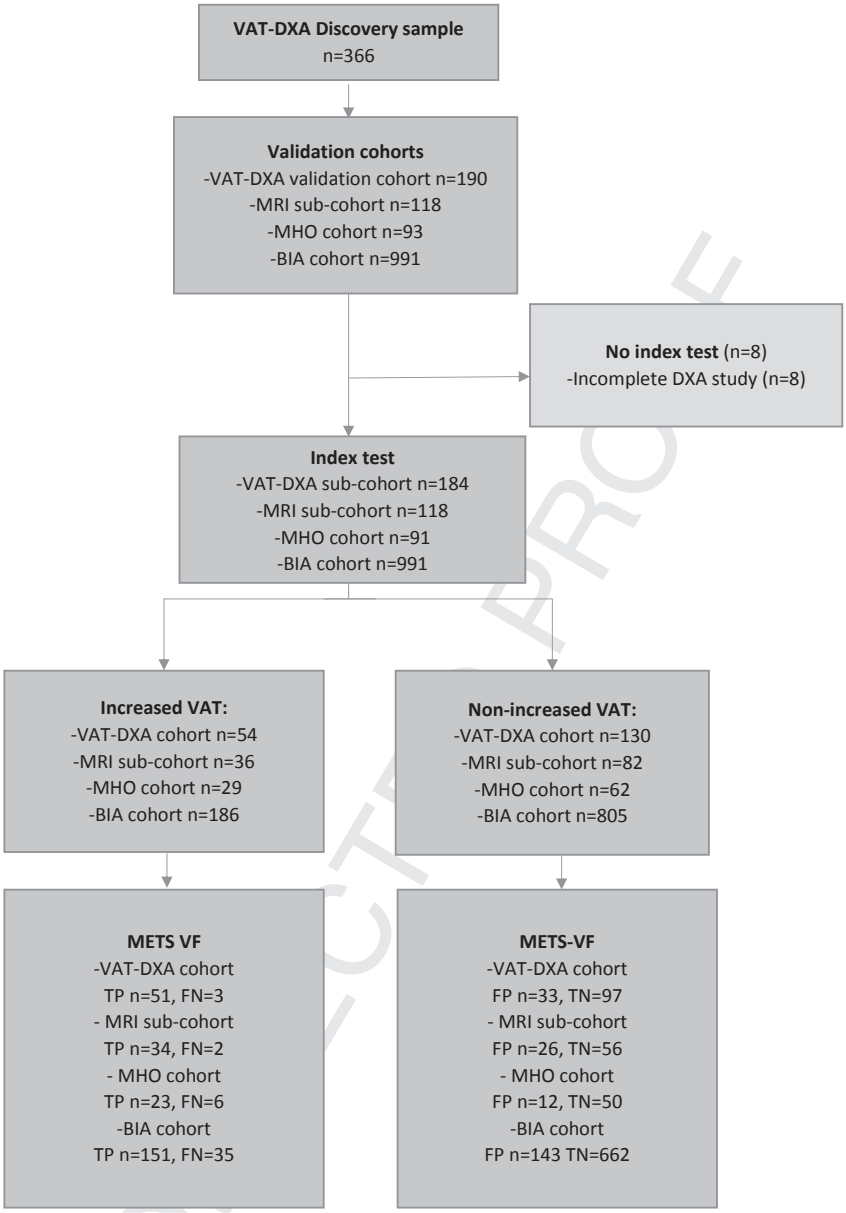

Fig. 1. STARD diagram representing evaluated cohorts for development and validation of the Metabolic Score for Visceral Fat (METS-VF) comparing across validation cohorts according to true and false positive and negative values. Abbreviations: MHO: metabolically healthy obese; MRI: Magnetic Resonance Imaging; VAT-DXA: Visceral Adipose Tissue assessed by dual X-ray absorptiometry, BIA: Bioelectrical impedance analysis; TP: True positive; FP: False positive; TN: True negative; FN: False negative.

Table 1
Biochemical and antropometrical characteristics of training and validation cohorts. Data is presented in mean (±SD) or as median (IQR) according to its distributions.

Parameter	Training DXA-cohort (n = 366)	Validation DXA cohort (n = 184)	MRI subcohort (n = 118)	MHO cohort (n = 91)	BIA cohort (n = 991)
Female sex (%)	287 (79.1)	122 (65.6)	68 (57.6)	81 (89.0)	654 (65.9)
T2D (%)	62 (17.1)	64 (34.4)	64 (54.2)	–	138 (13.8)
Age (years)	46.14 ± 14.07	46.37 ± 13.98	43.07 ± 15.31	40.04 ± 14.29	43.14 ± 14.72
Glucose (mg/dL)	101.84 ± 30.42	99.49 ± 23.06	104.21 ± 26.87	90.01 ± 6.98	106.43 ± 46.29
Insulin (μl/mL)	11.15 (6.87–17.05)	7.00 (5.07–10.12)	7.60 (5.27–11.42)	11.2 (6.9–15.4)	9.0 (5.9–14.3)
Total cholesterol (mg/dL)	179.55 ± 64.21	189.22 ± 37.25	180.67 ± 35.14	177.32 ± 33.44	197.86 ± 43.92
Triglycerides (mg/dL)	161.0 (109.0–261.0)	122.0 (87.5–164.25)	120.0 (85.0–163.25)	115.5 (85.8–134.5)	141.0 (97.0–194.0)
HDL-C (mg/dL)	48.69 ± 27.92	46.48 ± 27.62	45.19 ± 12.34	46.5 ± 7.90	46.01 ± 12.92
BMI (kg/m^2)	32.41 ± 8.14	27.61 ± 4.28	27.32 ± 3.73	36.22 ± 8.46	29.43 ± 6.97
Waist circumference (cm)	100.15 ± 18.24	95.59 ± 14.56	96.75 ± 15.37	105.73 ± 16.55	94.77 ± 16.30
METS-IR	54.06 ± 19.50	42.42 ± 8.56	42.34 ± 7.62	53.75 ± 13.09	46.07 ± 12.82

Abbreviations: T2D: type 2 diabetes; BMI: body mass index; METS-IR: metabolic Score for Insulin Resistance; MHO: metabolically healthy obese; MRI: magnetic resonance imaging; DXA: dual X-ray absorptiometry; BIA: bio-electrical impedance analysis.

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prevalence (34.9%). In this cohort, 61 subjects had normal weight (33.1%), 75 were overweight (40.8%) and 48 were obese (26.1%).

3.2. Mathematical modeling of METS-VF

To develop a novel VAT estimate in the discovery cohort, we observed that log-transformed METS-IR and WHtr had the highest observed R^2 using cubic fits for log-transformed VAT mass. Similarly, log-transformed age and male sex were associated to higher VAT mass. Using non-linear regression, we estimated $\widehat{\beta}$ coefficients for each predictor; the overall model explained 59.6% of the variability of log-transformed VAT-DXA (R^2=0.596, Table 2). The model included METS-IR ($\widehat{\beta} = 0.011$), WHtr ($\widehat{\beta} = 3.239$), age ($\widehat{\beta} = 0.546$) and male sex ($\widehat{\beta} = 0.319$) with a resulting equation defined as:

$$\begin{aligned}\text{METS}-\text{VF} = 4.466 &+ 0.011\left[(\text{Ln}(\text{METS}-\text{IR}))^3\right] \\ &+ 3.239\left[(\text{Ln}(\text{WHtr}))^3\right] + 0.319(\text{Sex}) \\ &+ 0.594(\text{Ln}(\text{Age}))\end{aligned} \quad (1)$$

where sex was a binary response variable (male = 1, female = 0) and age expressed in years. Since METS-VF essentially represents log-transformed VAT-DXA mass values, to estimate VAT mass the following transformation is required:

$$\begin{aligned}\text{VAT}\,(\text{g}) = e^{4.466+0.011\left[(\text{Ln}(\text{METS}-\text{IR}))^3\right]+3.239\left[(\text{Ln}(\text{WHtR}))^3\right]} \\ +0.319(\text{Sex}) + 0.594(\text{Ln}(\text{Age}))\end{aligned} \quad (2)$$

3.3. Validation of METS-VF, correlation and diagnostic performance against VAT-DXA

We conducted validation for METS-VF in a cohort of 184 patients who underwent DXA evaluation. In comparison to the discovery cohort, the validation cohort had higher rates of T2D and lower BMI. In the discovery cohort, METS-VF had a higher correlation with log-transformed VAT-DXA mass and was superior to other indexes with only EVA having a higher ρ value. These observations were replicated in the validation cohort, where the correlation for METS-VF with VAT-DXA was decidedly superior (Supplementary material). Furthermore, linear regression fits for METS-VF showed the largest decrease in AIC and highest r^2 compared to either METS-IR or WHtr alone or any other VAT surrogate. Finally, we evaluated DXA values >1389 g as analogous to >100 cm^2 in MRI-VAT due to lack of VAT-DXA percentile data in our population. In the discovery and validation cohorts we identified 159 (43.8%) and 54 (29.3%) subjects with increased VAT-DXA mass, respectively. In both cohorts, METS-VF had a higher AUROC compared to other indexes with significantly higher AUROC values in the validation cohort. We performed a semiparametric covariate-adjusted ROC curve analysis to evaluate the role of T2D in modifying performance of METS-VF; after adjustment, the AUROC for all indexes, including METS-VF decreased. Therefore, we estimated cut-offs separately for T2D and non-T2D subjects (Supplementary material).

Table 2
β-coefficients for estimation of log-transformed visceral fat mass using the Levenberg–Marquardt algorithm for non-linear regression.

Model	Parameter	β	Error	95% CI
$R^2 = 0.596$	Intercept	4.466	0.463	3.697–5.520
Residual = 80.36	Ln (METS-IR)^3	0.011	0.003	0.006–0.016
	Ln (WHtr)^3	3.239	0.286	2.652–3.779
	Ln (Age)	0.594	0.103	0.342–0.749
	Male sex (0/1)	0.319	0.082	0.145–0.467

Abbreviations: WHtr: waist to height ratio; METS-IR: metabolic score for insulin resistance; 95% CI: 95% confidence intervals.

Concordance of VAT estimated using METS-VF with actual VAT-DXA measures was evaluated using Bland–Altman analyses, demonstrating with <5% outliers. 95% confidence intervals for bias of agreement between VAT-DXA and estimated VAT using METS-VF was +75.38 g (95% CI 10.70–140.06) and stratified by sex comparatively higher in male (+197.72 g 95% CI 60.09–335.34) than female participants (+11.59 95% CI −54.53 to +77.72). Upper and lower 95% limits of agreement in the overall validation cohort were +932.41 g and −781.65 g, respectively (Supplementary material).

3.4. Validation of METS-VF against VAA-MRI

To evaluate METS-VF against the gold standard for VAT evaluation, we performed a second validation in a subgroup of 118 subjects from the validation cohort with VAA-MRI evaluation; we identified 35 subjects with VAA-MRI >100 cm^2. Subjects in this subgroup were younger and had higher rates of T2D compared to the overall validation cohort, BMI was lower than the discovery cohort but similar to the overall validation cohort; additionally, female predominance was lower compared to both training and validation cohorts (54.2%, Table 2). In this cohort, METS-VF had the highest correlation with VAA-MRI (ρ = 0.697 95% CI 0.597–0.779) compared to other VAT surrogates. We observed the higher AUROC for increased VAA-MRI for METS-VF (AUC = 0.842, 95% CI 0.771–0.913) compared to other VAT surrogates, particularly WHtr and WC. The estimated cut-off points stratified by T2D status were >7.18 for individuals without T2D (AUC = 0.922, 95% CI 0.924–0.993; 88.9% sensitivity, 88.6% specificity) and >7.0 for individuals with T2D (AUC = 0.778, 95% CI 0.654–0.890; 92.0% sensitivity, 63.8% specificity), which were similar to those observed for VAT-DXA.

3.5. Validation of METS-VF against VAT assessed by BIA

To extend our findings to a larger cohort, we performed a third validation in 991 subjects in whom we estimated EVAT using BIA. The rate of T2D in this cohort was significantly lower (13.8%) but subjects had similar age and sex distribution as the DXA validation cohort, except for BMI, which was leaning towards overweight and obese individuals. In this cohort, METS-VF had a high correlation with EVAT (ρ = 0.804 95% CI: 0.771–0.833) compared to VAI, LAP BMI, WtHI and METS-IR alone; EVA, DAAT and WC were slightly superior. Exploring the diagnostic capacity of METS-VF to detect increased EVAT >80th percentile, we found that METS-VF had an AUC of 0.895 (95% CI: 0.870–0.920); only WC and EVA had superior AUC for EVAT.

3.6. Performance of METS-VF in MHO subjects

We evaluated the performance of METS-VF in MHO subjects to investigate whether METS-VF could estimate VAT in subjects without significant metabolic disturbances. This cohort had female predominance (89.0%) with a mean age of 40.04 ± 14.29 years (Table 1). Using the VAT-DXA cut-off described earlier, we identified 29 subjects with increased VAT-DXA(31.9%). As seen in Supplementary Tables 2 and 3, METS-VF showed the higher correlation and AUROC compared to other VAT surrogates, outperforming VAI, LAP, WC and the WHtr.

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3.7. Association of METS-VF with adipokine measurements

To explore physiological correlates of increased VAT, we explored associations of METS-VF with adipokine profiles. We observed a correlation between METS-VF values, adiponectin ($\rho = -0.197$ 95% CI −0.349 to −0.012) and FGF-21 ($\rho = 0.230$ 95% CI 0.058−0.399) but not with leptin ($\rho = -0.013$ 95% CI −0.197 to 0.165) which was correlated with BMI ($\rho = -0.223$ 95% CI 0.033−0.390). Across METS-VF terciles, we observed significantly lower adiponectin values in the upper METS-VF tercile compared to middle and lower terciles ($p < 0.01$); in contrast, we observed significantly higher FGF-21 values in the upper METS-VF tercile. These observations of increasing FGF-21 levels and decreasing adiponectin levels across METS-VF terciles were also confirmed by trend analyses ($p < 0.05$, Fig. 2).

3.8. Prediction of incident T2D and hypertension using METS-VF

For prediction of incident T2D and hypertension, we included 9637 subjects from baseline evaluation, from which 6144 completed follow-up. Of note, subjects who developed T2D and/or hypertension had significantly higher METS-VF scores at baseline compared to those who did not ($p < 0.001$). Using Cox proportional-hazard regression analyses, we observed that subjects in the highest METS-VF quintile (METS-VF > 7.2) had a 3.8-fold higher risk to develop T2D. Using the METS-VF > 7.18 cut-off value, subjects had 2.4-fold higher risk of incident T2D (Fig. 3). When stratifying by BMI categories, subjects with METS-VF values >7.18 values and normal weight (BMI 18.5−24.9) had higher additional risk for developing T2D, compared to overweight and obese subjects.

Finally, we assessed prediction of incident hypertension in this cohort. Using Cox proportional-hazard regression analyses, we observed that subjects in the highest METS-VF quintile had 3.7-fold higher risk of incident hypertension. Using the METS-VF > 7.18 cut-off value, subjects had two-fold higher risk of developing hypertension (Fig. 3). As seen with T2D when stratifying by BMI category, individuals with normal-range BMI had higher additional risk of incident hypertension compared to overweight and obese subjects (Supplementary material).

4. Discussion

In the present work, a novel surrogate index to estimate intra-abdominal adipose tissue and VAT using DXA was generated, which incorporates a non-insulin-based IR index (METS-IR), anthropometric measures of body-fat distribution (WtHr), sex, and age. METS-VF had better performance compared to other VAT surrogate indexes compared to VAA-MRI and EVAT-BIA and had the better adjustment to estimate VAT-DXA compared to either of its components alone. In addition, it was proven that VAT estimation using METS-VF correlates with adipokine profiles as those expected in subjects with increased VAT. Finally, the clinical utility of METS-VF to predict incident T2D and hypertension independent of BMI was explored in our Metabolic Syndrome Cohort. METS-VF is a novel estimate of VAT which could be useful for evaluation of cardio-metabolic health primarily in clinical and epidemiological research settings.

As reported, METS-VF had the better correlation and performance with VAT-DXA compared to other surrogate VAT indexes; these observations were replicated in three validation cohorts, which assessed VAT using three distinct methods: DXA, MRI and BIA. The increased performance of METS-VF may be partly due to the fact that most anthropometric indexes including BMI, WC, WHR and WHtr capture variability from both subcutaneous and visceral adipose tissue, thus tending to overestimate visceral adiposity; therefore, including known-modifiers of VAT accumulation could offer more precise estimations and reduce the impact of

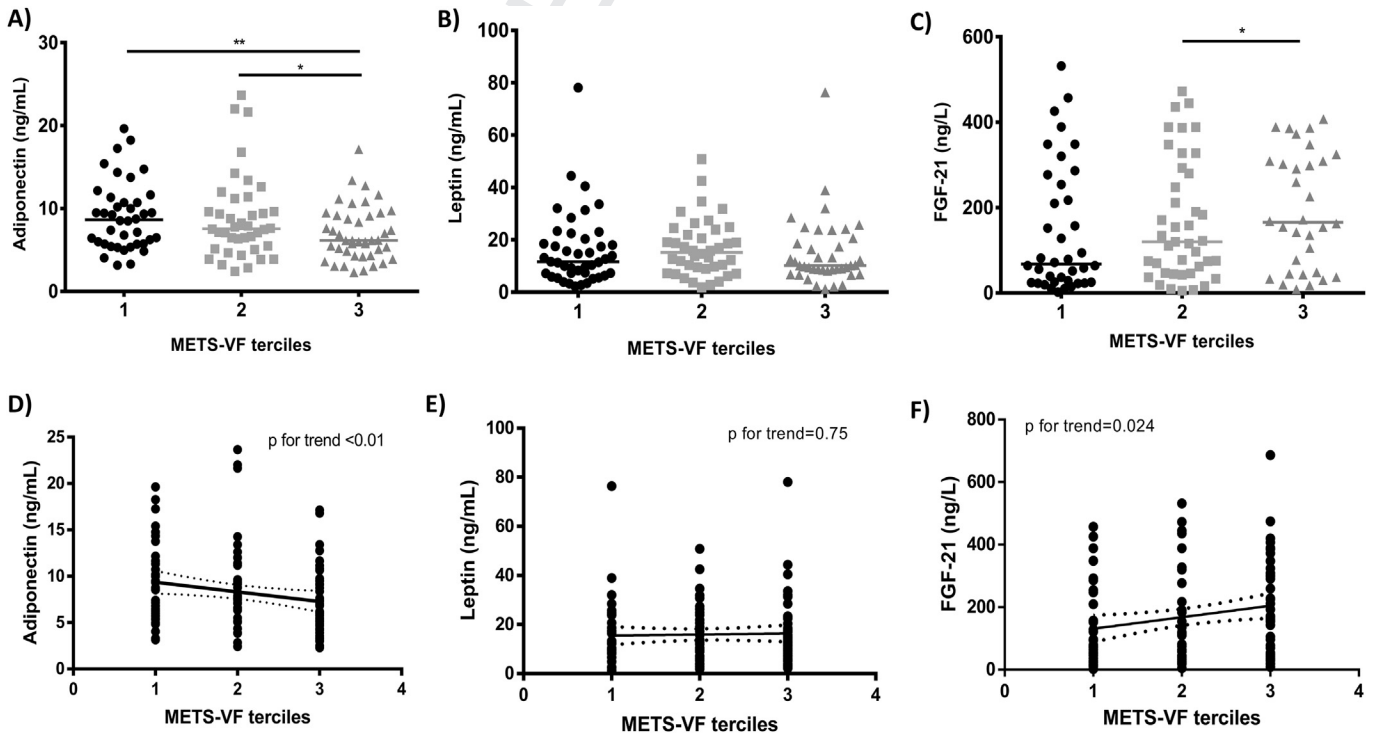

Fig. 2. Tercile comparison and trend analyses of increasing METS-VF terciles for fasting adiponectin (A, D), leptin (B, E) and FGF-21 (C, F) in the DXA validation sample (n = 184). Abbreviations: METS-VF: Metabolic Score for Visceral Fat; FGF-21: Fibroblast growth factor 21. *p-value < 0.05, **p-value < 0.01.

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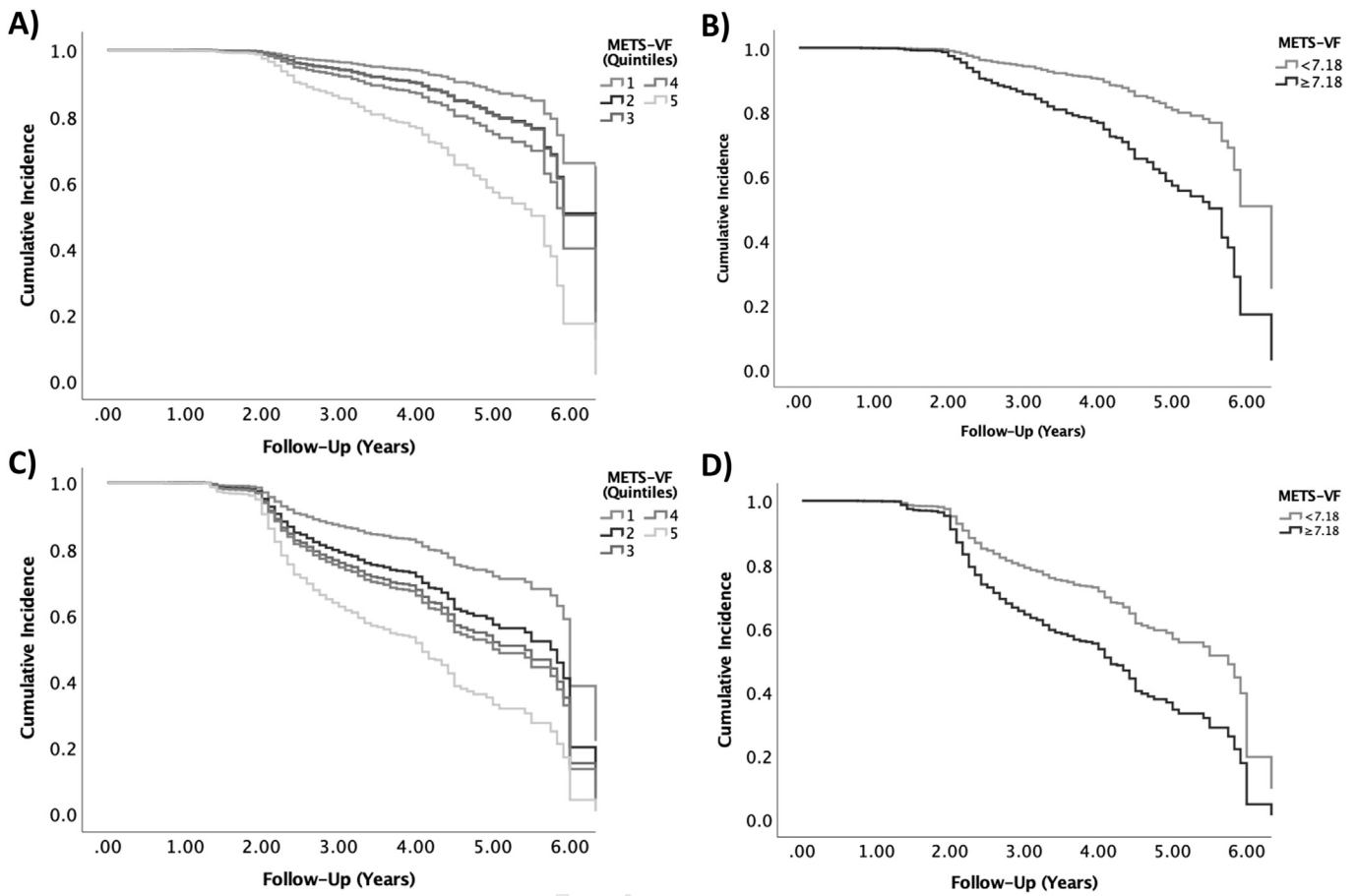

Fig. 3. Incidence of T2D comparing across METS-VF Quintiles (A) and cut-off point (>7.18, B) and incidence of hypertension across METS-VF terciles (C) and the identified cut-off value (D), adjusted for physical activity, family history of diabetes or hypertension and smoking in an open population cohort (n = 6144). Abbreviations: METS-VF: Metabolic Score for Visceral Fat; T2D: Type 2 diabetes.

subcutaneous adipose tissue on VAT estimation [18]. Both VAI and LAP were modeled considering laboratory measures and anthropometric variables; however, modeling methods for those surrogate scores differ and external validity of these indexes might be modified by ethnic-specific variations in body composition. This might also explain the underperformance of both indexes using CT-scan and MRI methods, as was previously reported in Japanese–American population [11]. In their recent study, Wander et al. developed the novel EVA index, which was aimed at improving estimations of visceral adiposity in Japanese–American subjects. In our study, we validated EVA against DXA and MRI; however, the METS-VF index showed superior performance in our population. This could be attributed to two significant differences between both indexes: First, the inclusion of an IR component, which could improve VAT estimation in subjects without significant laboratory disturbances attributable to VAT and the use of an anthropometric index such as WHtr which has demonstrated superior capacity to predict both VAT and cardio-metabolic risk in different ethnic groups [15,19].

To further strengthen the notion that METS-VF in fact estimated VAT, we aimed to demonstrate that adipokine profiles in subjects with increased VAT assessed by METS-VF were like those with increased VAT. Indeed, METS-VF showed a good correlation with lower adiponectin and higher FGF-21 levels, as expected in this group. Endocrine function of VAT mediates the relation between adipokines, IR and body composition [19,20]. Our model is based on the link between gender and age-specific variations in VAT along with IR and whole-body fat distribution. Several mechanisms have been proposed to explain the link between VAT and metabolic disturbances [21,22]. Anatomical disposition of VAT in interaction with IR increase liberation of free-fatty acids directly to the portal circulation, leading to dysregulations in glucose uptake, glycogen synthesis and glucose oxidation [23]. Low adiponectin levels and increased VAT lead to adverse metabolic profiles which could be compensated by increasing FGF-21 levels [24]. Therefore, METS-VF classifies subjects according to expected metabolic profiles of increased VAT which makes it a consistent and reliable estimate of VAT mass.

VAT has been traditionally associated with increased mortality owed to adverse cardio-metabolic outcomes [25,26]. In our study, individuals in the highest METS-VF quintiles had higher risk of incident T2D and hypertension after follow-up. This could be mediated by the consideration of increased age, IR status, pro-coagulative and pro-inflammatory states along with impairment in cytokine production in VAT [5]. Similar to T2D, IR has been proposed as one of the main pathophysiological mechanisms in which VAT may link the atherogenic state seen in subjects with cardiovascular events [27], mainly due to impairments in glucose homeostasis and increased atherogenic dyslipidemia, characterized by high triglycerides and apolipoprotein B and low HDL particles [1]. Subjects who developed T2D and hypertension had higher METS-VF values at baseline, suggesting a clinical application of the novel index as a useful and practical tool for primary care physicians to estimate VAT and its associated risk of cardio-metabolic

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outcomes. VAT may be considered as a secondary target to treat patients with metabolic syndrome components to decrease overall cardio-metabolic risk caused by IR [28]. The fact that METS-VF was associated to these outcomes independent of BMI as shown in our study demonstrates the role of estimating VAT in subjects in whom BMI estimation could be insufficient to predict cardio-metabolic risk, especially in young subjects [29]. A precise VAT clinical estimator such as ours could be a useful tool for screening and targeting control in at-risk individuals.

Our study had some strengths and limitations. METS-VF was modeled using a non-linear regression approach considering DXA our gold standard, which can feasibly measure whole-body fat mass and intra-abdominal fat and has been recorded to have substantial accuracy compared to MRI and CT. Furthermore, METS-VF was also validated against MRI, the gold standard for VAT assessment and separate cut-off points were estimated for individuals with T2D to account for the effect of T2D in increasing visceral adiposity [30]. Evaluation of our index went beyond assessment of VAT, it also included prediction of incident cardio-metabolic comorbidities and evaluation of adipokine profiles related to excess VAT. In addition, we demonstrated that the index performs better compared to other measures in MHO subjects in whom increased VAT is not directly correlated with metabolic abnormalities. Nevertheless, some limitations are to be acknowledged. These include a relatively small sample size in the MRI validation cohort and a small number of lean and healthy individuals in both training and validation samples, which could overestimate risk in lower-risk subjects and lead to higher bias in VAT estimation. In addition, since no previous study assessed population percentiles or outcome-driven cut-offs for VAT-DXA mass in Latino populations, we had to identify a cut-off compared to VAA-MRI in our validation cohort which could not reflect the most effective cut-off due to sample size constraints. Furthermore, the differences in T2D prevalence in the training and validation cohorts could impact performance of this measure in T2D patients, which calls for further evaluations in subjects with T2D and validation in a larger sample of healthy individuals to ensure its validity in a clinical setting.

In conclusion, METS-VF is a novel precise estimate of VAT and intra-abdominal fat. METS-VF was validated against three-different methods to estimate visceral adiposity and was shown to replicate the adverse adipokine profile observed in individuals with excess visceral adiposity; furthermore, our index also was shown to predict incident T2D and hypertension independent of BMI. Therefore, METS-VF could be a useful clinical surrogate of visceral adiposity for its use in epidemiological settings and an overall measure of cardio-metabolic health.

CRediT authorship contribution statement

Omar Yaxmehen Bello-Chavolla: Conceptualization, Methodology, Validation, Formal analysis, Software, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Neftali Eduardo Antonio-Villa:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Arsenio Vargas-Vázquez:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Tannia Leticia Viveros-Ruiz:** Conceptualization, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Paloma Almeda-Valdes:** Investigation, Writing - review & editing, Visualization. **Donaji Gomez-Velasco:** Data curation, Writing - review & editing. **Roopa Mehta:** Investigation, Writing - review & editing. **Daniel Elias-López:** Investigation, Writing - review & editing. **Ivette Cruz-Bautista:** Investigation, Writing - review & editing. **Ernesto Roldán-Valadez:** Investigation, Writing - review & editing. **Alexandro J. Martagón:** Investigation, Writing - review & editing, Visualization, Project administration. **Carlos A. Aguilar-Salinas:** Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing, Visualization, Resources, Supervision, Project administration, Funding acquisition.

Conflicts of interest

Nothing to disclose.

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Appendix A. Supplementary data

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The type 2 diabetes-specific dementia risk score (DSDRS) is associated with frailty, cognitive and functional status amongst Mexican community-dwelling older adults

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Abstract

Aim: The type 2 diabetes (T2D) specific dementia-risk score (DSDRS) was developed to evaluate dementia risk in older adults with T2D. T2D-related factors have been shown increase the risk of age-related conditions, which might also increase dementia risk. Here, we investigate the associations of DSDRS with frailty, disability, quality of life (QoL) and cognition in community-dwelling older adults with T2D.
Methods: We included 257 community-dwelling older adults with T2D to evaluate the association between DSDRS and Mini-mental state examination (MMSE), Isaac's set-test (IST), clock drawing test (CDT), quality of life (SF-36), risk of malnutrition (Mini-Nutritional Assessment or MNA), as well as frailty, Katz' and Lawton-Brody scores. We also assessed the phenotype and correlates of high-estimated dementia risk by assessing individuals with DSDRS $> 75^th$ age-specific percentiles.
Results: Mean age of participants was 78.0±6.2 years. DSDRS showed a significant correlation with MMSE test, IST, CDT, SF-36, MNA, Lawton-Brody and Katz scores, and an increasing number of frailty components. DSDRS was higher among frail, pre-frail, and subjects with limited ADL and IADL ($p < 0.001$). Participants with DSDRS $> 75^th$ age-specific percentiles had lower education, MMSE, IST, SF-36, MNA, Katz, Lawton-Brody, and higher frailty scores. High-estimated 10-year dementia risk was associated with ADL and IADL disability, frailty and risk of malnutrition. When assessing individual components of DSDRS, T2D-related microvascular complications were associated to all outcome measures.
Conclusions: The DSDRS is associated with frailty, disability, malnutrition and lower cognitive performance. These findings support that T2D-related factors have significant burden on functional status, QoL, disability and dementia risk.

Keywords Diabetes · Dementia · Frailty · Disability · DSDRS

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1 Introduction

Type 2 diabetes mellitus (T2D) is a main cause of morbidity and mortality in the Western World; the highest prevalence of T2D cases occurs in individuals over 60 years in whom it contributes to premature mortality and disability (Chaterjee et al, 2016). Consistent epidemiological evidence has shown an increased risk of incident all-cause, vascular and Alzheimer's disease dementia in individuals with T2D (Chaterjee et al, 2016; Bello-Chavolla et al, 2019). Screening of dementia risk has gained interest recently, particularly upon identification of modifiable risk factors for dementia to design strategies aimed at delaying or preventing disease onset (Carrillo et al, 2013). Risk stratification in individuals with T2D might be particularly useful, since dementia in individuals with T2D has an earlier onset, thus having significant impact on function and cognition in older adults (Carrillo et al, 2013; Biessels et al, 2014).
Recently, the diabetes-specific dementia risk score (DSDRS) was developed to evaluate dementia risk in American older individuals with T2D. DSDRS predicts of all-cause dementia by evaluating diabetes-specific risk factors including microvascular complications, hyperglycemic and hypoglycemic crises and diabetic foot along with traditional risk factors for dementia such as age, schooling, cardiovascular disease, and depression (Exalto et al, Lancet Diabetes Endocrinol 2014). Accumulated risk attributable to T2D-related factors has also been independently associated to age-related conditions including frailty, disability and cognitive impairment (Bello-Chavolla, Aguilar-Salinas et al, 2017). In addition, disability, frailty and cognitive impairment are associated to risk of malnutrition, late-life depression and functional impairment, leading to increase dependence and decreasing quality of life besides increasing dementia risk (Bell et al, 2017; Johansson et al, 2019). Therefore, the clinical utility of screening individuals using DSDRS might be approached with the aim of designing specific treatment regimens to improve quality of life (QoL) and functional status in at-risk older adults (Shih et al, 2018). Furthermore, since impaired cognition and dementia have significant negative impacts on T2D self-care, closer attention might be given to individuals identified at higher baseline risk (Hewitt et al, 2018).
Despite the practicality of the score, the functional and cognitive phenotype identified by the score has not been described beyond clinical features related to T2D. We hypothesized that individuals with higher DSDRS would have functional and cognitive impairment, which are likely to also impact T2D self-care and QoL. Therefore, the main objective of the present study was to determine the associations of the DSDRS with frailty, disability, and cognitive measures aiming to identify the cross-sectional phenotype which relates it to conditions linked to high-dementia risk in subjects with T2D.

2 Methods

2.1 Study population

Cross-sectional study of 257 older adults participating in the Coyoacán Cohort, an observational study conducted in a locality in Mexico City conducted between 2008-2009. Specific details for the design for this study have been published elsewhere (Bello-Chavolla et al, 2017; Ruiz-Arregui et al, 2013). Briefly, we included non-institutionalized individuals aged 70 years with established residence in Coyoacán registered at the "Food Support, Medical Care and Free Drugs Program" (FMDP), which is a government program that includes 95% of the community-dwelling older adults in Mexico City. The first evaluation consisted of face-to-face interviews for collection of self-reported data regarding socio-demographic characteristics, general health-related information, oral and mental health. Additionally, each participant underwent comprehensive geriatric assessment including physical performance tests, cognitive evaluation, nutrition, and medical assessment. Baseline data were collected between April and May 2008 when the questionnaire was administered to participants, and clinical evaluation and collection of biological samples were carried out between June 2008 and July 2009. In the original cohort, a sample of 1,294 was calculated to ensure a sample size that could estimate a prevalence of at least 14% of frailty among participants with = 0.05 and = 0.20. Among those contacted, acceptance rate was 86.9% and a total of 1,124 partticipants completed baseline evaluation and the initial interview, which included individuals with and without T2D. For the present study, we included participants with T2D without previous clinical diagnosis of dementia, defined as self-report of previously-diagnosed T2D and/or self-report of taking T2D medications ($n = 236$). To account for subjects who were not previously diagnosed with T2D, we included subjects with fasting glucose 126mg/dL ($n = 21$), who had enough information for their dementia-risk stratification using the DSDRS (overall, $n = 257$). The local Human Research Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán approved all proceedings regarding this study.

2.2 Definitions of potential correlates

- Frailty: We used a modified definition to what was proposed by Fried et al, as previously validated for this population using data from questionnaires and self-report as (Fried et al, 2001; Ávila-Funes et al, 2011) : a) Unintentional weight loss 5 kg in the last 12 months; b) Exhaustion was assessed by a positive answer in two questions from the Center for Epidemiologic Studies-Depression scale (CES-D) ("I felt that everything I did was an effort" and "I could not get going"); c) Low physical activity was defined as values <20th percentile adjusted by sex on the Physical Activity Scale for the Elderly questionnaire (PASE); d) Slowness was defined if participants answered "yes" or "can't do" to any of the following two questions: Because of a health problem, "do you have difficulty walking one block?" or "do you have difficulty with climbing several flights of stairs without resting?"; and e) Weakness was determined among participants who answered "yes" to the question, "Because of a health problem, do you have difficulty with lifting or carrying objects weighting over 5 kg, like a heavy bag of groceries?". Participants were categorized as frail if they fulfilled 3 criteria, pre-frail if they fulfilled 1-2 criteria, and non-frail if none.
- Depressive symptoms: Defined as a Geriatric Depression Scale score >5 (15-item version).
- Cognitive performance: Cognitive evaluation was comprised of an interview-based assessment, consisting of MMSE assessment and a questionnaire-based cognitive evaluation which included verbal fluency abilities with the Isaacs Set Test (IST) where four semantic categories were successively used (cities, fruits, animals, and colors) and the Clock-drawing test to assess visuo-constructional abilities. Low cognitive performance was based on a modified definition by Blaum et al, defined as scores <25th percentile in both the Mini-Mental State Examination and the IST semantic verbal fluency test or clock-drawing test, adjusted for sex, age, and schooling based on normative data from this population (Mokri et al, 2013; Blaum et al, 2002).
- Disability: Determined using Lawton-Brody Instrumental Activities of Daily Living (IADL) scale and Katz Index for the Activities of Daily Living (ADL). We defined limited ADL or IADL as having at least one impaired dominion in the Katz scale (limited ADL) or the Lawton-Brody scale (limited IADL; Millnac et al, 2016).
- Risk of malnutrition: Assessed by the Mini-Nutritional Assessment (MNA) questionnaire, scores <24 were indicative of at-risk of malnutrition.
- QoL: Assessed using the self-administered generic instrument SF-36 health questionnaire in the translated and validated version for Mexican population. Items are formulated as statements to evaluate eight specific health scales including physical functioning, physical pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue and general health perceptions. Scales are further classified in physical (PCS) and mental component scores (MCS).

2.3 Dementia risk calculation

We evaluated dementia risk using the DSDRS (Exalto et al, Lancet Diabetes Endocrinol 2014). Self-reported variables included duration of T2D from diagnosis in years, self-report of diabetic kidney disease (DKD) and diabetic retinopathy, history of insulin use, oral T2D treatment, diabetic foot or peripheral vascular disease, acute myocardial infarction, and stroke. Microvascular complications to estimate DSDRS considered the clustering of DKD, and/or diabetic retinopathy; this definition was also used in linear and logistic regression models. Acute metabolic event was defined as a previous episode of hyperglycemia which required hospitalization. High dementia-risk was defined as an estimated 10-year dementia risk $> 75^{th}$ age-specific percentile based on age categories described by the DSDRS.

2.4 Anthropometric and biochemical evaluation

Body mass index (BMI) was calculated as weight in kg/height in m^2. Blood was obtained between 8:00 and 9:00 am after 8-12 hour fast. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments Co.), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2). Lipid concentrations (cholesterol, triglycerides, and HDL cholesterol) were measured using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). LDL-cholesterol was calculated using Martin's equation.

2.5 Statistical analysis

2.5.1 Intergroup differences

To evaluate differences in socio-demographic, clinical and biochemical measures we used Student's t-test or Mann-Whitney U where appropriate. Frequency distribution of categorical variables is reported as frequencies and percentages and was compared between groups using chi-squared tests. Data are presented as mean ± SD or as median and interquartile range.

2.5.2 Correlation between DSDRS, cognitive tests, frailty, and disability components

To investigate the association between dementia risk and the evaluated scores, we tested the correlation of DSDRS with the MMSE, the IST, CDT, frailty, MNA, SF-36, Lawton, and Katz scores using Spearman's correlation; 95% confidence intervals were estimated using 1,000 bootstrap samples. To develop an explanatory model for DSDRS and identify independent predictors for dementia risk using these scores, we used step-wise multiple linear regression analyses with model selection carried out using Bayesian Information Criterion (BIC) minimization.

2.5.3 Logistic regression analyses

We developed an explanatory model for high-estimated 10-year dementia-risk to investigate the relation of subjects at higher risk with the investigated clinical phenotypes identified by the scores when transformed into categories. For this purpose, we used logistic regression analyses, treating high-dementia risk as the dependent variable and including as predictors frailty, ADL and IADL disability, risk of malnutrition and low cognitive performance; multiple logistic regression was carried out using step-wise models adjusted for years since T2D diagnosis, years of schooling and sex. Model diagnostics were conducted using R^2 and the Hosmer-Lemeshow test. Finally, we constructed ROC curves to estimate performance of DSDRS to identify phenotypes of frailty, low cognitive performance, limited ADL and IADL using probability estimates from regression modes; we also calculated sensitivity and specificity for each phenotype.

2.5.4 Contribution of DSDRS components to the observed associations

To investigate whether the association of DSDRS with cognition, disability, frailty and impaired QoL were driven by factors other than age, we fitted multiple linear regression models to evaluate which components of the DSDRS were primarily associated with the outcomes. Predictors included individual components of the DSDRS, including age, microvascular complications, depression, diabetic foot, acute metabolic events, cardiovascular and cerebrovascular disease. We included as dependent variables scores correlated to DSDRS, which included the frailty score, Lawton, Katz, MMSE, MNA and SF-36 PCS. Model diagnostics were conducted using R^2 and BIC; multicollinearity was assessed using tolerance and variance inflation factor (VIF). Predictors were tested on homoscedasticity and linearity assumptions; model diagnostics were conducted evaluating normality of residuals. Model parameters are expressed using β-coefficients and 95%CI. All statistical analyses were performed using the SPSS software (Version 22.0), R (Version 3.6.1) and GraphPad Prism (Version 6.0).

3 Results

3.1 Study subjects

We included 257 subjects with T2D, with a slight female predominance (54.1%), an average age of 78.0±6.2 years and a median of 10 years since T2D diagnosis. Insulin use was observed in 58 subjects (22.6%), 89 subjects had fasting glucose 130mg/dL (34.6%), 89 subjects were categorized as pre-frail (34.6%) and 32 subjects as frail (12.5%). In relation to microvascular complications, 62 subjects referred having a previous diagnosis of diabetic kidney disease (DKD) and 102 referred diabetic retinopathy (39.7%). Furthermore, 88 subjects (34.2%) referred having any degree of diabetic foot disease (**Table 1**).

The median of the DSDRS was 8.0 (range 6.0-10.0), which corresponds to an estimated 10-year dementia risk of 50% (34.0%-63.0%), which was unevenly distributed by sex, without significant differences in sex distribution across dementia risk categories ($p = 0.185$). When assessing sex-specific differences in DSDRS components, we identified that female participants had less years of education (6.0 [2.0-11.0], $p = 0.003$) and higher but non-significant rates of microvascular complications compared to men (55.4% vs 43.2%, $p = 0.051$) (**Figure 1**).

Parameter	Overall sample (N=257)	$\leq 75^{th}$ percentile (N=201)	$> 75^{th}$ percentile (N=56)	p-value
Female sex (%)	139 (54.1)	97 (52.7)	42 (57.5)	0.485
Age (years)	78.05±6.16	77.81±6.12	78.94±6.26	0.222
Years since T2D diagnosis	10.0 (3.0-20.0)	8.0 (2.5-19.5)	15.0 (10.0-23.0)	<0.001
Age at T2D diagnosis	64.93±12.56	65.90±12.32	61.45±12.87	0.019
Schooling (years)	6.0 (1.0-9.0)	6.0 (2.0-9.0)	3.0 (0.0-6.0)	<0.001
Glucose (mg/dL)	143.86±60.23	137.72±45.24	165.16±93.20	0.008
Triglycerides (mg/dL)	185.36±97.09	180.03±97.25	203.84±95.36	0.157
HDL-C (mg/dL)	42.05±12.64	42.17±13.16	41.65±10.73	0.760
Total Cholesterol (mg/dL)	194.52±42.01	195.02±42.56	192.79±40.47	0.814
BMI (kg/m^2)	26.99±4.02	27.11±4.02	26.55±4.02	0.362
MMSE score	20.83±5.45	21.80±4.84	17.26±6.09	<0.001
Isaac's set test score	23.78±6.87	24.79±6.39	21.41±7.42	0.006
Clock Drawing Test	2.0 (1.0-5.0)	2.0 (1.0-5.0)	3.50 (1.3-5.0)	0.176
Geriatric depression scale	2.0 (1.0-4.06)	2.0 (1.0-3.0)	5.0 (3.7-7.0)	<0.001
Frailty score	1.0 (0.0-2.0)	1.0 (0.0-1.0)	2.0 (1.0-3)	<0.001
Katz scale	5.21±1.41	5.43±1.18	4.43±1.84	<0.001
Lawton scale	5.30±1.28	5.46±1.11	4.75±1.66	<0.001
Mini-Nutritional Assessment	24.92±3.29	25.67±2.57	22.24±4.15	<0.001
SF-36 PCS	43.53±9.67	44.38±9.69	39.58±8.64	0.007
SF-36 MCS	52.64±9.59	53.58±8.90	48.01±11.49	0.009
Insulin use (%)	58 (22.6)	46 (25.0)	12 (16.4)	0.139
Stroke (%)	20 (7.8)	4 (2.0)	16 (28.6)	<0.001
Myocardial infarction (%)	27 (10.5)	14 (7.0)	13 (23.2)	<0.001
Microvascular complications (%)	128 (49.8)	76 (37.8)	52 (92.9)	<0.001
Diabetic foot (%)	88 (34.2)	53 (26.4)	35 (62.5)	<0.001
DSDRS	8.0 (6.0-10.0)	7.0 (6.0-9.0)	11.0 (9.25-12.0)	<0.001

Table 1: General characteristics of subjects included in the study, as well as a comparison between individuals with DSDRS above and below the 75^{th} age-specific percentile, defined as high 10-year dementia risk. Results are presented as either mean±SD or Median (IQR), according to variable distributions.

Abbreviations: T2D, Type 2 diabetes; DSDRS, Diabetes-specific dementia risk score; HDL-C, High-density lipoprotein cholesterol; BMI, Body-mass index; MMSE, Mini-mental state examination; ADL, Activities of daily life; IADL, Instrumented activities of daily life.

3.1.1 DSDRS, cognition, frailty, QoL and functional scores

We observed negative adjusted and unadjusted correlations between DSDRS and MMSE, IST, SF-36 PCS, SF-36 MCS and MNA. We also observed a positive and significant correlation between DSDRS and CDT, Katz, Lawton and the frailty score (Table 2). We did not observe a significant correlation between DSDRS, fasting glucose, triglycerides, HDL-C, total cholesterol or BMI. Using step-wise linear regression analyses we identified that frailty (β=0.0.364, 95%CI 0.042-0.687), MMSE (β=-0.140 95%CI -0.244 - -0.036) and MNA scores (β=-0.189 95%CI -0.312 - -0.065) explained 27.0% of the variability in DSDRS, adjusted for sex, years of schooling and years since T2D diagnosis (R^2=0.270, $p < 0.001$). Furthermore, we observed significantly higher DSDRS among frail participants compared with non-frail subjects and in subjects with disability (Figure 2).

3.1.2 DRDS, decreased cognitive performance, frailty, and disability

Subjects with high 10-year dementia risk had less years of education, higher fasting glucose levels, lower MNA, SF-36 PCS, SF-36 MCS, MMSE, IST, Katz and Lawton-Brody scores, higher frailty and GDS scores, and years of T2D exposure in comparison to DSDRS <75th age-specific percentile (Table 1). As expected, subjects with high 10-year dementia risk were also more likely to be frail (30.6% vs. 11.9%, $p < 0.001$), at risk of malnutrition (51.5% vs. 20.3%, $p < 0.001$), with disability (limited ADL 23.2% vs. 7.0%, $p < 0.001$;

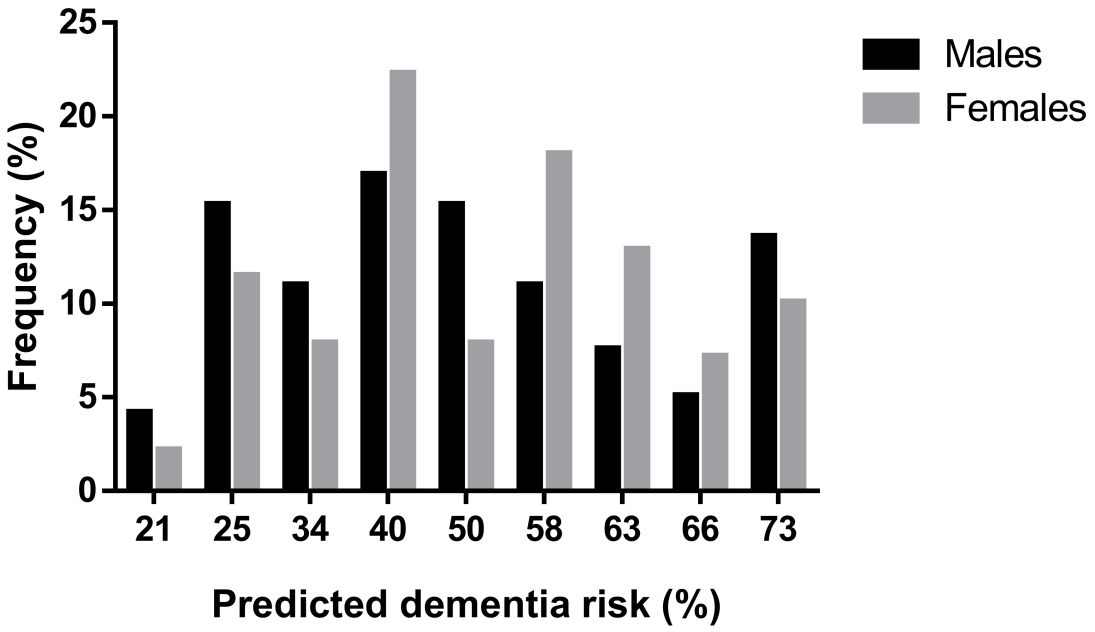

Figure 1: Modelo de riesgo de demencia en diabetes. La acumulación de factores de riesgo conforme evolucionan las alteraciones metabólicas asociadas a diabetes aumentan la progresión de deterioro neurológico y funcional. Adaptado de Bello-Chavolla et al [**?**].

Parameter	Unadjusted correlation (95%CI)	Adjusted correlation (95%CI)
MMSE score	-0.408 (-0.531 - -0.225)	-0.310 (-0.410 - -0.202)
IST score	-0.309 (-0.439 - -0.132)	-0.251 (-0.430 - -0.101)
Clock Drawing Test	0.290 (0.118-0.49)	0.264 (0.066-0.455)
SF-36 PCS	-0.384 (-0.490 - -0.261)	-0.276 (-0.404 - -0.147)
SF-36 MCS	-0.160 (-0.295 - -0.020)	-0.185 (-0.339 - -0.037)
Mini-nutritional assessment	-0.376 (-0.510 - -0.236)	-0.387 (-0.535 - -0.204)
Katz score	-0.312 (-0.448 - -0.144)	-0.306 (-0.417 - -0.182)
Lawton score	-0.227 (-0.374 - -0.066)	-0.357 (-0.483 - -0.216)
Frailty score	0.390 (0.265-0.495)	0.278 (0.063 – 0.440)

Table 2: Partial correlation analyses of DSDRS with evaluated scores, fasting laboratory and anthropometric measures in the sample, adjusted for sex, years of schooling and years since diabetes diagnosis.
Abbreviations: DSDRS, Diabetes-specific dementia risk score; MMSE, Mini-mental state examination; IST: Isaac's Set Test; MNA: Mini-nutritional assessment; SF-36 MCS: Mental Component Score of the SF-36 quality of life questionnaire; SF-36 PCS: Physical Component Score of the SF-36 quality of life questionnaire.

limited IADL 29.6% vs. 12.7%, $p = 0.003$) and low cognitive performance (20.5% vs. 8.7%, $p = 0.047$). When evaluating the role of the DSDRS score to identify frailty and disability we observed highest area under the curve (AUC) for detection of ADL disability (AUC 0.799 95%CI 0.712-0.886; sensitivity 85.7%, specificity 61.3%), IADL disability (AUC 0.738 95%CI 0.635-0.841; sensitivity 62.0%, specificity 75.7%), low cognitive performance (AUC 0.707 95%CI 0.587-0.828; sensitivity 83.3%, specificity 54.3%), and frailty (AUC 0.701 95%CI 0.605-0.797; sensitivity 71.9%, specificity 69.1%). Using logistic regression, high-estimated 10-year dementia risk was associated to ADL and IADL disability, frailty and risk of malnutrition, adjusted for sex, years of schooling and years since T2D diagnosis. In multivariable logistic regression analyses, frailty and risk of malnutrition were associated with high-estimated 10-year dementia risk, adjusted for sex, years of schooling and years since T2D diagnosis (R^2=0.290, χ^2=10.804, $p = 0.213$, Table 3).

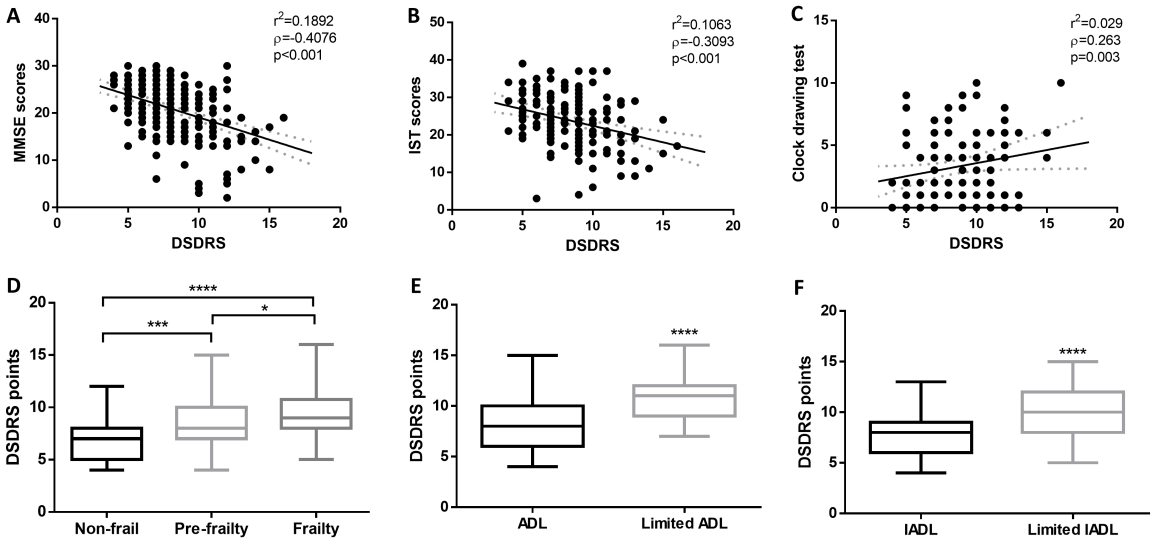

Figure 2: Correlation between increasing DSDRS and MMSE (A), IST (B) and Clock test scores (C). We also show comparisons of DSDRS according to frailty categories (D), and functional status regarding activities of daily life (E) and instrumented activities of daily life (F), demonstrating the role of DSDRS to discriminate functional and cognitive status. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.
Abbreviations: DSDRS, Diabetes-specific dementia risk score; MMSE, Mini-mental state examination; IST, Isaac's set-test; ADL, Activities of daily life; IADL, Instrumented activities of daily life.

3.1.3 Specific DSDRS components and outcomes

In the case of the frailty score, age, microvascular complications, diabetic foot, stroke and depression explain the observed associations. Older age, microvascular complications and cardiovascular disease were associated with Lawton scores whilst older age, microvascular complications and were associated with lower Katz scores. For MMSE we observed significant associations with age, schooling, depression and microvascular complications, whilst for MNA scores, we observed associations with diabetic foot disease, cardiovascular disease, stroke and depression. Finally, lower SF-36 PC scores were associated with microvascular disease, cardiovascular disease and depression (Table 4).

4 Discussion

Here, we demonstrate that the DSDRS is associated with measures of cognitive performance, frailty, risk of malnutrition, QoL, and ADL/IADL disability among community-dwelling older adults with T2D. Furthermore, we observed higher DSDRS in pre-frail and frail participants and among those with disability. Relying on these associations, the identified cross-sectional phenotype observed using the DSDRS is consistent with what would be expected for patients at higher risk of dementia, regardless of T2D status. Subjects at higher risk of dementia identified by the DSDRS would most likely be frail, have some degree of disability, decreased cognitive performance, risk of malnutrition and lower QoL. These findings strengthen the notion that T2D and T2D-related complications have significant burden on functional status, QoL, disability and, subsequently, on dementia risk.

4.1 Frailty and dementia risk by DSDRS

Older adults with T2D are at an increased risk of frailty; furthermore, interactions between frailty and hypoglycemia during T2D treatment have been reported to increase dementia-risk. This is significant, since

Model diagnostics	Parameter	$\hat{\beta}$	Wald	OR (95%CI)	p-value
R^2=0.207; χ^2=4.782, p=0.781	IADL disability	1.396	11.01	4.04 (1.77-9.21)	0.001
R^2=0.207; χ^2=7.522, p=0.481	ADL disability	1.001	4.48	2.72 (1.08-6.86)	0.034
R^2=0.035; χ^2=1.000, p<0.001	Low CP	0.988	3.73	2.69 (0.99-7.32)	0.053
R^2=0.219; χ^2=2.748, p=0.949	Frailty	1.184	7.58	3.27 (1.41-7.59)	0.006
R^2=0.116; χ^2=0.057, p=0.972	Risk of malnutrition	1.426	11.71	4.16 (1.84-9.42)	0.001
R^2=0.294; χ^2=10.804, p=0.213	Frailty	1.480	5.541	4.39 (1.28-15.06)	0.019
	Risk of malnutrition	0.998	4.099	2.71 (1.03-7.13)	0.043

Table 3: Simple and multiple logistic regression analyses of the association of evaluated scores and phenotypes with high-estimated dementia risk, defined as DSDRS $> 75^{th}$ age-specific percentiles. Analyses were adjusted for sex, years of schooling and years since diabetes diagnosis.
Abbreviations: DSDRS, Diabetes-specific dementia risk score; MMSE, Mini-mental state examination; ADL, Activities of daily life; IADL, impaired activities of daily life; CP: Cognitive Performance.

patients with increasing number of macro and microvascular complications might be assessed as requiring more intensive glycemic control which, in subjects with impaired functional status and frailty might increase dementia risk (Abdelhafiz et al, 2016). The role of frailty in increasing morbidity and impacting QoL in patients with T2D has previously been shown and has led to recommendations against intensive glycemic control in this population (Sheen et al, 2016; Schernthaner et al, 2018); in addition, frailty is related to vascular damage and might contribute to increased risk of vascular dementia in T2D (Ávila-Funes et al, 2012). DSDRS might prove useful to identify patients with impaired functional status, multiple comorbidities, and frailty, who might benefit from less intensive T2D treatment and might require treatment adjustments (Schernthaner et al, 2018). In our study, we did not assess hypoglycemic episodes or hypoglycemia risk, but the interaction between frailty and hypoglycemia in relation to DSDRS and its impact on dementia risk should be evaluated in future studies.

4.2 Microvascular complications and dementia risk by DSDRS

Microvascular complications, particularly diabetic retinopathy, are evaluated by DSDRS to discriminate subjects with increased dementia risk who might also have disability, impaired QoL and functional status (Chen et al, 2016). Diabetic retinopathy and neuropathy cause severe sensory impairments in older patients with T2D; furthermore, T2D has been associated to increased risk of bilateral sensorineural hearing loss in addition to established microvascular damage, which might contribute to further sensory loss (Corriere et al, 2013; Exalto et al J Alzheimers Dis, 2014). Sensory impairments, particularly in eyesight, hearing, and neuropathy have been associated with increased dementia risk and favor the development of disability and increased mortality; in addition, end-organ microvascular damage in T2D increases risk of falls and impairment of functional status (Li et al, 2018; Luo et al, 2018). The role of microvascular damage in the pathophysiology of dementia in diabetes has also been studied, but the evidence of this association is inconsistent and neurological changes have been observed in individuals with T2D without end-organ microvascular damage (Formiga et al, 2015). Thus, DSDRS might identify individuals at high risk of disability in IADL and ADL due in part to sensory impairment by recognizing a population with increased dementia risk in whom rehabilitation would be beneficial (Uremura et al, 2017). The role of depression, macro and microvascular factors and obesity in promoting disability in individuals with T2D has also been reported, and its evaluation by the score contributes to the identification of individuals with ADL and IADL disability (Fauth et al, 2013).

4.3 Cognition, microvascular complications and DSDRS

Older adults with T2D present lower performance on cognitive evaluations, particularly when affected by micro and macrovascular complications (Tabesh et al, 2018). Cognitive evaluations of individuals with T2D have demonstrated impaired domains in information processing speed, visuospatial functions, attention, executive functioning abstract reasoning (Ruis et al, 2009); furthermore, individuals with T2D present a higher rate of cognitive decline, directly dependent with glycemic control. Individuals with T2D and cognitive

Model	Parameter	$\hat{\beta}$	t	95%CI	p-value
Frailty score	Age	0.110	2.167	0.10-0.210	0.031
R^2=0.202, $p < 0.001$	Microvascular complications	0.385	2.220	0.043,0.727	0.027
	Diabetic foot	0.368	2.048	0.014,0.722	0.042
	Depressive symptoms	0.483	4.277	0.260,0.705	<0.001
	Stroke	0.305	1.985	0.002,0.608	0.048
Lawton-Brody score	Age	-0.118	-2.523	-0.211,-0.026	0.012
r2=0.049, p=0.001	Microvascular complications	-0.314	-2.003	-0.623,-0.005	0.046
	Cardiovascular disease	-0.496	-1.935	-1.001,0.009	0.054
Katz scores	Age	-0.161	-3.232	-0.259,-0.063	0.001
r2=0.165, p<0.001	Microvascular complications	-0.612	-3.665	-0.941,-0.283	<0.001
	Depressive symptoms	-0.407	-3.886	-0.614,-0.201	<0.001
MNA scores	Diabetic foot	-1.284	-2.737	-2.211,-0.357	0.007
r2=0.294, p<0.001	Cardiovascular disease	-3.074	-3.843	-4.655,-1.493	<0.001
	Stroke	-1.480	-3.566	-2.299,-0.660	<0.001
	Depressive symptoms	-1.221	-4.340	-1.777,-0.665	<0.001
MMSE scores	Age	-0.760	-4.341	-1.105,-0.415	<0.001
r2=0.283, p<0.001	Microvascular disease	-1.463	-2.259	-2.739,-0.188	0.025
	Schooling	-4.069	-4.781	-5.746,-2.393	<0.001
	Depression	-2.200	-5.848	-2.914,-1.459	<0.001
SF-36 PCS	Microvascular disease	-5.420	-4.268	-7.923,-2.916	<0.001
r2=0.142, p<0.001	Cardiovascular disease	-4.757	-2.354	-8.742,-0.772	0.020
	Depression	-2.371	-2.642	-4.140,-0.602	0.009

Table 4: Linear regression analyses of the association of individual DSDRS components with each evaluated score to assess individual contributions to their associations with DSDRS in community-dwelling older adults. *Abbreviations: DSDRS, Diabetes-specific dementia risk score; MMSE, Mini-mental state examination; MNA: Mini-nutritional assessment; SF-36 MCS: Mental Component Score of the SF-36 quality of life questionnaire..*

impairment also experience a higher rate of conversion to dementia, with earlier disease onset and increased disease progression in relation to T2D duration and microvascular complications, as has been shown in previous studies (van den Berg et al, 2010; de Bresser et al, 2010). The lower cognitive performance observed in individuals with increased DSDRS in our study might be attributable to the consideration of microvascular damage, age and glycemic control, which underlie associations with impaired cognition in T2D in most prospective studies. Furthermore, the impact of frailty and disability on cognition, both of which increase dementia risk, must also be considered (Ma et al, 2015; Zilkens et al, 2013). Since individuals with T2D are a population with high susceptibility to impaired cognition, our demonstration of lower cognitive performance when screening subjects using the DSDRS provides evidence for its utility in a cross-sectional setting. Future longitudinal studies should shed light on the role of DSDRS for prediction of cognitive impairment conversion to dementia in individuals with T2D.

Strengths and limitations

Our study had some strengths and limitations. First, we performed a wide range of evaluations, which allowed a thorough assessment of cognition, QoL, disability, and frailty in a sample of community-dwelling individuals with T2D in which most of these predictors had previously been validated. We also demonstrated that the DSDRS identifies subjects who could be considered for short-term interventions to improve function and ameliorate the negative effects of disability, frailty and T2D related complications. Furthermore, this is the first study in which DSDRS is used besides its original evaluation, demonstrating its utility in different populations and settings. Validation of DSDRS for functional and frailty status as demonstrated in this study allows to characterize the phenotype observed for subjects with high-estimated dementia risk score and extend the applications of DSDRS; nevertheless, prospective validation studies are required to both externally validate the score in different populations and replicate the observed associations for DSDRS in

this study. Amongst the limitations to be acknowledged is its cross-sectional setting, which limits the ability to establish causal relationships and the self-report of comorbidities and T2D complications, which might underestimate the true impact of the associations. The modified frailty definition which uses self-reported measures has previously been applied in other studies involving the Coyoacán Cohort study; furthermore, epidemiological studies in other settings with self-reported data have been conducted and yielded reproducible results (Aantos-Eggimann et al, 2009; Rothman et al, 2008). We believe this modified definition should not have a significant impact on the observed associations but additional evidence with frailty definitions using objective measures should be carried out in future studies. Furthermore, since the evaluated sample was representative of the community and previous reports have shown an increased rate of undiagnosed T2D in our population (Bello Chavolla et al, 2017), some cases of T2D with fasting glucose <126mg/dL could have been excluded, thus limiting information of such cases. In addition, since no specific dementia information was available for diagnosis in the Coyoacán Cohort study there exists a possibility for undiagnosed cases of dementia not identified by cognitive assessment, thus modifying the strength of these associations. Moreover, cognitive assessment did not include more extensive measures of executive function, which are highly sensitive to T2D-related cognitive changes and remain to be explored in future studies.

5 Conclusion

The DSDRS is associated with frailty, disability, risk of malnutrition, lower cognitive performance and impaired quality of life. Evaluation of this score in primary care facilities might prove useful for identification of subjects with T2D who might benefit from multidisciplinary interventions focusing on rehabilitation to improve upon IADL and ADL disability, frequent cognitive screening, nutritional counseling and evaluation of interventions to reduce burden related to frailty. The role of said interventions to delay onset of cognitive decline and dementia in high risk patients identified using the DSDRS should be evaluated in future studies.

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Conflicts of interest: Nothing to disclose.

Author Contributions

Omar Yaxmehen Bello-Chavolla: developed conceptualization and design of this study; contributed to interpretation of data, statistical analyses and manuscript drafting. He is the guarantor of the work, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Carlos Alberto Aguilar-Salinas: developed conceptualization and design of this study, contributed to interpretation of data and drafting and revision of the manuscript. José Alberto Ávila-Funes: developed conceptualization and design of this study; contributed to interpretation of data and drafting and revision of the manuscript.
Sponsor's Role: None.

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Rev Inves Clin. 2017;69:166-72 ORIGINAL ARTICLE

Geriatric Syndromes and Not Cardiovascular Risk Factors are Associated with Cognitive Impairment among Mexican Community-Dwelling Elderly with Type 2 Diabetes

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ABSTRACT

Background: The association of cognitive impairment and type 2 diabetes has been consistently shown in several studies, yet its association with geriatric syndromes has not been fully explored. **Objective:** To study the correlates of cognitive impairment among community-dwelling elderly with type 2 diabetes. **Methods:** Cross-sectional study of 135 diabetic persons aged 70 years or older participating in the Coyoacán Cohort Study in Mexico City. Baseline data included chronic illnesses, geriatric syndromes, and diabetes-related variables. The lowest quartile in both the Mini-Mental State Examination and the Isaacs Set Test, according to age and schooling, was used to identify participants with cognitive impairment. Multivariate logistic regression analyses were used to identify the correlates of cognitive impairment. **Results:** Mean age of participants was 77.7 ± 5.8 years. The prevalence of cognitive impairment was 14.1%. Univariate logistic regression analyses showed that diabetic nephropathy, depression symptoms, falls, and frailty were associated with cognitive impairment. Multivariate logistic regression analyses showed that urinary incontinence and frailty were independently associated with cognitive impairment. Cardiovascular risk factors and diabetes-related variables did not show significant association to cognitive impairment. **Conclusions:** Geriatric syndromes, but not cardiovascular risk factors, were independently associated with cognitive impairment among diabetic elderlies. Intentional evaluation of these conditions may be important to improve management of the elderly patient with type 2 diabetes and cognitive impairment. (REV INVES CLIN. 2017;69:166-72)

Key words: Geriatric syndrome. Cognitive impairment. Frailty. Urinary incontinence. Diabetes.

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INTRODUCTION

Type 2 diabetes mellitus (DM) is a significant public health burden worldwide, particularly in countries under epidemiological transition[1-3]. Care and management in elderly DM patients is complicated, especially given the simultaneous presence of comorbidity and geriatric syndromes. Of special importance is cognitive impairment (CIM), which is associated with reduced self-care and monitoring of DM as well as higher hospitalization records, among others[3]. The association between DM and CIM has been persistently reported in several studies. In comparison with non-diabetic populations, those with DM have more frequent cognitive impairment[4,5]. In addition, factors such as long evolution of DM, elevated HbA1c levels, or history of insulin treatment are considered risk factors for the development of CIM. Previous work has demonstrated that Mexican-American participants with DM had a greater risk of dementia and CIM in comparison with non-diabetic participants[6-8]. Similarly, DM patients also have a higher risk of geriatric conditions, including dementia, disability, falls, and urinary incontinence[9]. Despite the large evidence supporting these associations, the relation of geriatric conditions with the presence of CIM in DM patients is still largely unknown; furthermore, previous work suggests that cardiovascular risk factors have a more established role in association with CIM[10]. Therefore, this study aimed to determine the geriatric and cardiovascular correlates of CIM among community dwelling elderly diabetics.

METHODS

Study population

This is a cross-sectional analysis of a subset of participants from the Coyoacán Cohort, an observational study conducted in Mexico City. Specific details for the design of this study have been published elsewhere[10]. Briefly, to be eligible for recruitment, participants had to meet the following criteria: age 70 years or older, established residence in Coyoacán, not being institutionalized, and being registered at the Food Support, Medical Care and Free Drugs Program, which is a government program that includes 95% of the community dwelling elderly (≥ 70 years of age) in Mexico City. The first phase was composed of a face-to-face interview during which a wide range of information was collected, including self-reported data regarding sociodemographic characteristics, general health-related information, medication use, oral health (self-reported and clinically evaluated), and mental health. Each participant underwent a comprehensive geriatric assessment that included physical performance tests, cognitive tests, and nutrition and medical assessment as well. The Human Research Local Ethics Committee approved all proceedings regarding this study.

Sample

Patients with type 2 DM were selected from the Coyoacán Cohort and included in this study. Diabetes was defined as the self-report of previously diagnosed DM or self-report of taking DM medications. All considered participants underwent a Mini-Mental State Examination (MMSE) and a semantic verbal fluency test (Isaacs Set Test, IST). Cognitive impairment was defined as a score below the 25th percentile for this specific population, according to age and schooling in both tests, without functional impairment[10]. Patients who only completed either the MMSE or the IST were excluded from the study; no significant differences were found between patients included and excluded from the analysis.

Definition of potential correlates

Participants were categorized as "frail" if they fulfilled three or more frailty criteria; otherwise if they fulfilled one or two, they were considered to be pre-frail or non-frail if none[11]:

– Unintentional weight loss of 5 kg or more in the last 12 months;

– Exhaustion was assessed by the positive answer to two questions from the Center for Epidemiologic Studies-Depression scale (CES-D) :"I felt that everything I did was an effort" and "I could not get going";

– Low physical activity was defined according to the lowest quintile (adjusted by sex) on the Physical Activity Scale for the Elderly questionnaire (PASE);

– Slowness was defined if participants answered "yes" or "can't do" to any of the following two questions: Because of a health problem, "do you have difficulty

walking one block?" or alternatively, "Do you have difficulty with climbing several flights of stairs without resting?"; and

– Weakness was determined among participants who answered "yes" to the question, "Because of a health problem, do you have difficulty with lifting or carrying objects weighting over 5 kg, like a heavy bag of groceries?".

These definitions have previously been validated for this population[12].

Fall syndrome was defined as having > 2 fall episodes in the previous 12 months[13].

Depressive symptoms were determined when participants had a Geriatric Depression Scale score > 5 (15-item version)[14].

Polypharmacy was defined as taking > 3 different medications at the time of the study[15].

Urinary incontinence, visual deficit, myocardial infarction, stroke, hypertension, hypercholesterolemia, hypertriglyceridemia, osteoporosis, and smoking were determined by the self-report of each entity and considered individually as binary outcomes.

For diabetes-related variables, duration of DM from diagnosis in years as well as age at diagnosis were each individually assessed; self-report of diabetic nephropathy and diabetic retinopathy was assessed independently and then combined under "Any microvascular complications" for purposes of the analysis. Previous history of insulin use was treated as a binary variable according to self-report by the patient. Macrovascular complications include self-report of diabetic foot or peripheral vascular disease.

Body mass index (BMI; weight/height^2) was also calculated and included as a correlate.

Sociodemographic variables included age (years), sex, and schooling (education grade).

Statistical analysis

Characteristics of participants were described using arithmetic means and standard deviations (SD) or frequencies and proportions where appropriate. The following statistical procedures were used according to analyzed variables: chi-squared test for qualitative variables, Student *t*-test, and the *U* of Mann-Whitney tests were used where applicable for quantitative variables. In order to develop an explanatory model for CIM, we fitted multivariate logistic regression models, including several variable-blocks: sociodemographic variables, diabetes-related variables, cardiovascular risk factors, and geriatric conditions. Wald tests were used to eliminate from every model those variables judged not significant at the 20% level, and then the variables considered significantly associated with CIM were retained. Secondly, a new model including the variables significantly associated with CIM from previous models was run and the cut-off level at this time was 5% in order to select a set of variables to be included in a last full model. All comparisons were evaluated using 95% confidence intervals (CI) and the Nagelkerke R^2 was also reported. Statistical analyses were performed in SPSS software for Windows® (SPSS Inc., Chicago, IL, version 19.0).

RESULTS

The study sample included 135 participants. Mean age was 77.7 years (SD: 5.8) and 54.1% were female. Mean age of DM onset was 62.4 years (SD: 12.5), with a mean DM duration of 14.7 years (SD: 11.3), 19.3% were using insulin treatment, and 57% reported having at least one microvascular complication (28.1% diabetic nephropathy and 44.1% diabetic retinopathy). Of the participants, 18% had urinary incontinence, 46.7% had at least two falls in the last year, 20% reported depressive symptoms, 14% were frail, and 57.8% reported currently taking > 3 medications. Only 19 participants (14.1%) had CIM.

Table 1 presents the comparative analysis between participants according to the presence or absence of CIM. In comparison to subjects without CIM, those cognitively impaired had more diabetic nephropathy ($p = 0.014$) or the presence of any microvascular complication ($p = 0.046$), falls ($p = 0.047$), depressive symptoms ($p = 0.013$), and frailty ($p = 0.011$). The frequency of cardiovascular risk factors, including myocardial infarction, stroke, systemic hypertension, and hypercholesterolemia were not different between groups.

Table 1. Comparative analysis among participants without or with cognitive impairment

Variable	Overall (n = 135)	Without cognitive impairment (n = 116) (%)	With cognitive impairment (n = 19) (%)	p value
Women (%)	73 (54.1)	63 (54.3)	10 (52.9)	0.892
Age (Mean ± SD)	77.7 ± 5.8	77.7 ± 5.7	77.5 ± 7.1	0.414
Years of schooling (Median ± IQR)	6.0 ± 4.0	5.0 ± 4.0	6.0 ± 2.5	0.849
BMI (Mean ± SD)	26.3 ± 4.4	26.9 ± 4.3	24.6 ± 4.3	0.073
Myocardial infarction	15 (11.1%)	12 (10.3)	3 (15.8)	0.444
Stroke	14 (10.4)	12 (10.3)	2 (10.5)	0.999
Systemic hypertension	85 (63.0)	74 (63.3)	11 (57.9)	0.618
Hypercholesterolemia	58 (43.0)	51 (44)	7 (36.8)	0.561
Hypertriglyceridemia	35 (25.9)	19 (25)	6 (31.6)	0.544
Smoking	65 (48.1)	56 (48.3)	9 (47.4)	0.942
Osteoporosis	20 (14.8)	17 (14.7)	3 (15.8)	0.999
Previous diabetes treatment	127 (94.1)	111 (95.7)	16 (84.3)	0.084
Age at onset (Mean ± SD)	62.4 ± 12.3	62.6 ± 12.7	61.5 ± 11.3	0.742
Years of T2D duration (Mean ± SD)	14.7 ± 11.3	14.6 ± 11.2	15.5 ± 12.3	0.714
Insulin use	23 (19.3)	19 (18.6)	4 (23.5)	0.494
Diabetic nephropathy	38 (28.1)	28 (24.1)	10 (52.6)	0.010
Diabetic retinopathy	60 (44.4)	50 (43.1)	10 (52.6)	0.438
Any microvascular complication	77 (57.0)	62 (53.4)	15 (78.9)	0.037
Vascular problems	58 (43.0)	48 (41.4)	10 (52.6)	0.358
Urinary incontinence	24 (18.0)	18 (15.7)	6 (33.3)	0.070
Visual deficit	34 (54.0)	31 (57.4)	3 (33.3)	0.280
Falls	63 (46.7)	50 (43.1)	13 (68.4)	0.040
Depression symptoms	27 (20.0)	19 (16.4)	8 (42.1)	0.009
Frailty	16 (14.0)	11 (10.6)	5 (50.0)	0.001
Polypharmacy	78 (57.8)	67 (57.8)	11 (57.9)	0.991

BMI: body mass index; IQR: interquartile range; SD: standard deviation; T2D: type 2 diabetes.

Table 2 presents the univariate logistic regression analyses of CIM. The models found that diabetic nephropathy (p = 0.014), having any microvascular complication (p = 0.046), previous falls (p = 0.047), depression symptoms (p = 0.013), and frailty (p = 0.011, pre-frailty was non-significant) were associated with CIM, whereas urinary incontinence did not reach statistical significance (p = 0.070). Conversely, variables such as previous history of myocardial infarction or stroke, hypercholesterolemia, hypertension, hypertriglyceridemia, or smoking were not associated with CIM.

However, the multivariate logistic regression model showed that only urinary incontinence (OR: 13.9; 95% CI: 2.12-80.84; p < 0.001) and frailty (OR: 4.2; 95% CI: 0.75-22.74; p = 0.019) were independently associated with CIM among DM participants (Table 3). The model explained 31% of the variability observed in the composite measure of CIM (R^2= 0.319).

Table 2. Univariate regression analysis of variables associated to cognitive impairment in type 2 diabetes participants

Variable	OR	95% CI	p value
BMI	0.89	0.79-1.01	0.077
Previous myocardial infarction	0.61	0.16-2.42	0.487
Previous stroke	0.98	0.20-4.77	0.981
Systemic hypertension	1.28	0.48-4.44	0.622
Hypercholesterolemia	1.34	0.49-3.66	0.562
Diabetic nephropathy	3.49	1.29-9.54	0.014
Any microvascular complication	3.27	1.02-10.44	0.046
Falls	2.86	1.02-8.05	0.047
Depression symptoms	3.71	1.32-10.45	0.013
Frailty	4.89	1.12-21.30	0.011
Polypharmacy	1.01	0.38-2.69	0.991
Urinary incontinence	2.69	0.90-8.11	0.078

BMI: body mass index; OR: odds ratio.

Table 3. Multivariate regression analysis of variables associated to cognitive impairment in type 2 diabetes participants

Model	Variable	OR (95% CI)	p value
1 R2 = 0.363	BMI	0.93 (0.74-1.17)	0.538
	Diabetic nephropathy	1.57 (0.25-9.70)	0.627
	Urinary incontinence	10.22 (1.52-68.82)	0.017
	Frailty	3.27 (0.38-28.38)	0.047
	Depression	1.21 (0.11-13.67)	0.879
	Falls	1.86 (0.30-11.39)	0.503
2 R2 = 0.313	Frailty	4.12 (0.75-22.74)	0.019
	Urinary incontinence	13.09 (2.12-80.84)	0.006

BMI: body mass index; OR: odds ratio.

DISCUSSION

In this sample of community dwelling diabetic elderly persons, urinary incontinence and frailty were independently associated with cognitive impairment. Given that the adequate management of elderly DM patients is dependent on their functional status, investigating the presence of cognitive decline and additional geriatric syndromes within this population is of the utmost importance.

A previous study in elderly Mexican Americans with DM reported that the prevalence of CIM, urinary incontinence, and individual components of the frailty syndrome (muscle strength, slowness) was lower, which contrasts with the data herein reported[16]. However, its definition of cognitive impairment could potentially overestimate the prevalence of CIM (MMSE scores), especially considering that the scores were not adjusted for confounders such as age and schooling in comparison to the present study.

The association of DM and CIM has been consistently shown in several studies; the reported associations suggest a potential role of vascular and non-vascular mechanisms for CIM in DM patients[17,18]. Although the mechanisms of cognitive dysfunction associated to DM are still unclear, combined neurological dysfunction, inflammation, hyperglycemia, insulin resistance, and vascular dysfunction have been proposed to be either causal or contributing elements to the development of both Alzheimer's disease and vascular dementia. Additionally, DM-associated microvascular complications, especially diabetic nephropathy and retinopathy, have also been linked to brain stroke and small-vessel disease associated with cognitive dysfunction[19,20].

The association of frailty with CIM has been described in other studies[21,22]; however, the association of frailty and CIM in elderly DM patients has not previously been reported. Avila-Funes, et al. proposed that frailty is a major risk factor for the development of cognitive impairment and a possible prodromal stage of vascular dementia[21]. This suggests that, despite the lack of association between the traditional cardiovascular risk factors and CIM in our study, the presence of frailty may account as a mechanism for vascular-mediated cognitive decline. It is important to consider that in other studies, DM has also been associated to an increase in the risk of frailty[23]; moreover, frail DM patients have an increased risk of complications when compared to patients with comorbidities[24-27]. These may play a role as potential confounders to the established association in our study.

Additionally, we show that urinar incontinence was also independently associated with CIM in DM patients; however, a causal link cannot be established to be precise on its association, given that DM is also a known risk factor for the development of urinary incontinence and the latter may already be present before CIM onset. A recent study suggests that biochemical measures, mainly hyperglycemia, are not independent predictors of the presence of urinary incontinence in DM patients[28]. Hsu, et al. investigated the association of risk factors related to the presence of urinary incontinence in elderly DM subjects[29]. They concluded that dependence on ambulation and transferring as well as the presence of CIM is associated with the presence of urinary incontinence in patients with DM, which is consistent with our findings.

Among the correlates that were associated, though not independently with CIM, microvascular complications are the most extensively studied. Despite evidence of microvascular complications leading to CIM, glucose control has not shown significant benefit on cognitive outcomes[19]. The ACCORD-MIND trial evaluated the cross-sectional and longitudinal association of diabetic retinopathy on cognitive function and brain volume, suggesting that retinal microvascular damage leads to decreased gray matter and cognitive function in diabetic patients, but is not necessarily predictive of vascular-mediated cognitive dysfunction[30]. Our results imply that microvascular complications, in particular diabetic nephropathy, are more frequent in patients with CIM, but their association is not independent. Additionally, microvascular complications are related to age of DM onset and years of DM exposure, both of which were not different between the group with and without CIM. The role of diabetic nephropathy has not been extensively studied and thus requires further longitudinal confirmation.

In DM patients, vascular complications are an important cause of morbidity, and individuals with cardiovascular risk factors are traditionally at higher risk. A large recent study[31] suggested that metabolic syndrome, central obesity, diabetes mellitus, and other cardiovascular risk factors were associated to incident mild cognitive impairment and dementia; however, this observation is true for patients under the age of 75 years as those included in the population of the study. In populations older than 75 years, such as the mean age of our population, the association of cardiovascular risk factors, including metabolic syndrome, dyslipidemia, central obesity, and hypertension with incident cognitive impairment, has been inconsistent[31-33]. This observation is also supported by the observation that carotid stiffness is associated with cognitive impairment in individuals with diabetes, but was not the mechanism of cognitive dysfunction[34]. In our study, we did not find a significant association of cardiovascular risk factors and cognitive impairment, as has been shown for individuals over 75 years. This suggests that CIM in DM patients is not exclusively vascular-mediated and implies that other factors, such as frailty and urinary incontinence, may contribute to the presence of CIM. Nonetheless, these associations must be confirmed in longitudinal follow-up.

Our study had some limitations. First, this study is a cross-sectional analysis, thus limiting our ability to establish causal associations because we lack the possibility to establish a temporal relation between variables. Furthermore, because only a subset of the studied population completed cognitive evaluation, our study population was reduced and the group with confirmed CIM was relatively small. However, no significant differences were found between the initial sampled group and the analyzed group. Additionally, because our model only explains 31% of the variability observed in CIM, other variables may contribute as correlates to CIM that were not included in the present model for this work. Subjects classified in the cognitive outcome were not assessed according to the standardized method for CIM. Instead, normative data for performance below the 25th percentile in both the MMSE and in IST were used to identify subjects with CIM. We consider that this global assessment is sufficient for the evaluated spectrum, but the results must be interpreted with caution. Nevertheless, the considered correlates have previously been defined or analyzed in other studies involving the Coyoacán cohort, which makes their definition consistent.

In conclusion, in this cohort of community-dwelling elderly, frailty and incontinence, but not cardiovascular risk factors, are associated with a higher frequency of CIM. Geriatric assessment is not usually sought out during a routine examination by an endocrinologist in an elderly patient with DM. Furthermore, given the strong links of DM and vascular complications, cardiovascular risk factors are classically attributed as the cause of CIM in this population, largely overlooking the effect of geriatric comorbidities. This suggests that the contribution of geriatric conditions to the presence CIM in DM patients should be further investigated. Intentional evaluation of these conditions might be of importance to improve patient care and management of the elderly patient with DM and cognitive decline.

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Capítulo 4

Resultados adicionales

El presente capítulo contiene resultados adicionales en proceso de publicación obtenidos en el contexto del presente trabajo.

Revisión sistemática y meta-análisis de factores de riesgo asociados a demencia en DM2

A través de la revisión sistemática utilizando los criterios de búsqueda antes delineados, se identificaron 2,090 potenciales artículos que cumplían los criterios de inclusión por revisión de título y abstract. Tras la revisión preliminar, se excluyeron 1,762 artículos por una o más razones incluyendo el uso de diseños no prospectivos, estudios básicos o pre-clínicos, falta de datos primarios del estudio, evaluación de un desenlace primario diferente de la incidencia de demencia, falta de inclusión de pacientes con DM2 en la evaluación basal o la falta de evaluación cognitiva o de demencia exhaustiva. De los artículos restantes, 85 fueron incluidos en la evaluación cualitativa y 42 calificaron para su inclusión en meta-análisis para diferentes factores de riesgo individuales.

Todos los estudios incluidos eran prospectivos, evaluaban el papel de la DM2 y factores asociados en el riesgo de demencia, con tamaños de muestra variables, diferentes rangos de edad y diferencias en el tiempo de seguimiento e inconsistencias en los criterios de ajuste para los análisis multivariable. El desenlace primario se definió como la evaluación de diabetes como factor de riesgo para demencia. En la evaluación acumulativa utilizando meta-análisis de efectos aleatorios, se confirmó el riesgo incrementado de demencia por todas las causas asociado a DM2 en los estudios evaluados (HR 1.67, 95 %CI 1.65-1.68, I2=0 %), el cual era mayor para la demencia vascular (HR 2.41, 95 %CI 1.93-3.01) en comparación con la demencia por Enfermedad de Alzheimer (HR 1.12, 95 %CI 1.02-3.12). Identificamos además tres estudios realizados En un sub-análisis incluyendo estudios realizados en población mexicana y México-Americana; en nuestra población se obtuvo un riesgo acumulado mayor al reportado para todas las cohortes (HR 2.01 95 %CI 1.65-2.45) incluyendo los estudios ENASEM, 10/66 y SALSA ((41; 8; 42), **Figura 4.1**).

En la revisión sistemática, se identificó un reporte inconsistente de los factores de riesgo asociados al desarrollo de demencia en pacientes con DM2. En su mayoría,

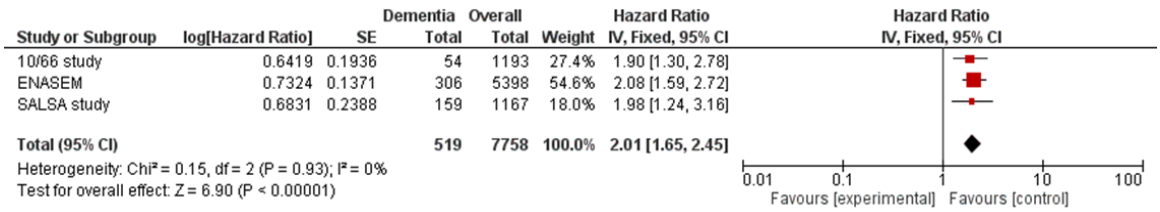

Figura 4.1: Forrest plot de meta-análisis de efectos aleatorios obtenidos de estudios realizado en población mexicana y México-Americana para el riesgo de demencia asociado a DM2.

el reporte de factores de riesgo específicos ha sido relegado a desenlaces secundarios sin certeza de un cálculo de poder suficiente para su identificación en la mayoría de los casos. Esto ha causado falta de consistencia en el reporte de factores de riesgo, así como definiciones operacionales sin adecuada certeza de reproducibilidad o únicamente obtenidas por auto-reporte. Algunos factores de riesgo previamente reportados incluyeron edad avanzada, sexo femenino para demencia de tipo vascular (16), pero masculino para demencia por todas las causas, uso de insulina (21; 43) uso de medicamentos anticolinérgicos, particularmente oxibutinina (38)), genotipo de riesgo para *APOE-4* y *HHEX_23* (rs1544210, (45)), hipertensión, enfermedad cardiovascular (16; 45), hiperglucemia de ayuno (46), episodios agudos de hipoglucemia (47), depresión y retinopatía diabética (24). De forma inconsistente, se ha reportado el incremento o reducción de riesgo asociado a hipertensión uso de metformina, sulfonilureas y pioglitazona (43; 48) aspirina (49) y enfermedad arterial periférica. En general, el reporte de los factores de riesgo no ha sido consistente en los estudios debido al poder estadístico de la muestra, la heterogenidad de la cohorte o la fortaleza de los desenlaces cognitivos estudiados. La implementación de una sistematización en el estudio de factores de riesgo asociados a demencia en DM2 es necesaria para la calibración de puntajes de riesgo. De la misma forma, poco se ha estudiado sobre factores de riesgo metabólico no ligados al manejo farmacológico o complicaciones micro o macrovasculares de diabetes.

Riesgo de demencia asociado a diabetes en la cohorte 3C de Bordeaux

En la cohorte de Bordeaux tras 14 años de seguimiento se identificaron 402 casos de demencia durante 23,126.65 años-persona, lo que representa una tasa de incidencia de 17.38 casos/1000 años-persona o 19.1 %. La prevalencia de DM2 en la evaluación basal fue de 11.2 %; en pacientes con DM2 se identificaron 65 casos de demencia por cualquier causa, con una tasa de incidencia de 29.24/1000 años-persona (25.9 %), que fue mayor al observado en pacientes sin DM2 (TI: 16.12/1000 años-persona o 18.0 %, $p < 0.004$). En la comparación utilizando modelos de Kaplan-Meier y funciones de incidencia acumulada se observó una mayor incidencia de demencia por cualquier causa (**Figura 4.2**, log-rank $p < 0.001$). En los modelos de regresión de riesgos proporcionales de Cox ajustados por sexo, edad, escolaridad y genotipo de riesgo de APOE-4

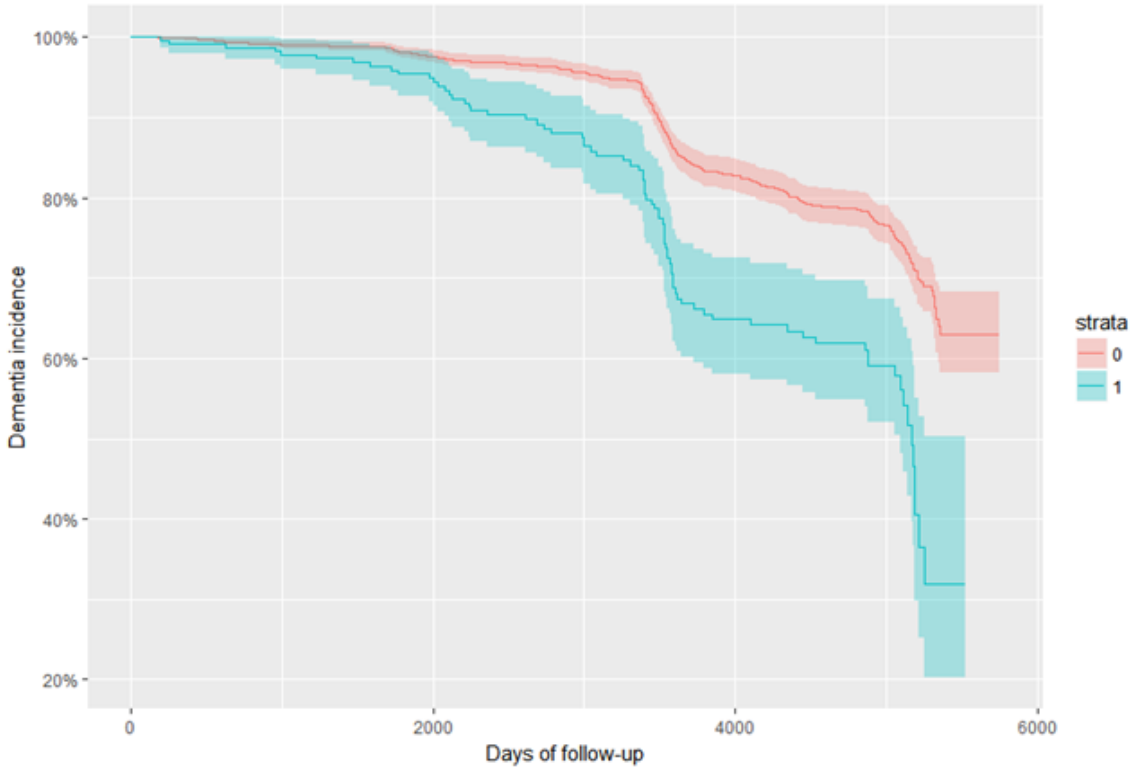

Figura 4.2: Modelo de Kaplan-Meier para casos de demencia incidente en la cohorte de Burdeos del *Three City Study*, comparando casos sin diabetes (0) y con diabetes (1) y sus respectivos intervalos de confianza al 95 %.

encontramos un mayor riesgo de demencia asociado a DM2 (HR 1.623 95 %CI 1.348-1.954, p<0.001) el cual también se confirmó en los modelos para riesgos competitivos ajustados por mortalidad (sHR 1.721 95 %CI 1.280-2.316, $p < 0.001$). En cuanto a las causas etiológicas de demencia también se encontró un riesgo aumentado para demencia de tipo vascular (sHR 1.439 95 %CI 0.0994-2.082, $p = 0.049$) y demencia de tipo Alzheimer (sHR 2.467 95 %CI 1.081-5.632, $p = 0.032$). Esto permitió confirmar que la asociación entre demencia en DM2 también era consistente en la cohorte 3C realizada en Bordeaux y que el modelaje de riesgo era factible.

Factores de riesgo asociados a demencia

En la evaluación de factores de riesgo individuales para el desarrollo de demencia en la cohorte 3C de Bordeaux se evaluaron antecedentes personales patológicos, mediciones antropométricas y bioquímicas, así como índices metabólicos de resistencia a la insulina, adiposidad visceral y masa magra para evaluar factores de riesgo asociados al desarrollo de demencia. En promedio, la edad de diagnóstico de demencia para individuos con DM2 fue más temprana en comparación con los individuos sin diabetes (83.105.78 vs. 81.905.54, p<0.001). Entre los factores de riesgo se identificó al uso de insulina como un factor de riesgo independiente para el desarrollo de de-

mencia en diabetes (HR 2.58 95 %CI 1.03-6.49), la resistencia a la insulina (HR 2.78 95 %CI 1.12-6.87), evento vascular cerebral (HR 4.66, 95 %CI 1.59-13.61), triglicéridos elevados (HR 1.36 95 %CI 1.06-1.75), un IMLG bajo (HR 0.65 95 %CI 0.46-0.92), pérdida de peso (HR 1.92 95 %CI 1.10-3.36) y los niveles elevados de colesterol (HR 1.01 95 %CI 1.00-1.01) ajustado por edad, sexo y escolaridad.
Algunos factores de riesgo observados en pacientes con y sin diabetes fueron depresión (HR 1.92 95 %CI 1.36-2.70 vs. HR 2.89 95 %CI 1.39-6.00), discapacidad motora (HR 1.52 95 %CI 1.19-1.93 vs. HR 2.10 9 %CI 1.17-3.79), edad, sexo y escolaridad. Ésta evaluación representa la primer aproximación comparativa a las diferencias en la presentación de factores de riesgo para demencia en pacientes con y sin diabetes y demuestra que algunos de los factores de riesgo identificados como asociados con un mayor riesgo de demencia en la literatura en población general no necesariamente se comportan como factores de riesgo en pacientes con diabetes. Sin embargo, es importante reconocer que pueden presentarse limitaciones de poder estadístico debido al tamaño muestral y el número de casos de demencia incidente de la muestra estudiada en esta fase para la cohorte de Bordeaux en 3C.

Modelos de riesgo para demencia incidente en DM2

Con el objetivo de evaluar la reproducibilidad de los factores de riesgo identificados en la cohorte de 3C en Bordeaux con respecto a las ciudades de Dijon y Montpellier, se realizó una evaluación de los factores de riesgo independientes descritos en la cohorte de Bordeaux utilizando el universo de completo de pacientes con diabetes de toda la cohorte de 3C (N=908). Para ésta definición se consideraron casos de diabetes como sujetos con reporte de diagnóstico previo de DM2, uso de medicamentos exclusivos para la DM2 o niveles de glucosa $\geq$ 126 mg/dL. En ésta cohorte se identificaron tras 14 años de seguimiento a 122 casos de demencia incidente por cualquier causa en los 3 centros de estudio (63 casos en Bordeaux, 46 casos en Dijon y 13 casos en Mintpellier). La tasa de incidencia acumulada durante 6,047.27 años de seguimiento de demencia en pacientes con DM2 fue de 20.17 casos de demencia por 1,000 años-persona (IC95 % 16.59-23.75) lo cual es una tasa inferior comparada con la observada únicamente para Bordeaux en DM2 aunque mayor comparado con la población general.
Al evaluar factores de riesgo para el desarrollo de demencia por cualquier causa, ajustado por edad, sexo y nivel de escolaridad se identificó un mayor riesgo de demencia para portadores de riesgo en APOE-ϵ4 (HR 1.72, IC95 % 1.10-2.71), resistencia a la insulina evaluada por METS-IR (HR 1.05, IC95 % 1.01-1.10), acumulación de tejido adiposo por METS-VF (HR 1.62, IC95 % 1.06-2.48) y obesidad visceral (METS-VF *geq* 7.18, HR 1.56, IC95 % 1.03-2.36), fasting triglycerides $\geq$ 150 mg/dL (HR 1.48, IC95 % 1.01-2.17), increasing levels of LDL-C adjusted by statin treatment (HR 1.006, IC95 % 1.001-1.012) and LDL-C $\geq$ 190 mg/dL (HR 1.41, IC95 % 1.02-1.94), discapacidad motora evaluada por la escala de Rosow-Breslau (HR 1.62, IC95 % 1.07-2.46), infarto agudo al miocardio previo (HR 1.74, IC95 % 1.01-3.00), evento vascular cerebral previo (HR 2.45, IC95 % 1.35-4.45), depresión (HR 1.79, IC95 % 1.11-2.87). Así mismo, identificamos un efecto protector de la actividad física sobre la incidencia de demencia (HR 0.71, IC95 % 0.52-0.97).

Tras el modelaje utilizando regresión de riesgos proporcionales de Cox identificamos como predictores al sexo femenino, edad, IMLG, METS-VF, años de escolaridad, discapacidad motora por Rosow-Breslau, actividad física, positividad para el alelo de riesgo en APOE-ϵ4 y antecedente de enfermedad vascular cerebral (50). Todos los predictores satisfacían los criterios para la evaluación por el modelo de riesgos proporcionales y el modelo tuvo buena concordancia (**Tabla 1**).

Modelo de riesgos proporcionales de Cox				
Coeficientes	$\hat{\beta}$	Error estándar	HR (IC95 %)	p-value
Sexo femenino	-2.0645	0.6381	0.13 (0.04-0.44)	0.0012
IMLG	-0.3830	0.1317	0.68 (0.53-0.88)	0.0036
METS-VF	1.4643	0.5189	4.32 (1.56-11.96)	0.0048
EVC	0.9259	0.9259	2.52 (1.27-5.01)	0.0081
Actividad física	-0.4322	0.1827	0.65 (0.45-0.93)	0.0180
APOE-ϵ4	0.4722	0.2356	1.60 (1.01-2.54)	0.0451
Escolaridad (años)	-0.1429	0.0611	0.87 (0.77-0.98)	0.0193
Discapacidad motora	0.5368	0.2251	1.71 (1.10-2.66)	0.0171
BIC= 1116.024	$R^2 =$ 0.088	$D_{xy} = 0.456$	c-statistic=0.728	p<0.0001

Tabla 1: Modelo de riesgos proporcionales de Cox para riesgo de demencia en DM2 utilizando codificación continua de variables predictoras.

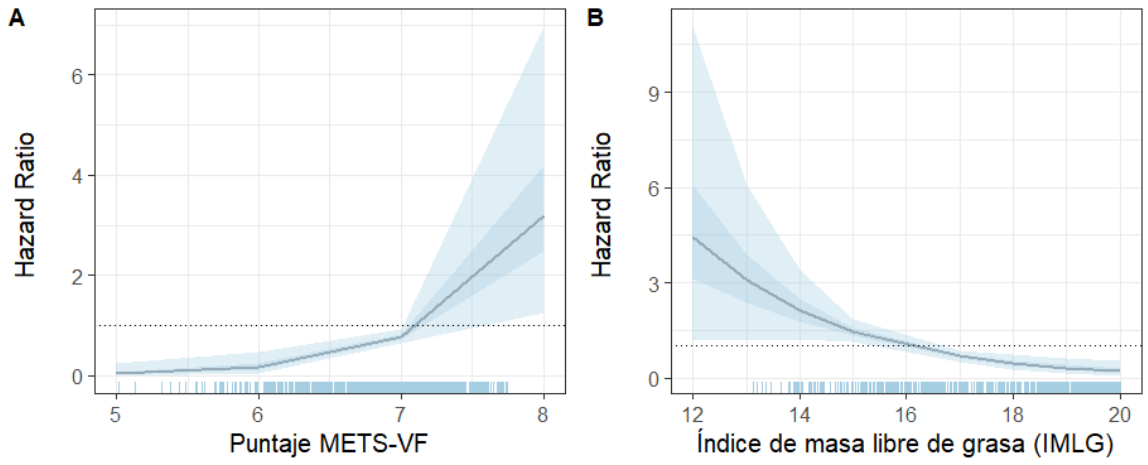

Figura 4.3: Modelo de Kaplan-Meier para casos de demencia incidente en la cohorte de Burdeos del *Three City Study*, comparando casos sin diabetes (0) y con diabetes (1) y sus respectivos intervalos de confianza al 95 %.

Posteriormente, se modelaron las variables cuantitativas continuas mediante una simulación utilizando el paquete `simPH` de `R`, para visualizar las modificaciones diná-

micas en los factores de riesgo con incrementos en las variables estudiadas. Con base en esto, se confirmaron los puntos de corte para las diferentes variables estudiadas (**Figura 4.3**). Se modeló nuevamente la regresión de Cox utilizando los puntos de corte para las variables cuantitativas continuas en 2 o 3 categorías, hasta maximizar el estadístico-c de Harrel y el criterio de información Bayesiano (BIC, **Tabla 2**). Se observó tan sólo un sutil descenso en la concordancia del modelo (0.728 a 0.711) con una disminución del BIC (ΔBIC 1116.24 a 1137.97) cuando se cambia el modelo continuo por el categórico, lo que permite determinar que el cálculo optimiza la predicción sin perder la información de riesgo estimada por el modelo.

Modelo de riesgos proporcionales de Cox dicotómico				
Coeficientes	$\hat{\beta}$	Error estándar	HR (IC95 %)	p-value
Sexo femenino	-0.5695	0.2516	0.56 (0.34-0.92)	0.0236
IMLG bajo	0.6723	0.2460	1.96 (1.21-3.17)	0.0063
METS-VF	0.8636	0.2810	2.37 (1.37-4.11)	0.0021
EVC	1.0460	0.3356	2.85 (1.47-5.49)	0.0018
Actividad física	-0.4575	0.1807	0.63 (0.44-0.90)	0.0113
APOE-ϵ4	0.5938	0.2333	1.81 (1.15-2.86)	0.0109
Escolaridad ($\geq$ 9 años)	-0.1429	0.0611	0.65 ()	0.0193
Discapacidad motora	0.5163	0.2190	1.67 (1.09-2.57)	0.0184
BIC= 1137.975	$R^2 =$ 0.084	$D_{xy} = 0.421$	c-statistic=0.711	p<0.0001

Tabla 2: Modelo de riesgos proporcionales de Cox para riesgo de demencia en DM2 utilizando codificación categórica de las variables predictoras ajustado por edad, escolaridad, sexo y años de diagnóstico de DM2.

La validación cruzada por bootstrap y validación k-veces del modelo categórico preservó la concordancia y confirmó la capacidad predictiva del modelo con la disminución del optimismo predictivo ($D_{xy} = 0.3681, R^2 = 0.0829$). El modelo identificado reconcilia algunos conceptos fisiopatológicos y funcionales que podrían alterarse en DM2 y establece un puntaje que podría facilitar la predicción de riesgo. Finalmente, los coeficientes $\hat{\beta}$ del modelo final (**Tabla 2**) pueden ser de utilidad como un estimador del riesgo asociado a demencia de acuerdo con la presencia de los diferentes factores de riesgo, sobre todo en la aplicación del modelo categórico.

Capítulo 5

Discusión, conclusiones y perspectivas

Discusión y conclusiones

En el presente trabajo, se generaron modelos predictivos para el estudio de diferentes desenlaces vinculados en estudios preclínicos y de intervención al deterioro de la función cognitiva y riesgo metabólico en DM2, en particular resistencia a la insulina y adiposidad visceral. Una de las principales interrogantes se enfocó además en la investigación del impacto de la diabetes en la funcionalidad en el contexto del riesgo de demencia en diabetes. En este sentido, se demostró que el score de riesgo propuesto por Exalto et al., el cual acumula factores de riesgo relacionados a la diabetes para la estimación del riesgo de demencia, identifica un fenotipo caracterizado por fragilidad, deterioro funcional, discapacidad y deterioro cognitivo, identificando potenciales fenotipos factibles para una intervención geriátrica temprana enfocadas a disminuir la carga de morbilidad asociada. Finalmente, se identificaron diferentes factores de riesgo metabólicos, funcionales y de comorbilides asociadas a la DM2 que podría ofrecer un tamizaje adicional para pacientes adultos mayores con DM2, en quienes el control glucémico a largo plazo no ha demostrado beneficios notables en la función cognitiva (28). Los factores de riesgo para demencia identificados en el presente trabajo son en su mayoría novedosos y no podrían haber sido explorados sin los indicadores desarrollados en el contexto del trabajo doctoral.

Los hallazgos presentados identifican también factores de riesgo que podrían ser dianas potenciales de intervención para aminorar el riesgo metabólico. La asociación identificada con resistencia a la insulina en la cohorte de Bordeaux en 3C no preservó significancia al introducir la obesidad visceral, en parte porque el score METS-VF requiere evaluación de METS-IR(37). Sin embargo, la resistencia a la insulina puede devenir en acumulación de grasa visceral y empeorar éste fenómeno por lo que la asociación con obesidad visceral podría ser una combinación de alteraciones metabólicas igualmente asociadas a resistencia a la insulina. Esto es consistente con hallazgos de algunos estudios han sugerido un potencial papel benéfico del tratamiento con sensibilizadores de la acción de la insulina como metformina y agonistas de PPAR-γ (51; 52). También indicaría un potencial benéfico de intervenciones encaminadas a

reducir la acumulación de tejido adiposo visceral.
A pesar de que el IMC bajo y la pérdida de peso acelerada se han asociado con un mayor riesgo de demencia en población general, el enfoque del presente estudio permitió observar la función predictiva de una estimación más precisa y diferencial de elementos de la composición corporal (36). Un estudio reciente realizado en Corea del Sur demostró que la asociación entre cambios en IMC y el riesgo de demencia tiene una relación en U; mientras que la pérdida de peso se asocia con un mayor riesgo, la ganancia ponderal también se asocia con mayor riesgo en pacientes con reciente diagnóstico de DM2(53). En línea con estos resultados, la presente investigación demostró que, mientras un IMLG bajo era predictor de un mayor riesgo de demencia en diabetes, la obesidad visceral es un factor de riesgo. Podría postularse que la pérdida acelerada de peso podría darse sobre todo a expensas de masa magra y/o muscular, induciendo un estado funcional adverso que podría devenir en discapacidad, fragilidad y alteraciones funcionales y estructurales en el SNC (55); esto particularmente importante en DM2 debido a la pérdida de masa muscular relacionada con la enfermedad (56). En cambio, durante la ganancia ponderal se presenta una acumulación desproporcionada de grasa visceral conforme aumenta la proporción de tejido adiposo total(57). Debido a que los pacientes con DM2 tienen una mayor acumulación de tejido adiposo visceral(56), la ganancia ponderal en estos pacientes modificaría la distribución de tejido adiposo hacia un fenotipo de obesidad visceral, el cual es un predictor de demencia incidente en nuestro estudio.
Nuestros resultados sugieren además el efecto protector de la actividad física sobre el riesgo de demencia en DM2. Un estudio previo había demostrado que el riesgo de demencia asociado a portadores de APOE-ϵ4 en DM2 se modificaba en pacientes con DM2 que hacían actividad física(58). Evidencias experimentales demuestran además que la actividad física mejora la sensibilidad a la insulina en el SNC en condiciones de aumento de peso asociado a dietas hipercalóricas; por lo tanto, una potencial terapia para reducción de riesgo podría ser la prescripción oportuna de actividad física con el objetivo de aumentar masa muscular y reducir la acumulación de tejido adiposo visceral (59). Esto también sugiere el importante papel de la rehabilitación en disminuir la carga asociada a discapacidad motora, la cual también resultó predictora de demencia en el presente estudio. Estudios basados en población han demostrado que las intervenciones multidiscplinarias ofrecen beneficios cognitivos, sobre todo posterior a eventos de alto riesgo como un evento vascular cerebral donde la rehabilitación ha demostrado disminuir el riesgo de demencia de tipo vascular. El presente modelo de riesgo identifica puntos de atención diferentes a los exclusivamente relacionados con control glucémico y complicaciones microvasculares en pacientes con DM2 y podría servir como punto de partida en combinación con el score de Exalto et al, el cual de igual manera identifica fenotipos con deterioro funcional en alto riesgo de demencia.

Perspectivas

Un estudio reciente en población Sueca, confirmado en población inglesa y alemana, sugiere la existencia de distintos patrones fenotípicos de diabetes que tienen además perfiles de respuesta a tratamiento y riesgo de complicaciones micro y ma-

crovasculares distintivos (4). Los *clusters* de diabetes podrían ser además factores predictores de deterioro funcional, cognitivo y con riesgos diferenciales en el riesgo de demencia. La asociación de obesidad visceral y resistencia a la insulina observados en el presente estudio podría sugerir que los fenotipos de diabetes relacionados a la resistencia a la insulina y con deficiencia de insulina podrían incurrir en un mayor riesgo de deterioro funcional y cognitivo, mientras que los fenotipos más leves relacionados a la edad y a la obesidad (en quienes predomina la acumulación de tejido adiposo subcutáneo) podrían tener un perfil de riesgo más bajo, independientemente del tiempo de exposición a DM2 que en nuestro estudio no jugó un papel determinante.

Durante la última parte de mi trabajo doctoral trabajé en simplificar la implementación de estos subtipos clínicos de diabetes utilizando algoritmos de inteligencia artificial con redes neuronales auto-normalizables; a pesar de haber alcanzado un avance significativo, este proyecto aún se encuentra en curso. La implementación de estas variantes fenotípicas de diabetes podría resultar de utilidad predictiva como un primer paso para la identificación de perfiles de riesgo que, aunado a factores adicionales propuestos para demencia en diabetes, podría mejorar el tamizaje preventivo y la identificación de sujetos en alto riesgo de demencia. Sería de mucho interés conocer el riesgo de demencia en estos subtipos de diabetes y saber si algunos factores de riesgo son también específicos a los subtipos. La especificación de patrones clínicos específicos es un paso en la dirección correcta para el fortalecimiento de la medicina personalizada en diabetes y, sobre todo, en el adulto mayor.

Capítulo 6

Otros manuscritos

A continuación, se presenta una lista de manuscritos que se publicaron durante el desarrollo del presente trabajo de investigación. Estos trabajos complementan algunos de los conceptos explorados en otros manuscritos además de explorar técnicas de análisis de información que permitieron el desarrollo del proyecyo doctoral.

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ORIGINAL PAPER

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Prediction of incident hypertension and arterial stiffness using the non–insulin-based metabolic score for insulin resistance (METS-IR) index

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Abstract

Hypertension is associated with insulin resistance (IR), metabolic syndrome (MS), and arterial stiffness. Non–insulin-based IR indexes were developed as tools for metabolic screening. Here, we aimed to evaluate the novel non–insulin-based Metabolic Score for IR (METS-IR) index for the prediction of incident hypertension and arterial stiffness evaluated using pulse wave velocity (PWV) analysis, compared with other non–insulin-based IR indexes. We evaluated two populations, a cross-sectional evaluation of high-risk individuals (n = 305) with a wide range of metabolic comorbidities

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and dyslipidemia in whom PWV measurement was performed and a 3-year prospective cohort of normotensive individuals (N = 6850). We observed a positive correlation between METS-IR and PWV in the cross-sectional cohort, which was higher compared with other non–insulin-based fasting IR indexes; furthermore, PWV values >75th percentile were associated with the upper tercile of METS-IR values. In the prospective cohort, we observed an increased risk for incident hypertension for the upper METS-IR tercile (METS-IR ≥ 46.42; HR: 1.81, 95% CI: 1.41-2.34), adjusted for known cardiovascular risk factors, and observed that METS-IR had greater increases in the predictive capacity for hypertension along with SBP and the Framingham Hypertension Risk Prediction Model compared with other non–insulin-based IR indexes. Therefore, METS-IR is a novel non–insulin-based IR index which correlates with arterial stiffness and is a predictor of incident hypertension, complementary to previously validated risk prediction models.

1 | INTRODUCTION

Clinical diagnosis of insulin resistance (IR) is useful for assessment of type 2 diabetes (T2D) risk, ectopic fat accumulation, visceral adiposity, and cardiovascular risk.[1] However, precise evaluation of IR requires one-stage euglycemic-hyperinsulinemic clamp (EHC), a method which is invasive, costly, and requires hospitalization. Therefore, surrogate insulin-based IR markers have been developed as predictors of IR and been proven predictive of cardiovascular disease (CVD) risk.[2,3] A limitation of such indexes is the required measurement of insulin, which has a high cost and variability depending on the utilized technique.[4] Recently, there has been crescent interest in developing non–insulin-based IR indexes including the TyG index and TG/HDL-C ratio. Components of such indices, including fasting glucose, triglycerides, and HDL-C, have been shown predictive of hypertension and CV risk in prospective studies.[5-7] A significant contribution of hypertension and CVD risk is explained by arterial stiffness, which implies degeneration of elastin fibers and deposition of collagen in arterial walls, inducing structural and functional modifications in the arterial wall.[8] The TyG index and the TG/HDL-C ratio have proven strong and consistent associations with hypertension, CVD risk, and arterial stiffness in several populations, suggesting a potential role for IR assessment in identifying arterial stiffness using non–insulin-based IR surrogates.[9,10]

The recently developed Metabolic Score for IR (METS-IR) offers higher concordance with EHC compared with other non–insulin-based IR indexes; furthermore, METS-IR includes evaluation of body mass index (BMI), which has shown strong predictive capacity for CVD risk.[11,12] Overall, METS-IR evaluates similar components to the metabolic syndrome (MS), which has been associated with age-related structural and functional changes in arteries and increased intima-media thickness, which confers an increased risk of hypertension and CVD.[13] Here, we aimed to investigate the correlation of METS-IR with pulse wave velocity (PWV) and other vascular health surrogates from PWV analysis. We also assessed the capacity of METS-IR to predict incident hypertension and its complementary role for the prediction of hypertension along with blood pressure levels and risk prediction models.

2 | METHODS

2.1 | Cross-sectional cohort

We evaluated subjects with high-cardiovascular risk conditions including obesity (BMI > 30kg/m^2), carbohydrate intolerance or prediabetes (2-hour glucose challenge ≥140 mg/dL but <200 mg/dL), and primary dyslipidemias including familial hypercholesterolemia and familial hypertriglyceridemia. Participants were instructed to not consume caffeinated beverages refrain from smoking ≤48 hours before evaluation. Upon evaluation, subjects were placed in a supine position for 10 minutes, and baseline supine brachial artery blood pressure (BP) and heart rate (HR) were recorded using a semiautomated cuff-based device (SphygmoCor XCEL, AtCor Medical Pty Ltd, USA). PWV measurements were taken after achieving hemodynamic stability, defined as two readings within systolic BP (SBP) of ±9 mm Hg, diastolic BP (DBP) ±6 mm Hg and HR ±8 beats/min. To assess PWV, carotid pulse waves were measured by applanation tonometry and femoral pulse waves were simultaneously obtained by a partially inflated cuff over the femoral artery at the leg midway between hip and knee. PWV was determined by calculating the ratio of corrected distance between pulse measuring sites to time delay between carotid and femoral pulse waves. Distance was measured with a non-stretchable tape from the suprasternal notch to the carotid site, from the femoral artery at the inguinal ligament to the proximal edge of the thigh cuff from the suprasternal notch to the proximal edge of the thigh cuff. Distances 1 and 2 were subtracted from distance 3 and used in the calculation of PWV.

2.2 | Metabolic syndrome cohort

The prospective MS cohort was developed to evaluate risk of MS components in incident T2D, hypertension, and cardiovascular mortality in an urban population living in 9 different cities in Mexico.[14] Inclusion criteria considered subjects aged 25-69 years, BMI ≥23 kg/m^2, without T2D, hypertension or other significant cardiovascular comorbidities, and obese individuals (BMI ≥ 30 kg/m^2) with at least one of the following conditions: BP ≥140/90 mm Hg, fasting glucose >100 mg/dL, total cholesterol >200 mg/dL, and triglyceride levels >150 mg/dL. Individuals with diagnosed T2D, coronary artery disease, cerebral vascular disease, alcoholism, taking corticosteroids, with liver disease, kidney dysfunction, or life-threatening diseases that would prevent follow-up were excluded. Subjects were interviewed to obtain medical history, sociodemographic information, dietary and physical activity habits, and anthropometric measurements. BP measurement was also performed using a manual sphygmomanometer after subjects remained seated ≥5 minutes and refrained from consuming caffeine before measurements. We obtained a 20mL blood sample after 9- to 12-hour fast for biochemical measures. These same evaluations were carried out after ≥2 years follow-up. Incident hypertension was defined as a construct of previous medical diagnosis of hypertension, taking antihypertensive medication and/or blood BP at levels consistent with any-degree of hypertension according to current ESC/ESH guidelines. Time to follow-up was estimated from recruitment up to last follow-up or hypertension diagnosis, whichever occurred first. We also used the Framingham Hypertension Risk Prediction Model to estimate the risk of incident hypertension.[15]

2.3 | Biochemical and anthropometric evaluations

In both evaluated cohorts, we obtained from all subjects a complete medical and family history, including use of medications. Subjects were weighed on calibrated scales, and height was determined with a floor scale stadiometer; BMI was calculated as weight in kg divided by the squared product of height in meters. Blood was obtained between 08:00 and 09:00 hours after 8- to 12-hour fast. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments Co.), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2), and A1c levels were measured by using high-performance liquid chromatography (HPLC) (Variant II Turbo, BIORAD). Lipid concentrations (cholesterol, triglycerides, and HDL cholesterol), apo A, apo B, uric acid, creatinine, and hepatic enzymes were measured using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). LDL-cholesterol was calculated with the Friedewald equation when triglycerides were <250 mg/dL. METS-IR was calculated using the formula $(LN((2*G_0) + TG_0))*BMI/(LN(HDL\text{-}C))$, where G_0 and TG_0 were fasting glucose and triglycerides, respectively.

2.4 | Statistical analysis

To evaluate inter-group differences, we used Student's *t* test and Mann-Whitney *U* test, where appropriate. Frequency distribution of categorical variables is reported as frequencies and percentages and was compared between groups using chi-squared tests. For measurements in follow-up studies, we used Student's paired *t* test and Wilcoxon's rank-sign tests, where appropriate. Logarithmic and inverse transformations were applied to approximate normality in variables showing nonparametric distribution. Data are presented as mean ± SD or as median and interquartile range.

2.4.1 | Prediction of incident hypertension using METS-IR

To evaluate the association of METS-IR and incident hypertension in the MS cohort, we performed survival analysis comparing across METS-IR terciles, quartiles, and cutoff value ≥50, using Kaplan-Meier curves compared with log-rank tests. Cox proportional risk regression analyses were used to evaluate the risk of incident hypertension across METS-IR percentiles, adjusted for age, sex, cholesterol levels (TC), waist circumference (WC), SBP, DBP, and smoking status. To evaluate increases in predictive capacity for hypertension risk using METS-IR, we estimated the Framingham Hypertension Risk Prediction Model and assessed predictive improvements with an omnibus test of model coefficients for changes across predictive models (X^2) and changes in *c*-statistic.

2.4.2 | Correlation of METS-IR with PWV and BP

In our cross-sectional PWV cohort, we tested METS-IR scores using trend analysis and linear regression against quartiles of PWV, SBP, and DBP, adjusted for age, sex, and smoking status. Finally, we evaluated whether METS-IR would predict PWV values >75th percentiles for this population adjusted for age, sex, smoking, and hypertension. Model diagnostics were conducted using the Hosmer-Lemeshow test. Statistical analyses were performed using R software version 3.4.3, Statistical Package for Social Science (SPSS) version 21.0 and GraphPad Prism, version 7.0.

3 | RESULTS

3.1 | Correlation between METS-IR values, arterial stiffness, and PWV

In the PWV cohort, we included 305 subjects, predominantly female (68.9%), with mean age of 49.86 ± 13.09, BMI of 29.01 ± 5.80, and SBP and DBP of 122.9 ± 15.19 and 72.47 ± 9.61, respectively. One hundred and sixteen subjects had prediabetes (38.0%), 75 had familial hypercholesterolemia under statin therapy (24.6%), 64 moderate-to-severe hypertriglyceridemia (21.0%), and 50 were metabolically healthy (16.4%); ninety were active smokers (29.5%), and 57 had treatment for

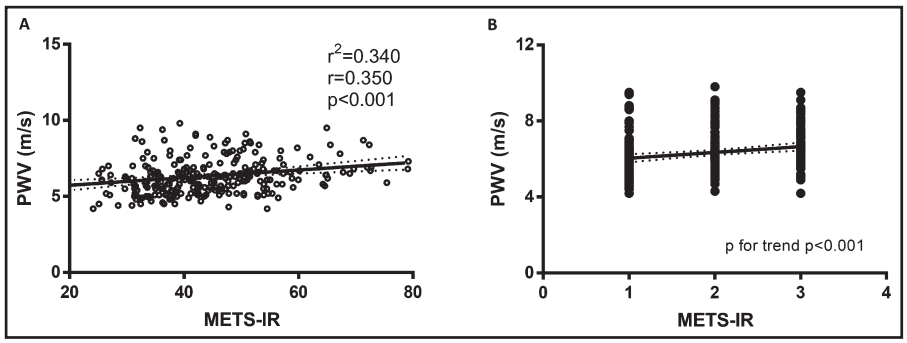

FIGURE 1 Correlation and linear regression between METS-IR and pulse wave velocity (A) and trend analyses for increasing METS-IR terciles (B) adjusted for age, sex, treatment for hypertension, and waist circumference

TABLE 1 Correlations between non–insulin-based IR indexes and PWV, SPB, and DBP. Age, sex, hypertension treatment, and smoking status were considered in the adjusted value

Index	METS-IR (ρ, 95% CI)		TG/HDL-C index (ρ, 95% CI)		TyG index (ρ, 95% CI)	
Parameter	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
PWV	0.253* (0.135-0.366)	0.350* (0.204-0.418)	0.301* (0.199-0.396)	0.289* (0.197-0.382)	0.260* (0.147-0.361)	0.238* (0.137-0.382)
Central SBP	0.219* (0.124-0.318)	0.263* (0.163-0.360)	0.062 (−0.034 to 0.161)	0.114* (0.000-0.212)	0.064 (0.029-0.167)	0.079 (−0.016 to 0.170)
Peripheral SBP	0.219* (0.120-0.315)	0.267* (0.169-0.373)	0.044 (−0.043 to 0.145)	0.076 (−0.037 to 0.181)	0.041 (−0.062 to 0.159)	0.062 (−0.035 to 0.161)
Peripheral DBP	0.316* (0.226-0.412)	0.309* (0.225-0.397)	0.152* (0.067-0.250)	0.138* (0.036-0.247)	0.161* (0.067-0.271)	0.133* (0.044-0.227)

Abbreviation: DPB, diastolic blood pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.
*$P < 0.05$.

hypertension (18.7%). Their biochemical values included the following: median fasting glucose of 94.0 mg/dL (IQR: 86-104), fasting triglycerides of 130 mg/dL (IQR: 92.0-180.5), total cholesterol of 206 mg/dL (IQR: 173-247), and HDL-C of 44 mg/dL (IQR: 38-54).

We observed a significant correlation between METS-IR and PWV that increased after adjustments for age, sex, and smoking; we also observed a trend of increasing PWV, SBP, and DBP values with increasing METS-IR terciles (Figure 1). Using linear regression, we observed that METS-IR predicts 34.0% of the variability in PWV measures (β = 0.290, $P < 0.001$), adjusted for sex, age, treatment for hypertension, and smoking status. When evaluating PWV >75th percentile using multiple logistic regression analyses, we observed an association with both METS-IR scores (OR 1.03 95% CI 1.01-1.06) and the upper METS-IR tercile (OR 2.49 95% CI 1.19-5.23) adjusted for age, sex, and smoking. Finally, we contrasted those observations evaluating the same parameters against the TG/HDL and TyG indexes and observed that METS-IR had the highest correlation compared with other indexes even after adjustment (Table 1).

3.2 | Prediction of incident hypertension using METS-IR in the MS cohort

For prospective evaluation, we included 6850 normotensive subjects from the MS cohort at baseline, from which 3974 subjects completed follow-up. We observed 592 cases of incident hypertension over 9549 accumulated persons-years, yielding an incidence rate of 61.99 cases per 1000 person-years or 14.9% in an average of 2.4 years of follow-up. Subjects who developed hypertension were older, had higher fasting glucose, insulin, LDL-C, apolipoprotein B and BMI, and lower HDL-C at baseline and follow-up (Table S1). Individuals who developed hypertension had significantly higher METS-IR scores at baseline and after follow-up in comparison with those who did not ($P < 0.001$). Both groups had an increase in METS-IR scores between visits, which remained larger in subjects who developed hypertension. We observed a low but significant correlation between METS-IR and baseline SBP (ρ = 0.095) and DBP (ρ = 0.056) adjusted for age, sex, smoking status, WC, TC, and family history of hypertension. The correlation was higher for baseline METS-IR and follow-up SBP (ρ = 0.138) and DBP (ρ = 0.126) and was particularly stronger for individuals with incident hypertension (ρ = 0.180 and ρ = 0.139, respectively).

We observed the highest rate of incident hypertension for the upper METS-IR tercile compared with lower terciles (log-rank test $P < 0.001$). This observation was confirmed in Cox proportional risk regression analysis, which showed progressively higher risk of incident hypertension for the upper (METS-IR ≥ 46.42) and middle METS-IR terciles (39.15 ≤ METS-IR < 46.42) in comparison with the lowest, adjusted for age, sex, smoking status, TC, WC, and family history of hypertension (Figure 2A, Table 2). Given the known role of diabetes and dysglycemia in increasing arterial stiffness and

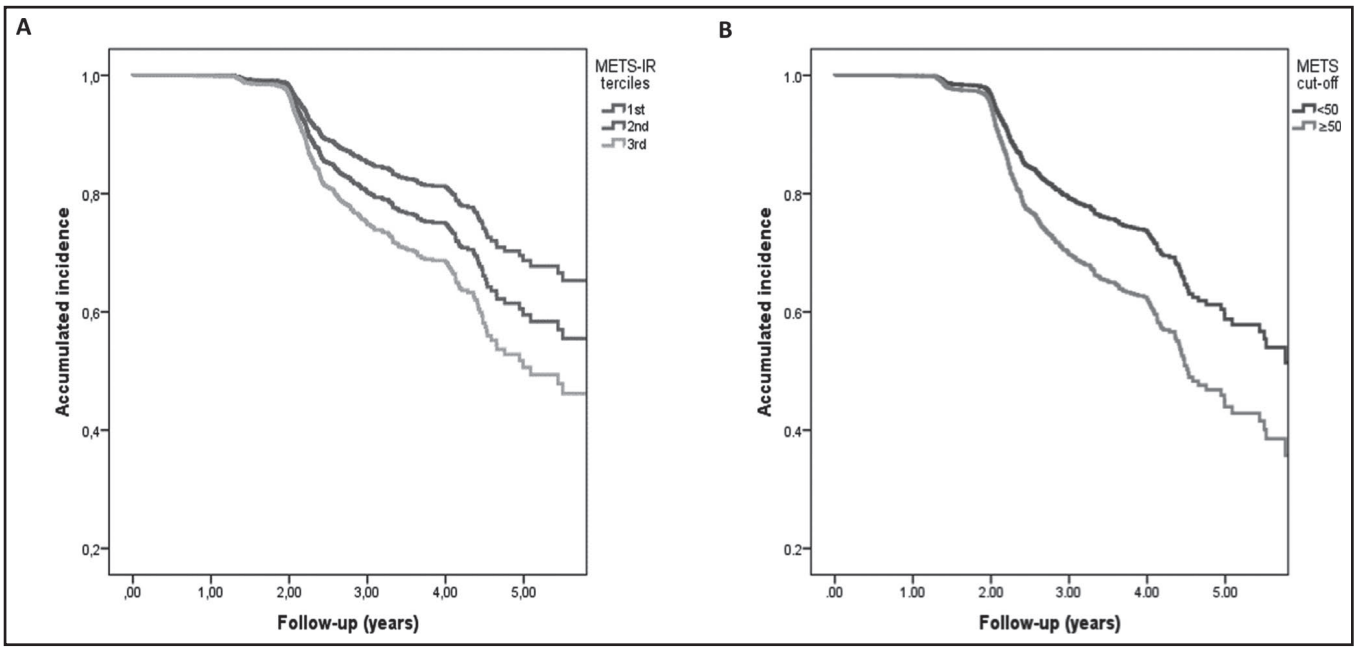

FIGURE 2 Incidence of hypertension comparing across METS-IR terciles (A) and divided by the METS-IR cutoff point (≥50, B), adjusted for age, sex, smoking status, cholesterol at baseline, abdominal circumference, and family history of hypertension

TABLE 2 Cox proportional hazard regression for risk of incident hypertension defined by ESC/ESH guidelines across METS-IR terciles and quartiles, adjusted for age, sex, smoking status, systolic and diastolic blood pressure, family history of hypertension, waist circumference, and total cholesterol

Model	METS-IR Percentile	β	Wald	HR	*P*-value	95% CI
1	Q2	0.073	0.271	1.076	0.603	0.816-1.419
	Q3	0.332	5.554	1.393	0.018	1.057-1.385
	Q4	0.593	15.304	1.810	<0.001	1.344-2.436
2	T2	0.296	6.939	1.345	0.008	1.079-1.677
	T3	0.554	18.172	1.740	<0.001	1.329-2.245

hypertension risk, we performed further adjustments by baseline hyperglycemia (fasting glucose ≥ 100mg/dL), which did not modify the strength of the association for the upper (HR 1.312 95% CI 1.060-1.624) or middle terciles (HR 1.817 95% CI 1.428-2.311). We also observed that subjects with METS-IR ≥50 had a higher adjusted risk to develop hypertension (HR: 1.44, 95% CI 1.17-1.78, Figure 2B). Next, we assessed which of the components of the score provided better predictive capacity for incident hypertension; the higher predictive accuracy was driven by BMI (HR 1.014 95% CI 1.007-1.021) and glucose (HR 1.043 95% CI 1.022-1.064). When comparing the information provided by the sum of the individual components of METS-IR compared to METS-IR alone, using METS-IR had a larger decrease using Akaike's information criterion (AIC) compared with individual components (9381.72 vs 9465.081). When assessing the insulin-based HOMA-IR index, it also proved predictive of hypertension (HR 1.067 95% CI 1.028-1.107) adjusted for age, sex, family history of hypertension, smoking, and blood pressure levels, but both the *c*-statistic (*c*-statistic = 0.635 vs 0.633) and the AIC (9381.72 vs 9487.50) demonstrated better predictive performance for METS-IR.

Finally, we evaluated the complementary use of METS-IR to predict hypertension in comparison with BP levels and the previously validated Framingham Hypertension Risk Prediction Model. When we included METS-IR at baseline along with SBP levels, we observed a significant change in predictive capacity for incident hypertension (X^2 = 8.74, *P* = 0.003); furthermore, we observed a significant increase when including METS-IR at baseline along with the Framingham Hypertension Risk Prediction Model (X^2 = 10.70, *P* = <0.001). The changes in *c*-statistic (AUC) were also superior for the combination of METS-IR and Framingham or SBP compared with either alone, and METS-IR had superior combined predictive performance in comparison with other non–insulin-based IR indexes and compared to the insulin-based HOMA-IR index as assessed by the AIC (Table 3).

4 | DISCUSSION

Metabolic Score for IR is a useful tool to identify cases with increased risk of incident arterial hypertension and arterial stiffness. This finding is in agreement with the well-known contribution of IR to the pathogenesis of atherogenesis, vascular changes, and hypertension.[16] First, we observed a linear correlation between METS-IR and

TABLE 3 Predictive performance of combined regression models comprising non–insulin-based fasting insulin resistance indexes complementary to Framingham hypertension risk equation in prediction of incident hypertension using *c*-statistics

	IR index + Hypertension risk score	*c-statistic*	AIC
METS-IR	Index	0.579	8695.89
	Index + BP	0.599	8674.47
	Index + Framingham	0.643	8584.60
TyG	Index	0.530	8828.20
	Index + BP	0.573	8805.33
	Index + Framingham	0.640	8695.84
TG/HDL-C	Index	0.518	8838.24
	Index + BP	0.594	8812.70
	Index + Framingham	0.643	8699.18
HOMA-IR	Index	0.571	8813.83
	Index + BP	0.596	8789.66
	Index + Framingham	0.647	8685.43

Abbreviations: AIC, Akaike's information criterion; HOMA-IR, Homeostasis model assessment for insulin resistance; METS-IR, Metabolic Score for Insulin Resistance; TG/HDL-C, triglyceride-high-density lipoprotein cholesterol; TyG, triglycerides-glucose product.

PW along with BP measurements in a cohort of high-risk individuals. METS-IR was also shown to be predictive of incident arterial hypertension, and we observed that the correlation between METS-IR and BP measures is higher in subjects with hypertension. Furthermore, METS-IR increased the predictive capacity for incident hypertension when combined with SBP/DBP and the Framingham Hypertension Risk Prediction Model and was superior to other previously validated non–insulin-based IR measures.

The correlation between METS-IR, arterial stiffness, and incident hypertension is supported by pathophysiological evidence. The most accepted hypothesis linking IR and arterial hypertension includes overstimulation of the sympathetic nervous system, increasing peripheral vascular resistance, and cardiac output leading to increases in systemic BP.[17] Decreased insulin action, glucotoxicity, and MS stimulate activity of the renin-angiotensin-aldosterone system, increasing tubular Na^+ reabsorption leading to volume expansion and BP changes.[18] Impaired insulin signaling also causes endothelial dysfunction and a decrease in activity of nitric oxide synthase, leading to systemic vasoconstriction.[19] Visceral adiposity and ectopic fat accumulation have also been shown to be predicted by METS-IR and are recognized risk factors for the development of CV disease and hypertension[20]; adjustments of the observed association for surrogates of abdominal obesity did not attenuate the observed and confirm an independent role for METS-IR in its prediction.

When we included METS-IR along with the Framingham Hypertension Risk Prediction Model, we observed significant increases in predictive capacity for incident hypertension. The Framingham Hypertension Risk Prediction Model has been validated in several population cohorts, and its predictive capacity for incident hypertension, morphological heart changes, and altered vascular function has not been shown to be superior to SBP alone.[21,22] Since components of the metabolic syndrome and IR also modify the prediction of incident hypertension, it is expected that METS-IR evaluation would be helpful to predict short-term hypertension risk.[23] Overall, this confirms that prediction of hypertension risk using METS-IR could be explained by the increased cardiovascular risk associated with both IR and MS. Individual components of the METS-IR score have also been linked independently to incident hypertension, including triglycerides and HDL-C as well as BMI as a marker of whole-body fat content.[24,25] As demonstrated by our results, besides BMI, glucose levels are also highly predictive of arterial stiffness and incident arterial hypertension; despite this, the better model assessed by decreased in AIC was comprised by METS-IR and not by its individual components. This is significant, since it confirms that METS-IR is useful as a complementary metabolic evaluation tool when assessing risk of arterial hypertension.

The relationship between increased risk of incident hypertension and vascular health explained by METS-IR is further strengthened by our observation of increased correlation with PWV. PWV is a surrogate marker of arterial stiffness, which has also been previously associated with HOMA-IR, the TyG index, and TG/HDL-C ratio[26,27]; in our study, we were able to demonstrate a superior correlation using PWV analysis for METS-IR in comparison with other non–insulin-based IR indexes in a cohort of high-risk individuals. The relevance of evaluating this association was demonstrated in a previous study, which showed that whereas endothelial function increases with or without IR, arterial stiffness increases only in relation to IR, especially in individuals with family history of T2D.[28] The mechanisms underlying this association are related to IR and hyperglycemia, which lead to nonenzymatic glycation of matrix proteins causing subendothelial accumulation of advanced end glycation products and arterial stiffening, leading to altered vessel hemodynamics.[29] The inclusion of individuals at high-cardiovascular risk allows us to extrapolate results to high-risk populations beyond hyperglycemia, but since statin therapy is known to have rheological impacts and reduce PWV,[30] additional evaluations of the predictive capacity of MET-IR for vascular health in untreated populations are warranted. In other populations, PWV has been shown to be a predictor of incident cardiovascular events and arterial calcification, which indicates that METS-IR could be a potential predictor of both and should be evaluated in future studies.[31] Although PWV is not a routine evaluation in primary care, our results show that METS-IR might be treated as subrogate of arterial stiffness and a predictor of incident hypertension.

Our study had some strengths and limitations. First, we evaluated a large cohort of normotensive but at-risk individuals, which allowed power for predictive modeling of incident hypertension and represents an adequate setting for validating the role of METS-IR to predict incident hypertension. We also had and noninvasive surrogate to assess arterial stiffness which provides pathophysiological evidence to complement our epidemiological observations. Limitations to be

acknowledged are the inclusion of high-risk subjects in the PWV cohort, which limits extrapolation of such results to the general population; furthermore, the sample of subjects included in the MS cohort was already at a higher risk compared with general population, which calls for further studies in lower risk populations to assess the utility of METS-IR in this subset of patients. Finally, all analyses were controlled for age, sex, and cardiovascular risk factors and there remains a possibility of residual confounding, particularly since the association between arterial stiffness and BP values is modified by age.[32]

In conclusion, METS-IR is a novel non–insulin-based IR index which predicts incident hypertension and its complementary risk prediction with SBP and the Framingham Hypertension Risk Prediction Model is stronger compared with other non–insulin-based IR indexes and HOMA-IR. Furthermore, METS-IR is correlated with PWV, SBP, and DBP in high-risk patients and is predictive of arterial stiffness. METS-IR can be used to evaluate cardio-metabolic risk complementary to routine evaluation and identify subjects at an increased risk of hypertension, which makes it useful in primary care practice as a screening tool to evaluate metabolic heath in at-risk individuals.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

OYBC, NEAV, AVV, AJM and CAAS: developed the conceptualization of the study. AJM, RM, OAC, DGV, PAV, MAMH, ICB, LMH, LEG, JJGG, UA, YOY, RCR, LSR, MEGS, JMMH, LMGR, FJGP, RR, and MTTL: conducted the studies in the different cohort centers, recruited patients, and processed biological samples. OYBC, NEAV, AVV, and CAAS: performed and interpreted statistical analyses and developed predictive models. OYBC, NEAV, and CAAS: also participated in manuscript drafting and processing. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final version of this manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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ORIGINAL ARTICLE

Bloodstream infection caused by *S. aureus* in patients with cancer: a 10-year longitudinal single-center study

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Abstract

Background *Staphylococcus aureus* bloodstream infections (SABIs) represent a significant cause of morbidity and mortality in cancer patients. In this study, we compared infection characteristics and evaluated epidemiology and risk factors associated to SABIs and 30-day attributable mortality in cancer patients.

Methods Clinical and microbiological data from patients with cancer and positive blood cultures for *S. aureus* were retrieved during a 10-year period at an oncology reference center. Analyses were performed according to type of malignancy and infection with methicillin-resistant *S. aureus* (MRSA). Data was evaluated using competing risk analyses to identify risk factors associated to 30-day mortality and used to create a point system for mortality risk stratification.

Results We included 450 patients and MRSA was documented in 21.1%. Hospital-acquired infection, healthcare-associated pneumonia, and type-2 diabetes were associated to MRSA. In patients with hematologic malignancies, MRSA was more frequent if hospital-acquired, but less likely in primary bacteremia. Variables associated to mortality included abdominal source of infection, hematologic malignancy, MRSA, glucose levels > 140 mg/dL, and infectious endocarditis; catheter removal and initiation of adequate treatment within 48 h of positive blood culture were protective factors. From our designed mortality prediction scale, patients with a score > 3 had a 70.23% (95%CI 47.2–85.3%) probability of infection-related death at 30 days.

Conclusion SABIs are a significant health burden for cancer patients. Risk factors for SABI-related mortality in this population are varied and impose a challenge for management to improve patient's outcomes. Risk stratification might be useful to evaluate 30-day mortality risk.

Keywords *Staphylococcus aureus* bloodstream infection · Cancer complications · Hematologic malignancies · Infection-related mortality in cancer · MRSA

Omar Yaxmehen Bello-Chavolla and Jessica Paola Bahena-Lopez contributed equally to this work.

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Introduction

Staphylococcus aureus (*S. aureus*) is the second leading cause of bloodstream infections (BSIs) [1, 2] with a significant proportion of cases developing severe complications [3]. Mortality attributable to *S. aureus* bloodstream infection (SABI) within 30 days of infection has been estimated up to 30% in the general population [4]. Methicillin-resistant *S. aureus* (MRSA) infection and an increased number of comorbidities in cancer patients have been consistently associated to worse outcomes [4, 5].

The use of chemotherapy, either alone or in combination with radiotherapy, and/or surgery are common procedures used for the treatment of cancer, with increasing disease-free and overall survival in most neoplasias during the last two decades [6]. However, these therapeutic modalities may lead to healthcare-associated infections, increasing morbidity, mortality, and health-related costs [4–6].

SABIs in cancer patients are a significant cause of morbidity and mortality in both neutropenic and non-neutropenic patients [7, 8]. Known risk factors for mortality attributable to SABI in this population include hematologic malignancies, MRSA infection, inadequate empiric therapy, hospital-acquired infections, and septic shock, amongst others. Data and clinical course of SABI in hematologic patients have been well documented [9]; however, data on solid malignancies has not been extensively evaluated and studies that compare clinical characteristics and outcomes between these groups of patients are scarce. Here, using data collected from our BSI surveillance program, we studied BSIs caused by methicillin-sensitive *S. aureus (*MSSA) and MRSA in cancer patients to evaluate incidence, risk factors, clinical course, and 30-day mortality related to SABIs in this population.

Methods

Study population and setting

The Instituto Nacional de Cancerologia (INCan) is a 146-bed teaching hospital for adult patients with cancer. We screened all positive, non-duplicate, blood cultures for *S. aureus* from 2006 to 2015 amongst all positive blood cultures collected at INCan during the study period. Electronic medical records were reviewed for clinical, microbiological, and outcome-related information. Subjects who had either insufficient data or who were determined to have a positive blood culture due to contamination were excluded. Follow-up was comprised from the moment at which the BSI was diagnosed via blood culture up to 1 year to record SABI relapses, infectious and oncologic-related complications, and mortality.

Microbiological identification and susceptibility testing

Blood samples were cultured in BD BACTEC™ blood culture media and plated in blood, chocolate, and MacConkey agar for microbiological identification. Identification of isolates was performed with Microscan (Siemens Laboratory Diagnostics) from 2008 to 2010. In 2011, the automated equipment was changed to BD Phoenix (Becton, Dickinson and Co.). Since 2014, isolates have been processed utilizing matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Susceptibility to antimicrobial agents was determined according to current Clinical Laboratory Standards Institute (CLSI) criteria. Susceptibility tests were identified by means of an automated microbiology system (BD, Phoenix 100, USA).

Definition of studied variables and outcomes

BSI was defined as laboratory-confirmed isolation of *S. aureus* from blood samples classified as (A) Central Line-associated BSI (CLABSI) if time to positivity between blood cultures taken from central catheter was ≥ 2 h from that of the peripheral line, along with signs of systemic infection with no other apparent source, and/or catheter-tip culture positivity for the same organisms after catheter removal, and/or signs and symptoms of catheter entry-site infection with cultures showing the same strain isolated from the blood. (B) Secondary BSI was diagnosed when there was another source of infection with bloodstream seeding related to urinary tract, skin, soft tissue, or abdominal infection, pneumonia, or any other source. (C) Primary BSI occurred when no underlying infection was diagnosed despite intense clinical and radiological workout. (D) Persistent bacteremia was defined by the continuous presence of bacteria in the bloodstream > 48 h after administration of appropriate treatment. All bacteremias were further classified as hospital-acquired, if diagnosis occurred ≥ 48 h after patients' admission, or healthcare-associated, if patients were under chemotherapy, were using a central venous catheter (CVC), or had undergone an invasive procedure in the last 30 days.

We also evaluated 30-day SABI-related mortality and 6-month all-cause mortality, from the moment of microbiological confirmation to death. Additional outcome measures included *S. aureus*-related intensive care unit (ICU) admission; relapse of *S. aureus* bacteremia, defined as a second case of bacteremia during 1-year follow-up in a patient with proven microbiological recovery; and infectious endocarditis (IE), defined by Duke's modified criteria as either confirmed or possible IE [10]. Adequate treatment for *S. aureus* infection was defined as initiation of treatment with specific/targeted antimicrobial agents (e.g., vancomycin, dicloxacillin) within the first 48 h of the first positive blood culture [11].

Healthcare-associated pneumonia was defined as an episode of radiographically confirmed pneumonia acquired ≥ 48 h of hospital stay; pneumonia acquired before that time frame was defined as community-acquired. Abdominal source was considered as any infectious foci originated in the abdominal cavity, as abscess, cholangitis, peritonitis, or organ and space surgical infection; in hematologic patients, abdominal source also included neutropenic colitis. Severe neutropenia was defined as < 500 neutrophils/mm^3 in a blood specimen at the date of positive blood culture.

Statistical analysis

Demographic and laboratory data were described using mean and standard deviation (SD) or with median and interquartile range as appropriate; categorical variables were described using frequencies. Chi-squared, Student's *t*, and *U* of Mann-Whitney tests were used where appropriate. First, we fitted logistic regression analyses to develop an explanatory model for MRSA infections; variables were removed from the model until maximizing the adjusted r^2 value for the dependent variable in both the overall population and in patients with hematologic malignancies.

We evaluated mortality differences between MRSA and MSSA infections using survival analysis, calculating time until death at 30 days or censorship as outcome variables and using log-rank tests for comparisons in Kaplan-Meier curves. Cox proportional risk regression models were fitted to evaluate risk factors associated to 30-day SABI-attributable mortality, adjusted using the Charlson Comorbidity Index [12] to account for the effect of age and comorbidity. To adjust for competing risk of death related to non-infectious causes, a second model was fitted using semi-parametric proportional hazards regression as proposed by Fine and Gray et al. [13]. We used beta coefficients from competing risk models to construct a point system and aggregated the score to evaluate subjects at baseline for 30-day mortality according to stratified scores. Comparisons were evaluated using 95% confidence intervals (95%CI) and a p value < 0.05 was considered statistically significant. Statistical analyses were performed in SPSS software for Windows® (SPSS Inc., Chicago, IL, version 21.0), GraphPad Prism version 6.0, and R version 3.4.1 using *cmprsk* and *survival* packages [13].

Results

Study subjects

A total of 5155 positive blood cultures were recorded during the study period, of which 521 (10.1%) were positive for *S. aureus*. Fifty-nine subjects were removed because of incomplete data and 12 because of blood culture contamination. After conducting initial file reviews, 450 patients completed inclusion criteria and were included in the analysis (Fig. 1). BSIs were classified as CLABSI in 258 patients (57.3%), secondary bacteremia in 118 (26.2%), and primary bacteremia in 74 patients (16.4%). Persistent bacteremia was observed in 51 (11.3%). Most of these infections were detected on ambulatory patients and classified as healthcare-associated (N = 335). We classified 115 cases (25.6%) as hospital-acquired, with higher rates of MRSA (48.7%). Amongst the evaluated subjects, 135 (30.0%) had an hematological malignancy, 167 (37.1%) had breast cancer, 36 (8.0%) had gastrointestinal malignancies, 21 (4.7%) had testicular cancer, and 91 (20.2%) had tumors in other body locations.

Evaluation according to MSSA vs. MRSA status

We identified MSSA in 355 (78.8%) subjects; 95 (21.1%) had confirmed MRSA. MSSA BSIs decreased over time, without a significant reduction in *S. aureus*-related mortality; however, we found a significant decrease in 30-day mortality rate for MRSA over the 10-year period (Fig. 2). We observed no significant differences in age and sex between patients with MSSA and MRSA; hematologic malignancies were significantly more frequent in subjects with MRSA in comparison to MSSA (42.1 vs. 26.8%, p = 0.004), while subjects with MRSA had lower rate of solid malignancies (57.9 vs. 73.2%, p < 0.001, Table 1).

Patients with MRSA had a significantly higher frequency of diabetes mellitus (18.9 vs. 10.4%, p = 0.024); as expected, most cases of MRSA (58.9%) were acquired during hospitalization, while MSSA was mostly healthcare-associated (83.4%) and related to CVC. When considering the source of infection, most cases of MRSA were associated to healthcare-associated pneumonia (20.0%), while catheter-related infections mostly yielded positive MSSA culture (60.0%). Patients with MRSA were also more likely to have neutropenia < 500 cells/mm^3 and coinfection with gram-positive cocci different than *S. aureus* or gram-negative bacteria. All of these neutropenic patients except three had hematological malignancy, and ten had received chemotherapy within the last 30 days (35.7%). All MRSA patients were treated as inpatients and had a higher length of hospital stay when compared to MSSA (p < 0.001), except for two patients who were terminally ill and opted for no further treatment.

In multivariate logistic regression analyses, we identified associations between hospital-acquired infection, healthcare-associated pneumonia, and diabetes with MRSA infection after adjustment by Charlson Comorbidity Index and glycemia > 140 mg/dL. In a second model, which only included patients with hematologic malignancies, we identified hospital-acquired infection as a risk factor for MRSA; primary bacteremia was less likely to be found in MRSA patients. The variability of the composite outcome measure explained by models 1 and 2 was 23.9 and 19%, respectively (Supplementary Material).

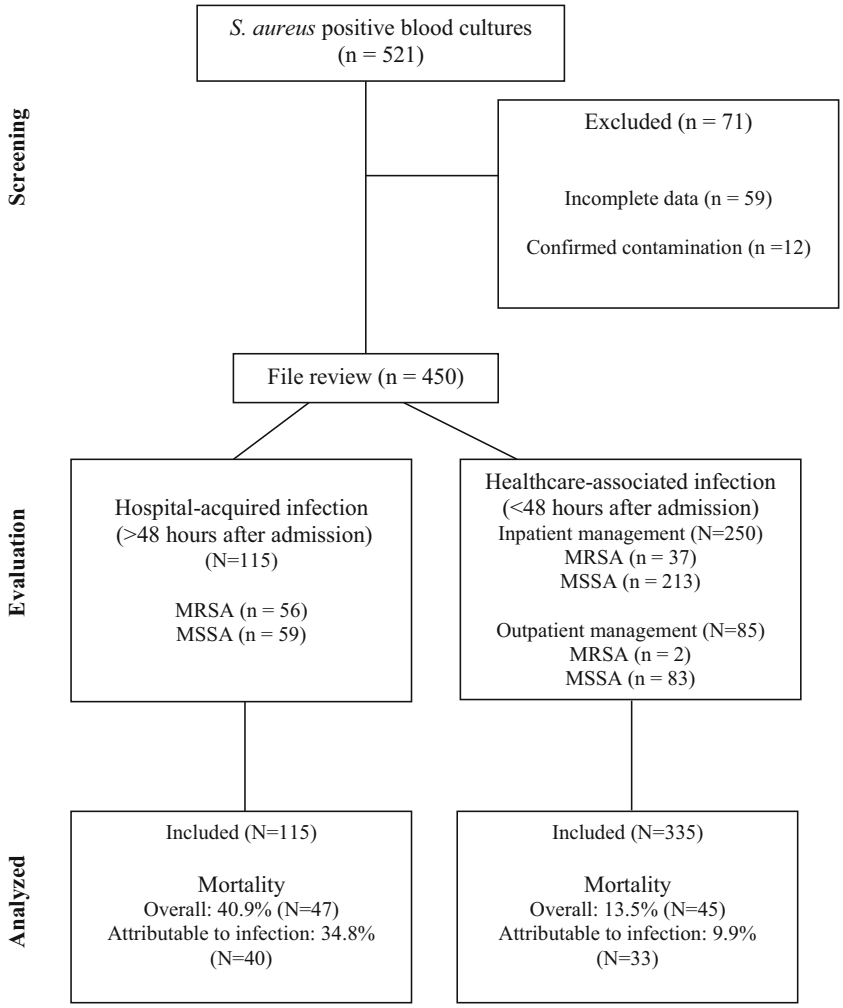

Fig. 1 Classification of *S. aureus* bloodstream infections according to type of bacteremia, management (inpatient or outpatient), and antibiotic susceptibility

Infection-related outcomes

We observed 124 all-cause deaths over 117,997 days of patient follow-up of which 92 occurred at 30 days, for an estimated 30-day mortality of 20.4%; 75 were SABI-related at 30 days (16.7%). Mortality was significantly higher in MRSA infection in both, solid and hematologic malignancies ($p < 0.001$); however, the difference in mortality was steeper for patients with hematologic malignancies (Fig. 3). MRSA patients had a higher rate of *S. aureus*-related admission to the ICU and higher rate

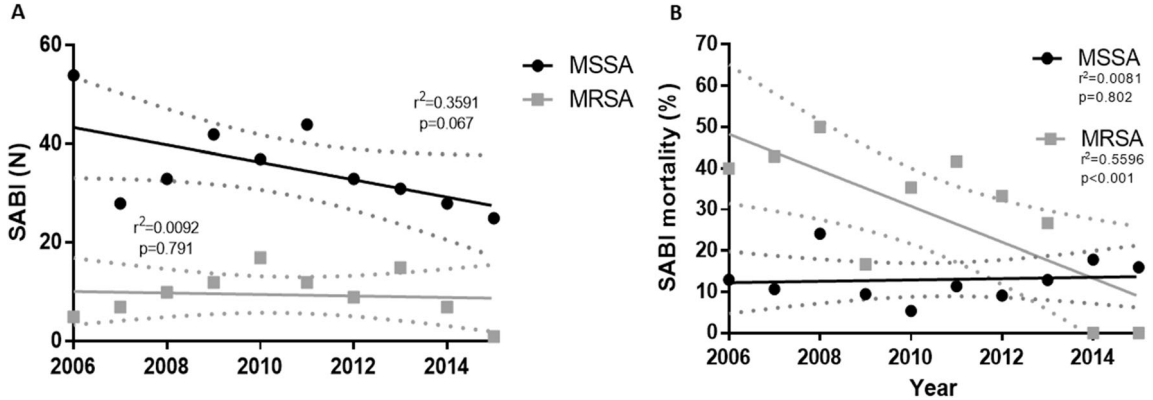

Fig. 2 **a** Yearly record of *S. aureus* bloodstream infections in the evaluated population according to antibiotic susceptibility. **b** Yearly mortality rate attributable to *S. aureus* bloodstream infections according to antibiotic susceptibility

Table 1 Clinical characteristics of studied subjects, considering microbiological characteristics of the SABI

Parameter	Total $N = 450$	MSSA $N = 355$	MRSA $N = 95$	P value
Age (years)	47 (17–87)	47 (17–87)	47.5 (17–75)	0.717
Male sex (%)	273 (60.7)	221 (62.3)	52 (54.7)	0.183
Charlson Comorbidity Index	4.0 (2.0–6.0)	4.0 (2.0–6.0)	3.0 (2.0–6.0)	0.184
Type of neoplasia (%)				
- Hematologic	135 (30.0)	95 (26.8)	40 (42.1)	0.004
- Solid	315 (70.0)	260 (73.2)	55 (57.9)	< 0.001
BSI site (%)				
- Healthcare-associated	335 (74.4)	296 (83.4)	39 (41.1)	< 0.001
- Hospital-acquired	115 (25.6)	59 (16.6)	56 (58.9)	< 0.001
Infectious foci (%)				
Community-acquired pneumonia	34 (7.6)	26 (7.3)	8 (8.4)	0.719
Healthcare-associated pneumonia	33 (7.3)	14 (3.9)	19 (20.0)	< 0.001
Soft tissue infection	28 (6.2)	21 (5.9)	7 (7.4)	0.603
Osteomyelitis	6 (1.3)	6 (1.7)	0	0.202
CVC	254 (56.4)	213 (60.0)	41 (43.2)	0.003
Abdominal	27 (6.0)	22 (6.2)	5 (5.3)	0.733
Unknown	68 (15.1)	57 (16.1)	11 (11.6)	0.279
Neutropenia (< 500 cells/mm^3, %)	86 (20.7)	58 (18.0)	28 (30.1)	0.011
Coinfections (%)				
- Gram-negative bacteria	87 (19.3)	56 (15.8)	31 (32.6)	< 0.001
- Gram-positive bacteria	31 (6.9)	20 (5.6)	11 (11.6)	0.042
Inpatient management (%)	365 (81.1)	272 (76.6)	93 (97.9)	< 0.001
Specific anti-*SA* treatment < 48 h (%)	354 (78.7)	289 (81.6)	65 (68.4)	0.006
CVC removal (%)	270 (71.8)	222 (74.2)	48 (62.3)	0.038
Days from infection to CVC removal	2.0 ± 3.0	2.0 ± 3.0	3.0 ± 5.0	0.023
Intensive care admission (%)	46 (10.2)	19 (5.4)	27 (28.4)	< 0.001
-Septic shock	22 (4.9%)	9 (2.5)	13 (13.7)	0.001
Relapse of *SA* bacteremia (%)	34 (7.6)	32 (9.0)	2 (2.1)	0.024
Secondary any-cause bacteremia at 6 months (%)	35 (7.8)	27 (7.6)	8 (8.4)	0.846
Infectious endocarditis (%)	7 (1.6)	6 (1.7)	1 (1.1)	0.905
30-day *SA*-related mortality (%)	75 (16.7)	45 (12.7)	30 (31.6)	< 0.001
6-month overall mortality (%)	124 (27.6)	83 (23.4)	41 (43.2)	< 0.001

CVC central venous catheter, *SA S. aureus*, *MRSA* methicillin-resistant *SA*, *MSSA* methicillin-sensitive *SA*, *BSI* bloodstream infection

of 30-day infection-related and all-cause mortality (31.6 and 43.2%, respectively). During the study period, fourteen cases of possible IE (3.1%) were recorded, seven were confirmed by using Duke's modified criteria, and all but one occurred in the MSSA group. Overall, 105 patients underwent echocardiographic evaluation to rule out IE.

Using Cox proportional risk regression analyses (Supplementary Material), we identified community-acquired pneumonia, abdominal source of infection, hematologic malignancy, MRSA, glucose levels > 140 mg/dL, and possible or confirmed endocarditis as risk factors for 30-day mortality; protective factors for mortality included catheter removal and initiation of adequate treatment for *S. aureus* < 48 h after positive blood cultures. After adjustment for competing risk of death using semi-parametric proportional hazard regression models considering non-infectious causes of 30-day mortality, community-acquired pneumonia and possible endocarditis were no longer associated to mortality (Table 2).

Prediction of 30-day mortality in SABIs

Finally, we evaluated the utility of the identified risk factors to stratify subjects in our cohort according to 30-day mortality risk. We used β-coefficients from the fitted regression model from competing risk data to approximate a point system using the magnitude of the coefficient. We observed that scores

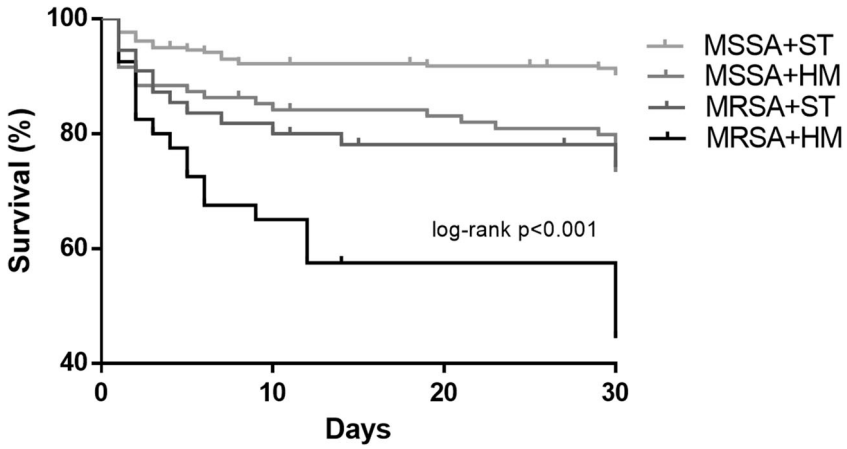

Fig. 3 Kaplan-Meier curve comparing survival in MRSA and MSSA bacteremias in patients with solid and hematologic malignancies. Abbreviations: Methicillin resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), solid tumor (ST), and hematologic malignancy (HM)

< − 2.0 had 3.34% (95%CI 1.11–7.86%) probability of infection-related mortality at 30 days, scores ≤ 0 but > − 2.0 had 12.71% (95%CI 8.77–17.4%), scores > 0 but ≤ 3.0 had 29.11% (95%CI 19.5–39.5%), and scores > 3.0 had 70.23% (95%CI 47.2–85.3%) probability of infection-related death at 30 days. The comparison between probability of death at each cutoff level was statistically significant in both the Kaplan-Meier (Fig. 4) and cumulative incidence analyses ($p < 0.001$ both).

Discussion

Our results demonstrate that SABIs imply significant disease burden for patients with cancer, increasing 30-day mortality in patients with hematologic malignancies and MRSA infections. MRSA was also associated to hospital-acquired infections and particularly to healthcare-associated pneumonia and in patients with diabetes. SABI-associated 30-day mortality at our institution was lower than those reported in other series (1–5), with significantly increased rates for the group of subjects with hematologic malignancies, MRSA infection, endocarditis, and hyperglycemia. The reduction in mortality associated to early catheter removal and initiation of specific antibiotic treatment for *S. aureus* has been observed in other series and demonstrates the significant role of hospital-level interventions in reducing the health burden associated to SABI. Finally, we demonstrated the utility of identified risk factors to stratify patients for infection-related 30-day mortality risk using a simplified score derived from our regression models.

Oncology patients are at high risk for SABIs [14, 15]. Previous reports have demonstrated that BSI and, particularly, SABIs are a significant cause for 30-day mortality in cancer patients [16]. Strategies to reduce SABIs are targeted at prediction of onset, including effective treatment and proper catheter management [17, 18]. We observed a decline over time in the rate of SABIs, which reflects the implementation and reinforcement of catheter care to reduce CLABSIs, and the reinforcement of hand hygiene programs. Over the study period, we also had significant improvements in our facilities for the isolation of neutropenic patients and an increase in the patients: infectious diseases physicians' ratio, all of which have led to improvement in the management and care of bloodstream infections, with positive changes in both, SABI incidence and SABI-related outcomes. In addition, improvement in diagnostic and therapeutic techniques, implementation of ambulatory chemotherapy regimens, and long-distance patient follow-up have

Table 2 Semi-proportional hazard regression adjusted for competing risk of death for 30-day mortality attributable to *S. aureus* bloodstream infections in cancer, adjusted by CCI

Variables	β	Wald	HR (95%CI)	*P* value
Abdominal source	1.292	2.41	3.64 (1.27–10.40)	0.016
Hematologic malignancy	1.118	3.93	3.28 (1.81–5.93)	< 0.001
MRSA	0.990	3.40	2.69 (1.52–4.76)	< 0.001
Glucose > 140 mg/dL	0.949	3.14	2.58 (1.43–4.67)	0.002
Catheter removal	− 0.868	− 2.95	0.42 (0.24–0.75)	0.003
Anti-staph treatment < 48 h	− 0.964	− 3.18	0.38 (0.21–0.69)	0.001
Infectious endocarditis	1.699	2.73	5.47 (1.62–18.49)	0.006

CCI Charlson Comorbidity Index, *MRSA* methicillin-resistant *S. aureus*, *HR* hazard ratio, *95%CI* 95% confidence interval

a

Variables	Point system
Abdominal source of infection	2.5
Hematologic malignancy	2
MRSA	2
Glucose >140mg/dL	2
Catheter removal	-2
Anti-staph treatment <48 hours	-2
Confirmed endocarditis	3

b

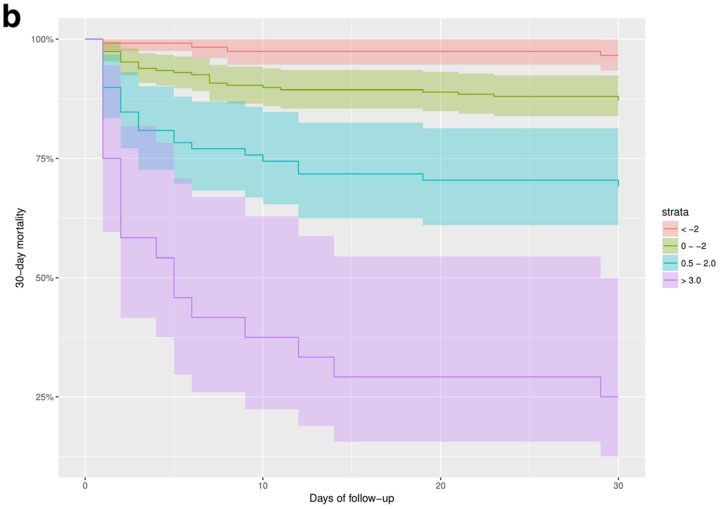

Fig. 4 **a** Score to evaluate prediction differences according to identified cutoff points using risk factors for 30-day mortality from competing risk data. **b** Cumulative incidence functions comparing strata of the predictive score to evaluate 30-day mortality risk

had significant effect on reducing infection-related mortality and disease burden.

MRSA-BSI is a cause of concern in oncology patients due to a higher mortality rate associated with methicillin resistance [19]. In our study, there was no reduction in MRSA incidence over time compared to MSSA; this could be explained by the higher frequency of MRSA in subjects with neutropenia, hematologic malignancies, and chemotherapy. Additionally, we observed an association of MRSA with diabetes, which has a higher prevalence in our population compared to worldwide estimates [20]. This association with diabetes is particularly interesting, given the known role of hyperglycemia with increased mortality in SABI for subjects with increased age and comorbidity independent of corticosteroid use, as confirmed in our study [21, 22]. Considering our observation that oncologic patients with diabetes have an increased rate of MRSA and that MRSA increases mortality in cancer, patients with diabetes and cancer should be closely monitored if SABI is suspected.

Subjects with hematologic malignancies are at an increased risk of acquiring BSI, infectious-related complications, and death, compared to patients with solid tumors [23, 24]. Venditti et al. found that non-neutropenic subjects with SABI and hematologic malignancies had a higher rate of early and late SABI-related complications compared to neutropenic patients [23]. This supports our observation that hematologic malignancy, but not severe neutropenia, was independently associated to higher 30-day infection-related mortality. We also observed that subjects with hematologic malignancies and MRSA have significantly higher 30-day mortality compared to subjects with solid tumors and MRSA infection [9, 24].

To quantify the role of mortality-associated risk and protective factors, we developed a scale which could be useful to predict 30-day mortality. An abdominal source of infection is considered a high-risk source as it increases the rate of treatment failure in patients with SABI [25]; in our study, subjects with an abdominal source of infection had significantly higher predicted mortality, even when adequate treatment for SABI was initiated and independently of the underlying malignancy. IE is a relevant comorbidity in SABI, causing increased early morbidity and mortality, particularly when effective treatment is not promptly initiated [26, 27]. In our scale, IE was considered the most significant predictor of mortality but in most subjects, catheter removal and early initiation of adequate treatment outweighed the associated risk. Risk estimation in SABI is relevant, since most risk factors for mortality are non-modifiable and patients at higher risk might require specialized and urgent care [27]. To evaluate the role our predictive score for infection-related mortality in SABI, external validation studies must be conducted, with special attention to patients with hematological malignancies.

Our study has some strengths and limitations. First, we evaluated a large and diverse cohort of oncologic patients that allowed comparison between solid and hematologic malignancies according to methicillin resistance. Second, due to our systematic epidemiologic surveillance system, we could collect sufficient and reliable clinical information over a 1-year period after the infection and were able to confirm the presence of comorbidity, cancer status, treatment, infectious source, and infection-related death from the patient's files. Third, we fitted all mortality models based on cumulative incidence from competing risk data,

which is a reasonable approach in oncologic patients and improves the performance of predictive models [13]. Amongst the limitations is the data collection process from clinical files, which is common in evaluation of BSIs in cancer but limits our ability to establish causal relationships for all variables, particularly in treatment-related data. Additionally, even though we performed adjustment using the Charlson Comorbidity Index, there exists the possibility of residual confounding for age and comorbidity in mortality models. Patients with hematological malignancies are a population that clearly differs from patients with solid neoplasia, in both burden of disease and life-threating complications, which calls for a targeted study in patients with hematologic malignancies to confirm our observations and improve mortality prediction.

In conclusion, our data demonstrates that SABIs are a significant burden in patients with cancer. MRSA infection was associated with hospital-acquired setting, pneumonia, and diabetes mellitus. For patients with hematologic malignancies, MRSA was more frequent if hospital-acquired and less likely in patients with primary bacteremias. Mortality rate in our institution decreased for MRSA infection and is lower to those reported in similar series. Risk factors for infection-related 30-day mortality include MRSA, hematologic malignancy, hyperglycemia, abdominal source of infection, and endocarditis. The reduction in mortality associated to early catheter removal and initiation of specific antibiotic treatment for SABI demonstrates the significant role of hospital-level interventions and the increasing supportive clinical care in cancer patients. Using these risk factors to stratify patients might be a useful approach to predict the probability of 30-day infection-related mortality.

Author contributions *Omar Yaxmehen Bello-Chavolla*: Research idea and study design, data acquisition, data analysis/interpretation, statistical analysis, manuscript drafting. *Jessica Paola Bahena-López*: Research idea and study design, data acquisition, data analysis/interpretation, statistical analysis, manuscript drafting. *Pamela Garciadiego-Fosass*: Research idea and study design, data acquisition, data analysis/interpretation. *Patricia Volkow*: Manuscript drafting and mentorship. *Alejandro Garcia-Horton*: Data acquisition, data analysis/interpretation. *Consuelo Velazquez-Acosta*: Data acquisition, microbiology analysis and review. *Diana Vilar-Compte*: Research idea and study design, data acquisition, data analysis/interpretation, manuscript drafting, supervision or mentorship.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval and informed consent For this type of study, formal consent is not required. All data was protected and confidentiality was guaranteed. This article does not contain any studies with human participants or animals performed by any of the authors.

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Rev Invest Clin. 2018;70:224-36 BRIEF REVIEW

Familial Combined Hyperlipidemia: Current Knowledge, Perspectives, and Controversies

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ABSTRACT

Familial combined hyperlipidemia (FCHL) is the most prevalent primary dyslipidemia; however, it frequently remains undiagnosed and its precise definition is a subject of controversy. FCHL is characterized by fluctuations in serum lipid concentrations and may present as mixed hyperlipidemia, isolated hypercholesterolemia, hypertriglyceridemia, or as a normal serum lipid profile in combination with abnormally elevated levels of apolipoprotein B. FCHL is an oligogenic primary lipid disorder, which can occur due to the interaction of several contributing variants and mutations along with environmental triggers. Controversies surrounding the relevance of identifying FCHL as a cause of isolated hypertriglyceridemia and a differential diagnosis of familial hypertriglyceridemia are offset by the description of associations with *USF1* and other genetic traits that are unique for FCHL and that are shared with other conditions with similar pathophysiological mechanisms. Patients with FCHL are at an increased risk of cardiovascular disease and mortality and have a high frequency of comorbidity with other metabolic conditions such as type 2 diabetes, non-alcoholic fatty liver disease, steatohepatitis, and the metabolic syndrome. Management usually requires lipid-lowering therapy directed toward reducing cholesterol and triglyceride concentrations along with cardiovascular risk protection. In recent years, the number of research studies on FCHL has been decreasing, mainly due to a lack of recognition of its impact on disease burden and comorbidity and the complexity in identifying probands for studies. This creates areas of opportunity to develop research for FCHL in epidemiology, genetics, pathophysiology, therapeutics, and cardiovascular risk management, which are discussed in depth in this review. (REV INVEST CLIN. 2018;70:224-36)

Key words: Familial combined hyperlipidemia. Genetics. Apolipoprotein B.

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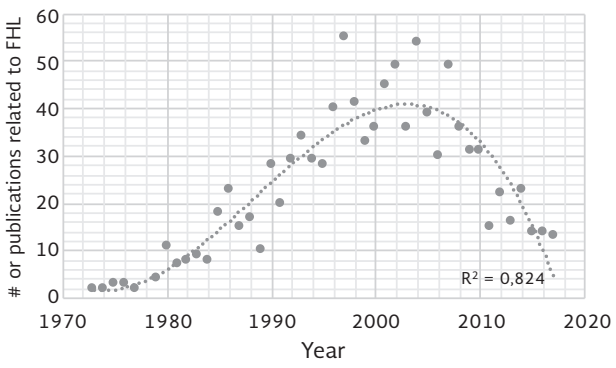
INTRODUCTION

Familial combined hyperlipidemia (FCHL) is the most prevalent primary dyslipidemia, occurring in up to 1-3% of the general population and in 20-38% of patients with previous history of myocardial infarction (MI)[1]. This disorder was simultaneously described by Goldstein et al.[2], Hazzard et al., and Kwiterovich et al., who independently described it in different cohorts[3]. FCHL is characterized by fluctuations in serum lipid profile and a rather heterogeneous clinical presentation which can be alternatingly identified with mixed hyperlipidemia, isolated hypercholesterolemia, or hypertriglyceridemia in combination with abnormally high levels of apolipoprotein B (apoB)[2,3]. Certain ethnic groups are particularly susceptible to FCHL, as demonstrated by Paramsothy et al. in a multiethnic cohort of 6814 participants in the United States, reporting a prevalence of 4.8% within Hispanics[4,5]. FCHL coexists with other metabolic diseases such as obesity, insulin resistance (IR), type 2 diabetes mellitus (T2D), hypertension, non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MS)[6]. FCHL cases with metabolic comorbidities have remarkably higher apoB plasma levels compared to cases with a similar severity of IR. In addition, subjects with FCHL have a greater susceptibility to developing T2D and are thus at a higher cardiovascular risk in comparison to matched controls[3,6].

Notably, the number of research studies focused on understanding the epidemiology, genetics, pathophysiology, and treatment of FCHL has been decreasing over the years as demonstrated by the number of related articles cited in PubMed since 2007 (Fig. 1).

Figure 1. Publication rate related to studies focused on familial combined hyperlipidemia in the scientific database PubMed until December 2017.



This could be attributable to the complex nature of the disease, heterogeneous clinical definitions, and inconsistent consensus in its defining traits, which makes comparisons across reports largely unfeasible, complicating precise estimates of FCHL epidemiology and its metabolic burden. In this review, we will focus on the most recent advances in understanding FCHL. We will also evaluate gaps in available knowledge and areas that lack sufficient information and call for further studies to describe fully comorbidity and cardiovascular risk associated to FCHL.

EVOLUTION OF FCHL DIAGNOSTIC CRITERIA

Different diagnostic criteria have been proposed for FCHL over the years[7]. Classically, the phenotype to establish the diagnosis of FCHL comprised either isolated hypercholesterolemia or hypertriglyceridemia or a mixed lipid profile along with the first-degree family history of premature coronary artery disease (CAD), excluding other causes of dyslipidemia[3]. More recent criteria have also included elevated apoB levels as highly suggestive of FCHL (Table 1)[8].

Due to the oligogenic nature of the disease, genetic testing is not yet a possibility[3], but diagnosis can be made based on a fluctuating lipid profile, increased apoB levels, and first-degree family history of mixed lipid disorders and premature cardiovascular disease (Fig. 2)[9-11]. Some limitations on these criteria include the low practicality of apoB measurements in everyday clinical settings in addition to interethnic differences in establishing the 90th percentile in both lipid and apoB measurements, which require population-specific percentiles that might not always be available.

GENETIC CHARACTERIZATION OF FCHL

Initial genetic characterizations of FCHL defined it as a primary lipid disorder with autosomal dominant inheritance[2]; however, recent data suggest that FCHL is an oligogenic entity with variable penetrance[11,12]. Establishing a unified causative genetic trait in FCHL is complex partly due to its clinical variability and the difficulties in comparing FCHL studies with inconsistent diagnostic criteria. Recent findings describe multiple genetic alterations contributing to the observed

Table 1. Changes in diagnostic criteria for FCHL throughout the years.

Year	Study/Author	TG (mmol/l)		CT (mmol/l)	ApoB (g/l)	Family history
1973	Goldstein	> 95th percentile	And	> 95th percentile	–	CAD < 60 years
1983	Brunzell	6.42 ± 1.19	And	2.53 ± 1.17	1.44 ± 0.36	CAD < 60 years
1999	EuroFam/ Pajukanta	> 90th percentile	Or	> 90th percentile	–	Mixed hyperlipidemia
1999	Dutch/Aouizerat	> 6.5	And	> 2.3	> 1.2	Differing hyperlipidemia in relative, CAD age < 60 years
2001	Consensus/ Sniderman	–	–	> 1.5	> 75th percentile	Hyperlipidemia in 1st degree relative
2003	British mapping/ Naoumova	> 95th percentile	And	> 90th percentile	-	Hyperlipidemia in 1st and 2nd degree relatives
2004	Dutch clinical/ Veerkamp	> 6.0	And	> 1.5	> 1.2	Hyperlipidemia in 1st degree relative
2004	Huertas-Vazquez	> 90th percentile	Or	> 90th percentile	> 90th percentile	CAD (MI) < 60 years in proband or 1st degree relative and One 1st degree relative TG or CT > 90th percentile
2004	Aguilar-Salinas	> 150 mg/dL	Or	> 200 mg/dL	> 90th percentile	CAD (MI) < 60 years At least three different family members: one with hypercholesterolemia, one with hypertriglyceridemia, and one with mixed hyperlipidemia
2005	GEM study/ Wyszynski	–	–	> 75th percentile	–	Index case and one relative with relevant profile
2014	Mata	> 200 mg/dL	And/or	< 240 mg/dL (LDL > 160 mg/dL)	–	Two or more family members with hypercholesterolemia, hypertriglyceridemia, or mixed hyperlipidemia

FCHL: familial combined hyperlipidemia, TG: triglycerides, CT: computed tomography, ApoB: apolipoprotein B, CAD: coronary artery disease, MI: myocardial infarction, LDL: low-density lipoprotein
*Adapted and modified from Wierzbicki AS (31).

clinical phenotype[2,3,11]. The fluctuating lipid profile characteristic of FCHL can be attributable to the interaction of cumulative large- and small-effect genetic variants that alter low-density lipoprotein-cholesterol (LDL-C) and triglycerides (TG) concentrations and contributing environmental factors (Fig. 3). These genetic alterations usually have independent segregation on different chromosomes, which impacts the degree of expression in affected family members, thus leading to heterogeneous clinical presentations even within the same kindred[11].

Consistent susceptibility loci have been reported among individuals with FCHL from different ethnic backgrounds and have been mapped to chromosomes 1q21-23, 11p14.1-q12.1, and 16q22-24.1[13]. An association of FCHL with the region in chromosome 1q21-1q23[1,14] has been consistently reported. This region includes several genes which might contribute to FCHL phenotype, including the upstream transcription factor 1 gene *(USF1)*[1]. *USF1* encodes a transcription factor that regulates nearly 40 genes implicated in lipid and lipoprotein metabolism, as well as immune

Figure 2. Proposed updated diagnostic algorithm for FCHL. TC: total cholesterol; TG: triglycerides; apoB: apolipoprotein B-100; CAD: coronary artery disease, FCHL: familial combined hyperlipidemia.

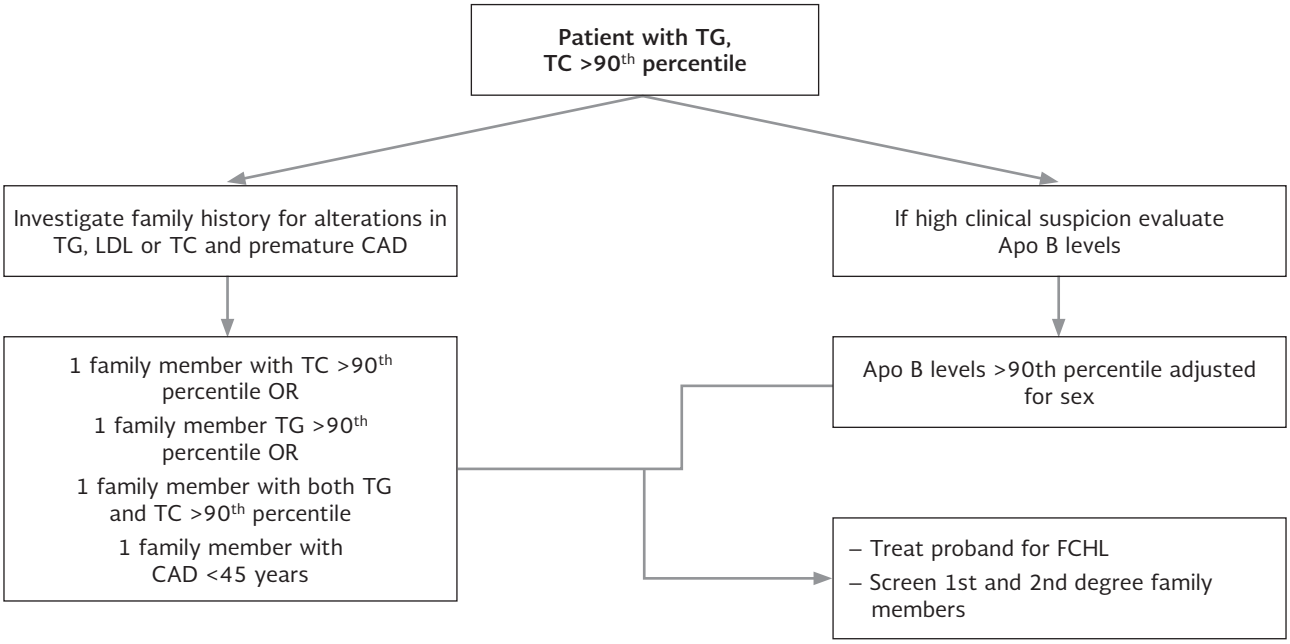
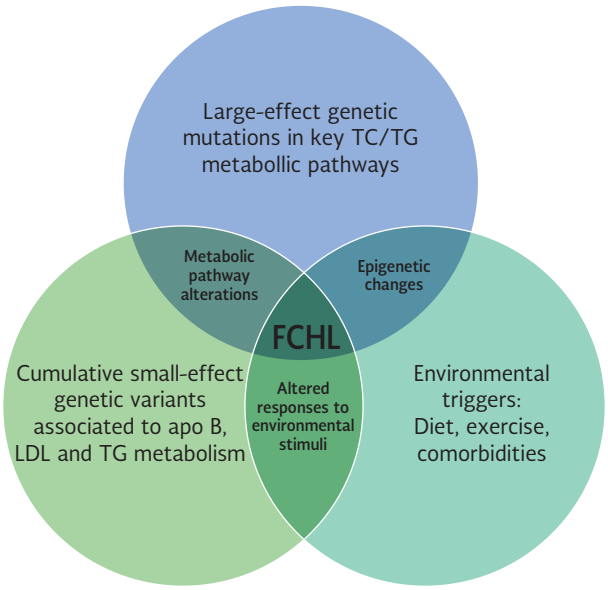


Figure 3. Genetics of familial combined hyperlipidemia (FCHL): The interplay of large-effect genetic mutations, cumulative small-effect genetic variants, and environmental triggers contribute to developing the FCHL phenotype.



response, and is located 1.5Mb away from *TXNIP*, a gene linked to mixed hyperlipidemia in mice[1,3]. *USF1* encodes for a basic helix-loop-helix leucine zipper transcription factor located in chromosome 1q23.3, which binds to a palindromic E-box sequence. *USF1* was first described by Sawadogo et al. as a key component in adenovirus replication[15] and its role as a regulator of lipid and glucose metabolism was later reported. USF1 has been shown to regulate expression of L-pyruvate kinase, fatty acid synthase, and glucokinase, as well as apoA-V, apoC-III, apoA-II, apoE, hormone-sensitive lipase, and other enzymes involved

in lipid and carbohydrate metabolism[16-19]. Pajukanta et al. characterized *USF1* as the major genetic trait of FCHL which was further demonstrated by Huertas-Vázquez et al. in Mexican population[1,20].

Several single-nucleotide polymorphisms (SNP) have been associated with FCHL. A haplotype for *USF1* associated with susceptibility for both FCHL and FHTG was identified in Finnish and Mexican families, with a stronger association for FCHL[20]. The SNP rs3737787 has been associated with differences in the expression of the target genes for *USF1* in adipose tissue and lymphoblasts, as well as higher TG concentrations in Mexican and Finnish populations, and is the SNP most consistently associated to FCHL. A comparison of the expression of USF1 in muscle and adipose tissue identified 13 genes that are regulated by *USF1,* including *FASD3, FABP2, FOLH1, MADD, NR1H3, CETP, LCAT, APOE,* and *PLTP*[21]. Overall, GWAS have confirmed that subjects with FCHL have a high polygenic lipid score for associated LDL-C and TG variants and confirm the polygenic nature of the disease[1,10].

Mutations in *LDLR* and *PCSK9* have been associated to increased LDL-C levels in FCHL; however, identification of these mutations is not specific of FCHL and demonstrates the difficulty of distinguishing mutations associated with the FCHL phenotype and those with increased LDL-C levels. Minocci et al. reported that up to 5% of cases with FCHL and predicted dysfunctional *LDLR* had to be reclassified as familial hypercholesterolemia with elevated TG levels, whereby additional genetic variants and environmental factors were responsible for the elevated TG concentrations[11,22]. An additional example of these interactions is the loss-of-function in lipoprotein lipase (LPL), APOA5 and GCKR, which have known to contribute to elevated TG levels, and which interact with additional genetic variants in other genes that increase LDL-C concentrations in FCHL[22]. Genome-wide scans have also demonstrated a strong link between the angiopoietin-like protein 3 gene *(ANGPTL3)* and plasma TG levels in FCHL[23]. ANGPTL3 is a secretory protein that affects plasma TG levels by reversibly inhibiting the catalytic activity of LPL; studies in both animal and human models have shown that inactivation of *ANGPTL3* leads to a decrease in TG, high-density lipoprotein-cholesterol (HDL-C), and LDL-C levels, which might diminish the risk of atherosclerotic cardiovascular disease[14]. However, the role of alterations in *ANGPTL3* in FCHL patients and its potential therapeutic role have not been determined[24].

Additional SNPs have been identified for specific populations in relation to FCHL and metabolic comorbidities. Huertas-Vázquez et al. demonstrated that the rs7903146 and rs12255372 variants in *TCF7L2* are associated with TG concentrations and T2D in Mexicans with FCHL as well as the 20q12-q131 locus, which is explained by *HNF4*α variants in Mexican and Finnish subjects with FCHL[25]. Two novel associations have been recently described for apoB levels at rs1424032 in 16q21, a highly conserved non-codifying region, and rs1349411 12p13.31, which included the *APOBEC1* gene, implicated in the edition of apoB mRNA in the small intestine[1]. Alterations in several metabolic pathways have been identified as potential candidates to further describe metabolic alterations in FCHL. Altered pathways in FCHL have been reported in the *APOA1-C3-A4-A5* gene cluster, which has been linked to HDL-c and TG levels, as well as *LPL, LCAT,* and *TNFRSF1B*[10,11,22]. SNPs in some of these loci have been linked to both TG and cholesterol fluctuations, with recent reports also suggesting a role for a highly disruptive p.Tyr125Cys SNP in *SLC25A40,* which encodes a mitochondrial solute transporter evaluated in Seattle kindred[26].

PATHOPHYSIOLOGY OF FCHL

FCHL comprises both hyperapobetalipoproteinemia and normal or elevated apoB synthesis[27,28]. Alterations in both secretion and degradation of apoB particles have been encountered in FCHL patients and have been linked to IR, decreased apoB clearance rate, and increased expression of molecules that downregulate the LDL receptor[2,12,30]. Imbalance between de novo lipogenesis and β-oxidation is a hallmark of FCHL, resulting in hepatic fat accumulation and very low-density lipoprotein (VLDL) overproduction[31]. Adipose tissue dysfunction has been linked with an increase in free-fatty acid (FFA) levels and efflux of FFA toward the liver, leading to an increased rate of lipoprotein synthesis[2,3]. It is known that increased levels and production of apoC-II and C-III are determinants of kinetics and plasma concentrations of TG-rich lipoproteins (TRLs), including VLDL1 and 2[32]. The *APOCIII* gene has also been linked to states of IR and T2D, both of which are frequent in FCHL

patients. FCHL has also been characterized by lower intestinal cholesterol absorption and higher cholesterol synthesis independent of body mass index (BMI) in comparison to primary hypercholesterolemia of genetic origin[33]. Unfavorable lipid profiles and increased postprandial lipemia have been linked to higher cardiovascular risk in FCHL[34]. A study by Almeda-Valdes et al. determined that the incremental area under the curve of postprandial lipemia in FCHL patients is determined by fasting apoB-48 levels[35,36] and potentiated by the presence of abdominal obesity. This study also proposed that apoA-V was associated with VLDL and chylomicron production in FCHL subjects.

The role of *USF1* in the pathogenesis of FCHL has not been completely explained. Inactivation of *USF1* in mice leads to protection for diet-induced dyslipidemia, obesity, NAFLD, and atherosclerosis; the proposed mechanism has been linked to increased TG uptake by brown adipose tissue through an LPL-dependent mechanism, which increases adrenergic response and thermogenesis[16]. *USF1* knockout mice *(USF1$^{-/-}$)* preserved a normal lipid profile when exposed to a high-fat and -carbohydrate diet; in addition, this group showed an enhanced insulin sensitivity and reduced liver steatosis compared with *USF1$^{+/+}$* type mice[16]. The findings of *USF-1* downregulation in animal models are similar to those observed in humans in whom improved insulin sensitivity, atheroprotective lipid profiles, and decreased atherosclerosis were associated with reduced *USF1* mRNA expression[16]. Plaisier et al. compared *USF1* expression patterns in subcutaneous adipose tissue from FCHL patients compared to healthy controls, demonstrating higher USF1 expression in affected subjects[1]. Wu et al. developed two overexpression *USF1* models in mice; both liver and systemic *USF1* overexpression models showed adverse metabolic phenotypes including obesity, worsened lipid profile, and higher glucose/insulin ratio[37]. These observations suggest a role for *USF1* in the pathophysiology of FCHL; however, identification of precise mechanisms requires functional studies on human subjects with FCHL with and without *USF1* variants that can be later confirmed in controlled studies in animal models.

In FCHL, there is an upregulation of thioredoxins, which are disulfide reductases responsible for regulating redox reactions, and confers an increased oxidative stress with reduced glutathione levels associated to IR. Both the increase in oxidative stress damage and IR contribute to atherosclerosis and potentially to increased cardiovascular risk in FCHL patients[38]. Most FCHL patients have increased sdLDL and apoB levels for all levels of IR in comparison to controls, adjusted by HOMA-IR and BMI[9]; this supports the concept that the etiology of the lipid phenotype in FCHL is a result of additive effects of genetic determinants with modulation by BMI and IR. Cardiovascular risk has also been associated to IR in FCHL. Subjects with FCHL have been reported to have increased vascular inflammation and metabolic activity in spleen, bone marrow, and liver as measured by ^{18}F-fluorodeoxyglucose positron-emission tomography/computed tomography imaging[39]. In addition, Carratala et al. showed that subjects with FCHL had increased plasminogen activator inhibitor type 1 (PAI-1) levels, which correlated with IR, MS components and increased carotid intima thickness, all of which are markers of increased cardiovascular risk[17,39-42].

Along with TRL, diminished lipoprotein clearance associated to IR-mediated decreased LPL activity, leads to sdLDL and intermediate-density lipoprotein particle accumulation, both of which are highly atherogenic and easily oxidized, contributing to its entry into subendothelial pathways[3,43]. Decreased adiponectin levels in the setting of IR have been linked to higher levels of apoB and VLDL particles, further contributing to atherogenesis[44], which might be feasible in the setting of FCHL. Fibroblast growth factor 21 (FGF-21) is also implicated in the metabolism and kinetics of TRLs, causing an increase in insulin-induced CD36 and LPL-mediated catabolism of TRLs in white and brown adipose tissue and a reduction of serum TG concentrations. It could be hypothesized that the FGF-21 physiologic activity may be decreased in FCHL, but this has not been shown in animal or human subjects[45]. There is also evidence that PCSK9 concentrations are elevated in FCHL and contribute to the impaired catabolism of apoB[12]. PSCK9 induces degradation and downregulation of the LDL receptor through resistin and other pro-inflammatory cytokines[2,12] and is as well one of the factors contributing to the hyperapolipoproteinemia in FCHL and a possible target for therapies as discussed later.

COMORBIDITIES AND CARDIOVASCULAR RISK IN FCHL

Metabolic comorbidities in FCHL

FCHL has been associated to numerous metabolic diseases and comprises metabolic and biochemical abnormalities not unlike T2D, NAFLD, and the MS. FCHL has also been linked to an increased cardiovascular risk, particularly with CAD[3]. MS shares several pathophysiological alterations with FCHL, including elevated TG levels, impaired glucose tolerance, increased cardiovascular risk, and the comorbid presence of obesity and hypertension. However, in contrast to MS, FCHL subjects consistently show apoB levels > 90th percentile, while in patients with MS apoB may be high, normal, or even decreased. FCHL onset occurs earlier and hereditary traits are more evident, while for MS lifestyle plays a more prominent role than genetics[3]. In a recent study, Skoumas et al. examined the relationship between FCHL and MS, demonstrating that apoB levels were higher for FCHL patients, despite many similar features in both[11].

FCHL has been shown to carry an increased risk of incident T2D, conferring a higher metabolic and cardiovascular burden for patients with the disease. However, FCHL studies that evaluate cardiovascular risk often omit population with comorbid T2D, making excess risk estimations unfeasible[1,40]. The shared genetic background in FCHL has also been suggested by the evidence of association of FCHL with variants in *HNF4*α and *TCF7L2*[1,25,40,46]. FCHL has been shown to share common pathophysiological mechanisms with T2D including muscle and adipose tissue IR, as well as impaired insulin-mediated suppression of hepatic VLDL production[40].

An increased risk of hepatic steatosis has been observed in FCHL, with consistent associations for both NAFLD and non-alcoholic steatohepatitis (NASH) and up to 20-37% of the variability in intrahepatic fat content attributable to genetic factors in FCHL[40,47,48]. Increased hepatic visceral fat explains the change in serum TG levels in relation to changes in alanine aminotransferase levels for FCHL patients[48]. Brouwers et al. described that fatty liver occurrence was significantly higher for FCHL patients and their normolipidemic family members when compared to their spouses, who were used as control subjects, and determined that subcutaneous and intravisceral fat were predictors of intrahepatic fat content[2]. Recent studies suggest that genetic polymorphisms in *USF1* (rs6427573 and rs2516839), which have been linked to FCHL, have an increased independent risk of NAFLD when compared to controls in Chinese population[49]. Due to the role of *USF1* in the transcriptional regulation of hepatic lipogenesis, mice with *USF1* overexpression could be used to understand the role of USF1 in the setting of hepatosteatosis and IR and epigenetic studies could contribute to the understanding of the role of post-translational modifications in the setting of hepatosteatosis in FCHL patients[17]. Identification of additional mutations that explain intrahepatic fat accumulation, NAFLD, and NASH in FCHL patients remains largely unexplored and requires further evaluation and validation in other ethnic groups.

Cardiovascular risk in FCHL

FCHL is strongly associated with premature CAD, with up to 10-14% of patients with premature CAD having comorbid FCHL[22]. A patient diagnosed with FCHL has 1.7-10-fold higher risk of CAD compared to the average population 20 years after the initial diagnosis[22,50]. Wiesbaue et al. demonstrated that 38% of premature MI survivors had FCHL, and a similar study including 706 participants with FCHL reported a CAD prevalence of 15.3%, describing that disease presentation was independent of age, sex, or presence of T2D[6]. Cardiovascular risk in patients with hypertriglyceridemia is also increased, especially in the setting of older age, tobacco use, and hypertension and decreased HDL-C levels[11]. Among FCHL patients, males are more susceptible to inherit and develop the lipid disorder independent of lipid profile, which might also account for the increased risk[24]. Elevated expression of CD11b, a marker of fasting and postprandial leucocyte activation, has been previously reported for FCHL subjects and has been associated to increased cardiovascular risk in subjects with FCHL and comorbid T2D[51-55].

THERAPEUTIC APPROACH IN FCHL

As of the writing of this review, no specific clinical trials, guidelines, or algorithms have been developed for the management of FCHL. However, some

guidelines such as the 2016 ESC/EAS Guidelines for the Management of Dyslipidemias suggest that it would be managed as a particular primary lipid disorder and an atherogenic dyslipidemia[56,57]. However, the recommendation is lacking since it considers the oligogenic nature of the disease but does not offer specific comments regarding the particular cardiovascular risk in FCHL and how it might interact with metabolic comorbidities which also increase CV risk; furthermore, the guideline does not offer precise recommendations regarding family studies and early initiation of treatment in susceptible individuals.

A reasonable initial step in the management of a patient with FCHL includes controlled interventions targeting modifiable cardiovascular risk factors including smoking, alcoholism, overweight, and obesity[12]. Mateo-Gallego et al. showed that a weight loss of 5% of total weight in overweight adults with FCHL significantly reduces TG and non-HDL cholesterol levels at 3 and 6 months[58]. This justifies the role of weight loss in overweight patients with FCHL to complement lipid-lowering therapy in FCHL[59]. Evidence and recommendations regarding management of specific risk factors in FCHL are insufficient and call for the development of intervention-based evaluations aiming at describing the role and magnitude of these treatments and their impact on lipid profile and metabolic burden.

The decision of using either a statin, a fibrate, or a combination of both as the first-line therapy in FCHL is highly dependent on the predominant lipid alteration at diagnosis. However, it has been shown that the use of statins in comparison to fibrates as the first-line therapy significantly improves the lipid profile and increases the likelihood of reaching lipid targets in patients with FCHL[60,61]. Furthermore, statins have been shown to decrease significantly the levels of total cholesterol, LDL-C, apoB, non-HDL-C, and VLDL particles and remnants in comparison to fibrates, which are more effective at decreasing TG and increasing HDL-C levels in FCHL patients[61]. The effect of statin therapy on lipoprotein kinetics was evaluated by Le et al., who demonstrated that rosuvastatin significantly decreases LDL-C, apoB-100, and TG levels and increases the fractional catabolic rate of LDL and apoB-100 in a dose-dependent manner and thus cholesterol biosynthesis but does not have effect on apoB-100 production or HDL kinetics. This indicates that high-intensity statins in FCHL patients are effective at decreasing LDL apoB-100 levels and thoroughly modify lipoprotein profile[62]. As mentioned earlier, the inhibition of apoB production may also have beneficial roles in preventing hepatosteatosis and improving beta-oxidative pathways, which suggests a potential role to investigate the therapeutic effect of VLDL reduction in FCHL patients[42].

FCHL confers an increased risk of premature cardiovascular disease partly due to a rise in the accumulation of atherogenic particles, which may require the use of moderate- to high-intensity statin. Rosuvastatin increases the catabolism of sdLDL apoB-100 levels without changes in the conversion of TRL apoB-100 to sdLDL, though at a lower rate than large buoyant LDL-C[63]. Additional cardiovascular risk has been associated to increased postprandial lipemia in FCHL patients; despite its efficacy in sdLDL and LDL-C reduction, statin therapy has not shown modifications on postprandial lipemia for most FCHL patients, except for MTP-493G/T carriers, in whom a greater reduction of postprandial lipemia has been associated with the use of atorvastatin in comparison to non-carriers[36]. A particular concern of statin therapy in FCHL patients is the associated increase in incident T2D risk with statin use. Skoumas et al. showed that the risk of incident T2D did not increase with statin use or statin intensity in FCHL patients; these findings were confirmed by their group after a 10-year follow-up in which no significant differences in T2D incidence with statin use were observed between subjects with FCHL and controls. These observations suggest that the risk/benefit analysis for statin use in FCHL should have considerations similar to the rest of the population in terms of the statin-associated incident T2D risk[45,36].

Current guidelines suggest that FCHL patients should be managed according to LDL levels to decrease cardiovascular risk as recommended by European guidelines, by which these levels are considered a condition of high CV risk[64-66] (Table 2). However, patients with FCHL often present hypertriglyceridemia, which may decrease the reliability of LDL-C estimation by the Friedewald equation; thus, alternative goals focusing on non-HDL cholesterol and apoB levels are required. Sniderman et al. conducted a meta-analysis to investigate whether apoB or non-HDL-C increased the predictive power of LDL-C. They reported that during a

Table 2. 2016/European Society of Cardiology/European Atherosclerosis Society for treatment of lipid disorder recommendations.

CV risk	Features	LDL-C target concentration
Low risk	SCORE < 1% for 10-year risk of fatal CVD	<190 mg/dL lifestyle intervention, consider drug if uncontrolled
Moderate risk	SCORE is > 1% and < 5% for 10-year risk of fatal CVD	100-<155 mg/dL, Lifestyle intervention, consider drug if uncontrolled
High risk	Markedly elevated single risk factors, in particular cholesterol > 8 mmol/L (> 310 mg/dL) (e.g., in FH and FCHL) or BP > 180/110 mmHg. Most other people with DM (some young people with type 1 diabetes may be at low or moderate risk). Moderate CKD. SCORE > 5% and < 10% for 10-year risk of fatal CVD	70-< 100 mg/dL Non-HDL-C < 130 mg/dL, ApoB < 90 mg/dL
Very high risk	Documented CVD, clinical or unequivocal on imaging, previous myocardial infarction, coronary revascularization, coronary artery bypass graft surgery, stroke and transient ischemic attack, and peripheral arterial disease. DM with target organ damage, severe CKD. SCORE > 10% for 10-year risk of fatal CVD	< 70 mg/dL Non-HDL-C < 100 mg/dL

CV: cardiovascular, CVD: cardiovascular disease, HDL-C: high-density lipoprotein cholesterol, T1D: type 1 diabetes, T2D: type 2 diabetes, CKD: chronic kidney disease, SCORE: systematic coronary risk estimation, FH: familial hypercholesterolemia, FCHL: familial combined hyperlipidemia.

10-year period, a strategy focused on controlling non-HDL-C could prevent 300,000 more cardiovascular events than one directed to LDL-C; and a strategy focused on controlling apoB could prevent 500,000 more cardiovascular events than one directed to LDL-C[67]. Therefore, targets focused on non-HDL cholesterol < 130 mg/dL and apoB < 90 mg/dL should be considered in patients with FCHL[65].

Evidence beyond the use of statins for cholesterol management in FCHL patients has not been extensively evaluated. Ezetimibe can be added to treatment in cases where LDL-C decrease is refractory to statins monotherapy to improve the prognosis in endpoint CAD event as shown in the IMPROVE-IT study[68]. Recent analyses suggest to reconsider the use of ezetimibe and bile acid sequestrants in primary prevention in patients with true statin intolerance and in those patients in whom the goal levels of LDL-C cannot be achieved with maximum statin doses[69]. Evidence from large trials has shown that iPCSk9 is highly effective at reducing LDL-C and non-HDL-C levels; they are currently indicated for patients who do not reach lipid target after receiving maximum statin doses and with additional therapy, or patients who have statin intolerance[70,71]. Despite its promising role, the effect of additional LDL-C reduction with iPCSk9 should be evaluated in terms of its effect in cardiovascular risk reduction for FCHL patients and its impact on specific lipoprotein metabolism and kinetics.

Because FCHL is also characterized by hypertriglyceridemia, specific measures to control TG concentrations should be taken for adequate management (Table 3). Non-pharmacologic therapy includes glycemic control, avoidance of medication that increases lipid levels, limitation of alcohol intake, avoidance of simple carbohydrates, low-fat diet (< 30% of total daily caloric intake), and weight loss in patients who are overweight or obese[66,72]. With regard to pharmacological therapy, the efficacy of fibrates, Ω-3 fatty acids, and statins has been demonstrated in clinical trials for the management of FCHL patients[73-75]. A meta-analysis by Guo et al. demonstrated that the concomitant use of a fibrate and a statin is recommended for the management of FCHL subjects[76], and an algorithm proposed by Ellis et al.[12] suggests that pharmacologic treatment for elevated TG levels in FCHL patients with levels > 180 mg/dL should be started after reaching targets of LDL-C and apoB by statin treatment with or without ezetimibe.

Evidence from randomized, controlled clinical trials should be generated in FCHL population to assess the

Table 3. Therapeutic goals and treatment strategies in hypertriglyceridemia with FCHL.

TG level	Therapeutic goal	Therapeutic strategies
Borderline high (150-199 mg/dL)	Achieve LDL-C target, and apoB levels	Non-pharmacologic strategies
High (200-499 mg/dL)	Achieve LDL-C and apoB target, non-HDL-C goal	Non-pharmacologic strategies
		If treatment for LDL-C (statin) does not achieve goal, consider:
		Fibrate, niacin, Ω-3 fatty acids
Very high (> 500 mg/dL)	Reduce triglycerides to prevent acute pancreatitis. Achieve LDL-C target and non-HDL-C. Investigate secondary causes of elevated TG levels, elevated TG unlikely due to FCHL	Pharmacologic treatment
		Fibrates are preferred or Niacin, Ω-3 fatty acids, and non-pharmacologic therapy

TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, FCHL: familial combined hyperlipidemia, TG: triglycerides

Table 4. Areas of opportunity to improve recognition, standards of care, and research in familial combined hyperlipidemia.

Areas of opportunity in FCHL
1. Lack of recognition of FCHL and its associated comorbidities by primary care physicians
2. Lack of recognition of associated cardiovascular risk and measures to decrease risk burden
3. Imprecise diagnosis often leads to a lack of family screening, which, in turn, delays treatment in affected individuals
4. Under treatment associated to lack of precise diagnosis or unrecognized possible metabolic and cardiovascular complications
5. Patients are not thoroughly followed, which impairs the ability to influence outcomes and decrease morbidity
6. Studies lack consistent definitions, which makes comparisons across studies difficult
7. Comparative studies of combined treatment strategies are required to improve outcome-oriented treatment algorithms
8. GWAS and EWAS are required to investigate common and rare genetic variants for FCHL in other populations
9. Metabolomics, proteomics, systems biology, and epigenetic studies are required to further the understanding of the pathophysiology of FCHL
10. Follow-up studies are required to evaluate cardiovascular and metabolic risk and assess methods for risk prediction in FCHL

FCHL: familial combined hyperlipidemia

efficacy of new treatments and determine the specific role of statin treatment intensity and fibrate use to improve therapeutic indications. Treatment evaluations should also focus on prevention of metabolic and cardiovascular complications in prospective long-term follow-ups. Given the shared genetic and pathophysiological features between FCHL and T2D, the role of insulin-sensitizing therapy should be evaluated in physiological assessments for human subjects and randomized, controlled clinical trials to determine its utility[40].

In summary, FCHL is a common disorder and the most prevalent primary dyslipidemia in the western world. Despite the extensive accumulated knowledge, FCHL is not usually considered as a first diagnostic choice because of a lack of awareness of its existence among primary care physicians. Therefore, FCHL is frequently undiagnosed, mostly due to its shifting clinical variability and the heterogeneity of diagnostic criteria, which leads to underreporting of its prevalence in epidemiological studies. This is significant since treatment is often delayed in affected family members that have not been evaluated. Areas of opportunity to improve recognition, standards of care, and research related to genetics, pathophysiology, cardiovascular risk, and management abound and call for further studies to confirm previous findings and increase awareness of this often neglected lipid disorder (Table 4). FCHL is a well-defined oligogenic

Table 5. Comparison between FCHL and familial hypertriglyceridemia as differential diagnosis of primary lipid disorders with elevated TG levels.

Features	FCHL	Familial hypertriglyceridemia
Former designations	Familial mixed hyperlipidemia, familial combined hyperlipoproteinemia, familial combined hypercholesterolemia-hypertriglyceridemia	Type 4 hyperlipidemia
Main lipoprotein disturbances	Elevated LDL-C and VLDL	Elevated VLDL
Typical onset	Adolescence	Adult age
Clinical features	Family history of coronary artery disease, cholesterol and triglyceride levels > 90th percentile, ApoB > 90th percentile, one family member with TC > 90th percentile, one family member with TG > 90th percentile, one family member with TC and TG > 90th percentile	TG levels > 200 mg/dL, Normal or decreased LDL levels, decreased HDL levels, Normal or decreased apoB levels, cholesterol:triglyceride ratio 1:5 when TGs reach 1000 mg/dL
Association with CVD	+++	++
Prevalence	1/40	1/20
Contribution of secondary factors	Obesity, IR, Metabolic Syndrome, T2D, NASH	Obesity, T2D, hypertension, pancreatitis, hyperuricemia, IR
Genetic features	Oligogenic	Variable
Genetic causes	*TXNIP, RXRA, CRABP2, ATF6, USF1, ANGPLT3, TCF7L2, APOA5, APOE*	*LPL, APOCII, APOA5, GPIHBP1, LMF1*
Current treatment	Statins, ezetimibe, fibrates	Fibrates, niacin, omega-3 fatty acids, fish oil
Future treatments	PCSK9 inhibitors, mipomersen	

HDL: high-density lipoprotein, LDL-C: low-density lipoprotein cholesterol, FCHL: familial combined hyperlipidemia, TG: triglycerides, VLDL: very low-density lipoprotein, ApoB: apolipoprotein B, TC: total cholesterol, T2D: type 2 diabetes, NASH: non-alcoholic steatohepatitis, IR: insulin resistance.

primary lipid disorder with a fluctuating lipid profile, increased cardiovascular risk, and comorbidity with other metabolic conditions such as T2D, NASH, and MS. It is unlikely that a similar phenotype could be found by chance in all patients who have been diagnosed with FCHL, thus, making the case for FCHL as an isolated lipid disorder. FCHL is a critical differential diagnosis in the setting of hypertriglyceridemia or a mixed dyslipidemia, particularly in distinguishing between common hypertriglyceridemia, mixed dyslipidemia, FCHL, and FHTG (Table 5).

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ORIGINAL ARTICLE

Optimization of kidney dysfunction prediction in diabetic kidney disease using targeted metabolomics

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Abstract

Aims Metabolomics have been used to evaluate the role of small molecules in human disease. However, the cost and complexity of the methodology and interpretation of findings have limited the transference of knowledge to clinical practice. Here, we apply a targeted metabolomics approach using samples blotted in filter paper to develop clinical-metabolomics models to detect kidney dysfunction in diabetic kidney disease (DKD).

Methods We included healthy controls and subjects with type 2 diabetes (T2D) with and without DKD and investigated the association between metabolite concentrations in blood and urine with eGFR and albuminuria. We also evaluated performance of clinical, biochemical and metabolomic models to improve kidney dysfunction prediction in DKD.

Results Using clinical-metabolomics models, we identified associations of decreased eGFR with body mass index (BMI), uric acid and C10:2 levels; albuminuria was associated to years of T2D duration, A1C, uric acid, creatinine, protein intake and serum C0, C10:2 and urinary C12:1 levels. DKD was associated with age, A1C, uric acid, BMI, serum C0, C10:2, C8:1 and urinary C12:1. Inclusion of metabolomics increased the predictive and informative capacity of models composed of clinical variables by decreasing Akaike's information criterion, and was replicated both in training and validation datasets.

Conclusions Targeted metabolomics using blotted samples in filter paper is a simple, low-cost approach to identify outcomes associated with DKD; the inclusion of metabolomics improves predictive capacity of clinical models to identify kidney dysfunction and DKD-related outcomes.

Keywords Metabolomics · Type 2 diabetes · Diabetic kidney disease · Filter paper · Amino acids · Acylcarnitines

Abbreviations

DKD	Diabetic kidney disease
DBS	Dried blood samples
T2D	Type 2 diabetes
eGFR	Estimated glomerular filtration rate
A1C	Glycosylated hemoglobin
ACEI/ARB	Angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
U/B ratio	Ratio or urinary divided by blood concentration of measured metabolites
ARG	Arginine
CIT	Citrulline
GLY	Glycine
ALA	Alanine
LEU	Leucine + isoleucine
MET	Methionine
PHE	Phenylalanine
TYR	Tyrosine
VAL	Valine
ORN	Ornithine

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PRO	Proline
SA	Succinylacetone
C0	Free carnitine
C2	Acetylcarnitine
C3	Propionylcarnitine
C4OH\C3DC	3-Hydroxybutyryl + malonyl carnitine
C5OH\C4DC	3-Hydroxyisovaleril + methylmalonyl carnitine
C5DC\C6OH	Glutaryl + 3-hydroxyhexanoyl carnitine
C6DC	Adipylcarnitine
C4	Butyrylcarnitine
C5	Isovalerylcarnitine
C5:1	Tiglylcarnitine
C6	Hexanoylcarnitine
C8	Octanoylcarnitine
C8:1	Octenoylcarnitine
C16	Decanoylcarnitine
C16:1	Decenoylcarnitine
C16:1OH	Decadienoylcarnitine
C16OH	Dodecanoylcarnitine
C10	Dedecenoylcarnitine
C10:1	Tetradecanoylcarnitine
C10:2	Tetradecenoylcarnitine
C12	Tetradecadyenylcarnitine
C12:1	3-Hydroxy-tetradecanoylcarnitine
C14	Hexadecenoylcarnitine
C14:1	Hexadecenoylcarnitine
C14:2	3-Hydroxy-hexadecanoylcarnitine
C14OH	3-Hydroxy-hexadecenoylcarnitinae
C18	Octadecanoylcarnitine
C18:1	Octadecenoylcarnitine
C18:1OH	Octadecenoylcarnitine
C18:2	3-Hydroxy-octadecanoylcarnitine
C18OH	3-Hydroxy-octadecanoylcarnitine

Introduction

Diabetic kidney disease (DKD) is a diabetes complication whose clinical diagnosis is made based on the presence of albuminuria and/or reduced estimated glomerular filtration rate (eGFR) in the absence of signs or symptoms of other primary causes of kidney damage [1]. DKD imposes significant burden in patients by increasing mortality risk and posing barriers for allotransplantation in advanced stages [2, 3]. Early DKD alterations include glomerular hypertrophy, mesangial expansion and basal membrane thickening, with late changes characterized by nodular sclerosis. Unfortunately, identification of histological alterations is costly, invasive and has not shown independent correlation with clinical outcomes [4, 5]. This has led to development of biomarkers to improve identification and screening of DKD. Existing kidney dysfunction indicators in DKD include serum creatinine and albuminuria, both of which have major limitations [6–8]. Serum creatinine concentration changes until there is an important loss of renal function. In addition, renal function is overestimated by the amount of tubular secretion of creatinine and varies per age, gender, muscle mass and metabolism, body weight, protein and water intake [5]. Albuminuria onset demonstrates established glomerular dysfunction but is not exclusive of DKD, despite being required for diagnosis [2, 7]. Additional kidney function markers in DKD include cystatin-C and the neutrophil gelatinase-associated lipocalin (NGAL), which have been shown to correlate with kidney dysfunction in T2D; nevertheless, their clinical usefulness remains to be further studied [7, 8]. Therefore, development of novel biomarkers that correlate with kidney dysfunction and improve identification of DKD at earlier stages is still an unmet necessity [2, 8].

The study of the metabolome aims to identify small-molecule profiles of complex biological samples instead of individual metabolites to improve the informative capacity of biochemical analyses [9–12]. The utility of metabolomics in DKD has been proved by different groups, who have reported abnormal plasma concentrations of amino acids and acylcarnitines associated with risk of progression of kidney disease [13–15]. Targeted metabolomics is an alternative for the study of relatively high number of samples, with the advantage that it can be performed on dry biological samples blotted in filter paper, which is a low-cost approach to store and handle biological samples [15]. To the best of our knowledge, this approach has not been applied in studies of DKD. This work aims to propose the complementary use of metabolomics to improve kidney dysfunction prediction in DKD.

Materials and methods

Subjects and study setting

We performed a cross-sectional evaluation of 200 T2D individuals who were recruited from our Diabetes Clinic and healthy subjects from the Metabolic syndrome cohort of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), which is a cohort study that includes a large number of subjects with and without T2D. We divided the study population into three groups: (1) Healthy normotensive, non-obese subjects with normal kidney function, (2) T2D subjects without DKD (T2Dnon-DKD) with diabetes diagnosis duration ≥ 10 years and (3) T2D with DKD (T2DDKD) with ≥ 10 years diabetes duration and any grade of diabetic retinopathy to confirm microvascular disease. We excluded subjects with other causes of kidney disease, previous acute ischemic heart disease or any condition that may alter albumin excretion or creatinine

clearance. T2D was diagnosed according to standardized ADA recommendations. Albuminuria was defined by urinary albumin > 30 mg/24 h and DKD by the presence of either albuminuria and/or eGFR < 60 mL/min/1.73 m^2. The Human Research Ethics Committee of the INCMNSZ approved all proceedings in the study.

Biochemical and anthropometric measurements

Twenty-four hour urine sample recollection was performed the day prior to biochemical evaluation; patients received a container with 200 µl of protease inhibitor (aprotinin protease inhibitor 500 KIU/mg, Thermo Fisher Scientific) and were instructed to collect urine during a 24 h period. After recollection, blood was drawn between 8:00–9:00 am after an overnight fast of 8–12 h. Glucose, total cholesterol, triglycerides, uric acid, serum and urine creatinine, HDL cholesterol, and albuminuria were determined by enzymatic colorimetric commercially available reagents using the Synchron CX Delta (Beckman Coulter); A1C levels were measured by high-performance liquid chromatography. For GFR estimation (eGFR), we calculated creatinine clearance using a 24 h creatinine measurement [16, 17].

In the same visit, a complete medical and family history was obtained from all subjects, including evaluation of dietary protein intake from 1 month and the day prior to urine recollection. Patients were weighed on calibrated scales and height was determined with a floor scale stadiometer. We consigned all medications used by patients; none of the subjects were receiving carnitine or fatty acid supplements during the evaluation.

Metabolomic analyses

Fasting capillary blood and 24 h urine samples obtained the day of biochemical evaluation were collected in filter paper cards (Protein Saver 903 cotton cards, Whatman-GE, USA), dried and conserved in refrigeration until analysis, performed as previously described [18, 19]. Eleven amino acids, free carnitine and 30 acylcarnitines were measured with a commercial kit (NeoBase Non-derivatized MS/MS kit; PerkinElmer Waltham Massachusetts). From each sample, a 3 mm diameter disk was punched with an automatic device (Dried blood spot punch Wallac 1296-071) into a 96-well sample plate and 190 µL of extraction solution containing a mixture of 22 stable isotope-labeled internal standards were added. The plate was covered with aluminum foil, incubated with agitation (30 °C at 650×*g* for 30 min). 30 µL of sample extracts were directly injected by a 2777 C Waters auto-sampler (Waters Corp., Milford, MA and HPLC pump Waters 1525 µ) to the electrospray tandem mass spectrometry equipment (Quattro Micro API tandem MS using multiple reaction monitoring (MRM) mode), with a flow rate of 1.5 mL/min and an analysis time of 1.5 min. Metabolites were quantified by reference to appropriate internal standards with the MassLynx® software. Low and high analytical controls were included in each plate in triplicate; additionally, a blank sample (extraction solution with internal standards) was included in each plate. Intra- and inter-plate variation coefficients were calculated based on repetitive measurements of the analytical control sample. Inter- and intra-assay variation coefficients ranged from 5 to 9%.

Statistical analysis

Inter-group comparisons

We used Kolmogorov–Smirnov test to explore distribution of each variable. Log and inverse transformations were applied to approximate normality in variables showing non-parametric distribution. Data are presented as mean ± SD or as median and interquartile range, where appropriate. Categorical variables are reported as frequencies and percentages; frequency distribution of categorical variables between groups was compared using chi-squared tests. To evaluate inter-group differences in individual metabolites, we compared metabolite concentrations across groups using ANOVA and Fisher's LSD for multiple post hoc comparisons. To evaluate the association between metabolites, eGFR and albuminuria, we performed partial correlation analysis adjusted for BMI, A1C, gender, ACRI/ARB use, SBP, DBP and dietary protein intake.

Linear clinical-metabolomics models

We developed manual step-wise multiple linear regression models using eGFR and albuminuria as dependent variables to evaluate the association of clinical, biochemical and metabolomic variables adjusted for age, sex, A1C, ACEI/ARB use, protein consumption, SBP and DBP. Variables were removed from the model until the best fitting model with the maximum adjusted r^2 was achieved; to confirm the improvement in the informative capacity of the model we used the Akaike information criterion (AIC) in both models. Models were also tested for multicollinearity using both tolerance and variance inflation factor (VIF). The models were validated using cross-validation derived from a training and validation samples randomly split from the original cohort to correct for over-optimism. Variables selected to enter regression analyses were those correlated significantly with albuminuria and eGFR.

Binary logistic clinical-metabolomics models

Variables associated with eGFR and albuminuria in linear regression analyses were included in binary logistic

regression models to detect decreased eGFR (< 60 mL/min) and albuminuria (> 30 mg/24 h), adjusted for age, sex, A1C levels, ACEI/ARB use, protein intake, SBP and DBP. The models were also validated using cross-validation. The performance of the models to be concordant with clinical outcomes was assessed using the area under a receiver operating characteristic (ROC) curve (Harrel's *c statistic*) of estimated probabilities obtained from regression analyses and goodness of fit was assessed using the Hosmer–Lemeshow test. A $p < 0.05$ was considered statistically significant. All statistical analyses were performed using Statistical Package for Social Sciences software (SPSS, version 21.0) and R software (Version 3.4.5).

Results

Clinical and biochemical characteristics of studied subjects

Two-hundred subjects were evaluated as follows: 43 subjects in the control group, 102 in the T2DnonDKD group and 55 in the T2DDKD group (Table 1). Recruitment process and sample size calculation are outlined in Supplementary Material. DKD subjects were significantly older, mostly male and had higher triglyceride, creatinine, A1C and lower HDL-c concentrations compared to other groups ($p < 0.001$). Seventy-four subjects were treated with ACEI/ARB, 19 subjects had overt DN (albuminuria > 300 g/24 h) and 24 subjects had eGFR < 60 mL/min/1.73 m^2.

Metabolite concentrations between groups and correlation with albuminuria and eGFR

We observed significant differences between T2DDKD and other groups in concentrations of blood C0, citrulline, tyrosine and C6, urinary C10:1, U/B proline, C6, C8, C10:1 and C10:2 ($p < 0.001$, Table 2). Next, we explored correlation between albuminuria, eGFR and metabolomic variables, which are shown in Table 3.

Linear clinical-metabolomics models

Using step-wise linear regression, we constructed a clinical model to predict albuminuria using A1C, serum creatinine and dietary protein intake, adjusted for age, sex, ACEI/ARB use, SBP and DBP (Table 4); when we introduced metabolomics, we found significant associations for serum citrulline, C0, C10:2 and urinary C12:1, which increased the explained variability of the model and decreased the AIC, thus improving informative capacity of the model. When using eGFR as the dependent variable, significant clinical associations included A1C, years of T2D exposure and uric acid; the inclusion of metabolomics increased the explained

Table 1 Clinical and biochemical characteristics of studied patients

Parameter	Healthy individuals ($N = 43$)	T2DnonDKD ($N = 102$)	T2DDKD ($N = 55$)	*P*
Female sex (%)	28 (65.1%)	69 (67.6%)	20 (36.4%)	< 0.001
Age (years)	54.65 ± 9.08*	60.40 ± 8.22	61.71 ± 8.82	< 0.001
BMI (kg/m^2)	25.21 ± 3.51*	28.17 + 3.95	27.11 + 3.87	< 0.001
Waist/hip ratio	0.89 ± 0.15*	0.92 ± 0.07	0.94 ± 0.08	0.015
Systolic BP (mmHg)	105.09 ± 13.79*	125.51 ± 16.79	130.6 ± 19.38	< 0.001
Diastolic BP (mmHg)	70.25 ± 8.87*	74.4 ± 9.98	76.71 ± 11.30	0.004
Fasting glucose (mg/dL)	95.34 ± 9.94*	160.58 ± 57.84	161.03 ± 78.83	< 0.001
A1C (%)	5.58 ± 0.36*	8.5 ± 2.03^{+}	9.35 ± 2.24	< 0.001
Triglycerides (mg/dL)	129.0 (80.0-198.0)*	168.0 (111.3-241.3)	184.0 (126.0-230.0)	0.02
Total cholesterol (mg/dL)	203.28 ± 39.70*	184.32 ± 41.77	193.55 ± 41.21	0.08
HDL-C (mg/dL)	55.53 ± 15.61*	46.14 ± 13.08	46.24 ± 15.41	0.002
LDL-C (mg/dL)	118.35 ± 28.40*	100.23 ± 33.91	105.19 ± 30.45	0.02
Serum creatinine (mg/dl)	0.75 ± 0.18	0.73 ± 0.18	1.17 ± 0.84$^{\&}$	< 0.001
Albuminuria (mg/24 h)	4.02 (0.0-5.90)	7.05 (2.75–13.17)	120.0 (37.8-554.4) $^{\&}$	< 0.001
Uric acid (mg/dL)	5.23 ± 1.28	5.18 ± 1.17	6.21 ± 1.56$^{\&}$	< 0.001
Diabetes duration (yr)	–	16.5 ± 7.31	20.09 ± 8.82	< 0.001
eGFR (ml/min/1.73 m^2)	90.44 (79.5-106.7)	99.8 (76.4-120.3)	73.3 (52.6-104.7)$^{\&}$	< 0.001

Values are means ± SD, unless indicated otherwise

T2D Type 2 diabetes mellitus, *DKD* diabetic kidney disease, *BMI* body mass index, *BP* blood pressure, *A1C* glycosylated hemoglobin, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *eGFR* estimated glomerular filtration rate

*$p < 0.001$ Healthy vs. T2DnonDKD and T2DDKD, $^{+}$T2DnonDKD vs. T2DDKD, $^{\&}$T2DDKD vs. healthy and T2DnonDKD

Table 2 Levels of amino acids and acylcarnitines in blood, urine and the urine/blood ratio

Sample	Metabolite concentration (μM)	Healthy subjects median ± IQR	T2DnonDKD median ± IQR	T2DDKD median ± IQR
Blood	Citrulline	24.8 (20.2–30.2)*	15.2 (11.4–20.5)[&]	19.5 (16.2–27.0)
	Methionine	5.1 (3.8–6.1)*	3.1 (2.3–4.4)	3.2 (2.6–4.5)
	Phenylalanine	42.9 (38.5–47.2)*	35.8 (31.2–40.3)	36.2 (30.9–41.2)
	Tyrosine	56.3 (51.0–67.2)*	50.0 (40.8–60.7)[&]	46.0 (39.3–53.2)
	Valine	141.8 (122.1–160.1)[#]	119.2 (102.4-139.3)	120.0 (100.0–141.2)
	Ornithine	34.0 (30.0–42.4)*	14.7 (10.0-28.7)	16.8 (12.6–27.6)
	Carnitine	33.6 (28.0–38.7)[#]	34.1 (27.7–41.9)[&]	40.7 (32.1–47.6)
	C4OH:C3D	0.04 (0.03–0.05)*	0.04 (0.03–0.06)	0.05 (0.04–0.06)
	C5DC\C6OH	0.10 (0.09–0.12)[#]	0.13 (0.10–0.16)	0.14 (0.11–0.17)
	C6	0.04 (0.03–0.04)[#]	0.03 (0.027–0.040)[&]	0.04 (0.03–0.05)
	C8	0.08 (0.06–0.10)	0.07 (0.05–0.10)[&]	0.08 (0.06–0.14)
	C8:1	0.12 (0.09–0.14)[#]	0.13 (0.09–0.17)[&]	0.15 (0.10–0.22)
	C10:2	0.01 (0.01–0.02)[#]	0.01 (0.01–0.02)[&]	0.02 (0.01–0.02)
	C14:1	0.05 (0.04–0.07)*	0.04 (0.03–0.05)	0.05 (0.03–0.06)
	C14:2	0.02 (0.02–0.03)*	0.02 (0.01–0.02)	0.02 (0.01–0.02)
	C18:1OH	0.02 (0.02–0.03)[#]	0.02 (0.02–0.03)	0.02 (0.02–0.03)
Urine	Glycine	1855.1 (1042.9-3242.4)[#]	1614.2 (1025.7–3048.0)	1356.4 (716.5-2229.1)
	Proline	28.1 (22.9–37.5)*	51.3 (38.9–81.8)	65.1 (35.7-130.1)
	C10:1	1.13 (0.81–2.09)*	1.00 (0.71–1.49)[&]	0.90 (0.65–1.48)
	C12:1	0.76 (0.64–1.05)[#]	0.73 (0.55-1.00)[&]	0.58 (0.42–0.88)
Urine/blood	Citrulline	0.70 (0.41–1.01)*	1.12 (0.82–1.85)[+]	1.24 (0.78–1.90)
	Methionine	3.67 (2.37–5.28)*	6.23 (4.18–9.48)	5.93 (3.83–9.66)
	Ornithine	0.87 (0.62–1.19)*	2.08 (1.18–3.80)	2.05 (1.26–3.18)
	Proline	0.20 (0.14–0.27)*	0.32 (0.24–0.51)[&]	0.40 (0.26–0.81)
	C6	30.0 (18.6–75.2)*	25.6 (15.5–40.6)	16.7 (9.8–36.5)
	C8	18.1 (10.1–30.9)[#]	17.3 (9.3–23.4)	12.3 (7.5–19.1)
	C10:1	69.0 (40.5-103.9)[#]	64.4 (43.9–88.3)[&]	34.4 (21.0-61.5)
	C10:2	279.3 (167.9-370.6)[#]	196.52 (144.9-345.5)[&]	148.3 (76.4–201.0)
	C12:1	16.8 (11.5–21.1)[#]	18.6 (14.4–25.1)[&]	13.4 (11.1–17.6)
	C14:2	15.2 (12.3–22.0)*	27.4 (17.6–37.3)	20.6 (14.6–31.5)

Post hoc analysis by Fisher LSD. *p* values shown are differences between the group with DKD and the other groups

T2D Type 2 diabetes mellitus, *DKD* diabetic kidney disease, *IQR* interquartile range

*p value < 0.05 for healthy vs. T2DnonDKD and T2DDKD

[+]$p < 0.05$ for healthy vs. T2DnonDKD

[#]$p < 0.05$ for healthy vs. T2DDKD

[&]$p < 0.05$ for T2DnonDKD vs. T2DDKD

variability of the model, with significant associations for serum citrulline, C8:1, C10:2, urinary C10:1 and U/B proline (Table 4), which increased the $r^2$and decreased the AIC of the model.

Logistic clinical-metabolomics models

When evaluating specific outcomes (Table 5), decreased eGFR was significantly associated with BMI and uric acid levels. The inclusion of metabolomics (C10:2) increased the explained variability and AUC of the model. Similarly, albuminuria > 30 mg/day was associated to years of T2D exposure, A1C, uric acid, creatinine and protein intake; the inclusion of serum C0, C10:2 and urinary C12:1 increased the r^2 and AUC of the models. Finally, we developed a clinical-metabolomics model for DKD, which included BMI, A1C, uric acid, age, C0, C10:2, C8:1 and urinary C12:1, which had a higher r^2 and AUC compared to the clinical model.

Table 3 Partial correlations between microalbuminuria and eGFR with biochemical and metabolomic variables adjusted by blood pressure, A1C, gender, age, and body mass index

		Albuminuria		Glomerular filtration rate	
Blood	Variable	*r*	*p* Value	*r*	*p* Value
	Citrulline	0.293	< 0.001	− 0.282	< 0.001
	C0	0.272	< 0.001	− 0.234	< 0.001
	C5DC/C6O	0.045	0.53	− 0.26	< 0.001
	C6	0.233	< 0.001	− 0.232	< 0.001
	C8	0.15	0.037	− 0.161	0.025
	C8:1	0.15	0.037	− 0.438	< 0.001
	C10:2	0.312	< 0.001	− 0.474	< 0.001
	C14:1	0.124	0.085	− 0.173	0.016
	C14:2	0.111	0.124	− 0.181	0.012
	C18:1OH	0.199	0.005	− 0.133	0.065
Urine	Glycine	− 0.122	0.09	0.208	0.004
	Proline	0.152	0.035	− 0.266	< 0.001
	C10:1	− 0.225	0.002	0.312	< 0.001
	C12:1	− 0.242	0.001	0.245	0.001
Urine/blood	Proline	0.154	0.032	− 0.257	< 0.001
	C6	− 0.142	0.048	0.214	0.003
	C8	− 0.165	0.022	0.22	0.002
	C10	− 0.195	0.007	0.255	< 0.001
	C10:1	− 0.29	< 0.001	0.439	< 0.001
	C10:2	− 0.288	< 0.001	0.392	< 0.001
	C12:1	− 0.312	< 0.001	0.383	< 0.001
	C14:2	− 0.204	0.004	− 0.207	0.004

Clinical-metabolomics model validation

We then evaluated both the linear and binary logistic regression models using training ($N = 118$) and validation datasets ($N = 82$) to correct findings for over-optimism and validate the findings. We observed an increase in r^2 and a decrease in AIC with the inclusion of metabolomics without evidence of multicollinearity for all models, which was replicated in both datasets (Table 6).

Discussion

Our study highlights the development of clinical-metabolomics models related to kidney dysfunction in DKD. Here, we replicate previously reported abnormalities in metabolomics linked to DKD using a validated method of dried biological samples (DBS) blotted in filter paper. This method may facilitate sample handling and could be applied in large-scale efforts to identify new metabolomics-based biomarkers of DKD. Targeted metabolomics has recently been introduced in the study of diverse models of disease and its use in DKD has previously been reported in some animal models and more recently in human subjects. Differences in plasma and/or urine metabolomics between T2DDKD and controls suggest that this condition is associated with abnormalities in glycolysis and lipid and amino acid pathways [20–25]. At this time, identification of specific biomarkers of DKD using metabolomics is a topic that remains largely unexplored [26].

First, we evaluated clinical-metabolomics profiles that explain the variability in identifying kidney dysfunction in our cohort. We observed that the inclusion of metabolomic and clinical variables improved the explained variability of linear models for albuminuria and eGFR and yielded predictive improvements. In addition, performances of estimated probabilities from clinical models are improved with the inclusion of metabolomics to detect decreased glomerular function, albuminuria and DKD. These observations are consistent with the expected course of kidney dysfunction in DKD, since models included A1C, BMI and years of T2D exposure, as well as protein intake for albuminuria and markers of kidney dysfunction including serum creatinine and uric acid, which is a byproduct of purine metabolism and is elevated in the setting of cellular hypoxia, oxidative stress and inflammation, processes which have been linked to kidney dysfunction, particularly albuminuria, in DKD [27, 28]. Overall, our results demonstrate that inclusion of metabolomics improves the detection threshold of glomerular dysfunction over traditional clinical variables and confirms the significance of studying metabolomics to evaluate kidney dysfunction patients with DKD [29–32].

As our observations confirmed, altered metabolic pathways in amino acid biosynthesis might be relevant in DKD [33]. Citrulline concentrations are decreased in subjects with T2D regardless of the presence of DKD. However, we observed higher serum concentrations in T2DDKD compared to T2DnonDKD. Elevated levels of citrulline and other urea cycle metabolites have been shown to be related to kidney disease progression in T2D [22, 25, 26]. A possible alteration of citrulline to arginine conversion has been proposed as an explanation, given that these metabolites normally compete with endothelial nitric oxide synthase to increase nitric oxide production, stabilizing endothelial function [34]. In the case of eGFR, increased urinary and U/B proline were also identified in T2DDKD subjects, which indicates increased proline production. High proline levels have been related to insulin deprivation and products of proline metabolism have been linked to glomerular dysfunction in advanced chronic kidney disease [34, 35]. Finally, we observed altered blood concentrations of phenylalanine in patients with DKD, which is similar to previous reports of low tyrosine levels in patients with type 2 diabetes and advanced CKD [36]. Plasma elevations of acylcarnitines in patients with albuminuria has previously been described [37, 38], in our work we only found differences in U/B medium

Table 4 Multiple linear regression analysis showing independent variables associated with albuminuria and glomerular filtration rate

	Model	Parameters	Parameters	β	Standarized β	t	p value	95% CI
Albuminuria	Clinical	$R^2=0.318$ $F=10.772$ $P<0.001$ AIC = 224.26	A1C	0.191	0.208	3.016	0.003	0.066–0.317
			T2D duration	0.041	0.201	2.542	0.012	0.009–0.073
			Protein intake	−0.776	−0.217	−3.257	0.001	−1.246 to −0.306
	Clinical + metabolomics	$R^2=0.460$ $F=13.376$ $P<0.001$ AIC = 183.57	A1C	0.256	0.279	4.408	< 0.001	0.142–0.371
			T2D duration	0.031	0.152	2.062	0.041	0.001–0.060
			Protein intake	−0.616	−0.172	−2.855	0.005	−1.041 to −0.190
			Citrulline	2.508	0.217	3.126	0.025	0.213–3.087
			C0	2.441	0.137	2.058	0.002	1.290–5.438
			C10:2	2.256	0.175	2.703	0.025	0.001–0.020
			C12:1 U	−2.187	−0.213	−3.365	< 0.001	−3.982 to −1.577
eGFR	Clinical	$R^2=0.395$ $F=13.146$ $P<0.001$ AIC = −392.14	A1C	0.034	0.180	2.750	0.007	0.010–0.059
			T2D duration	−0.008	−0.196	−2.622	0.010	−0.014 to −0.002
			Uric acid	−0.116	−0.372	−5.902	< 0.001	−0.155 to −0.077
	Clinical + metabolomics	$R^2=0.650$ $F=24.056$ $P<0.001$ AIC = −490.0	A1C	0.041	0.218	4.085	< 0.001	0.021–0.061
			Uric acid	−0.065	−0.209	−4.101	< 0.001	−0.097 to −0.034
			Citrulline	−0.333	−0.136	−2.688	0.008	−0.577 to -0.088
			C8:1	−0.537	−0.233	−4.314	< 0.001	−0.782 to −0.291
			C10:2	−0.003	−0.205	−4.054	< 0.001	−0.005 to −0.002
			C10:1 U	0.462	0.300	6.083	< 0.001	0.312–0.611
			Proline U/B	−0.329	−0.259	−5.400	< 0.001	−0.449 to −0.209

Models adjusted by age, sex, BMI, SBP, DBP, A1C, T2D duration and ACEI/ARB use

T2D Type 2 diabetes, *BMI* body mass index, *SBP* systolic blood pressure, *DBP* dyastolic blood pressure, *A1C* glycosylated hemoglobin, *eGFR* estimated glomerular filtration rate

and large chain acylcarnitines between T2DDKD and the other groups. Previous reports have suggested that accumulation of various acylcarnitines in plasma demonstrates impaired metabolite clearance due to CKD and other authors have proposed that an increase of urinary acylcarnitines is associated with early kidney damage, reflecting alterations in the β-oxidation pathway, which has also demonstrated alterations in murine models of diabetic nephropathy [39–44]. The observed progressive increase of serum carnitine concentrations in our study ranging from controls, T2DnonDKD and T2DDKD subjects has been formerly documented as related to impairment of acylcarnitine excretion and decreased carnitine clearance, indicating mitochondrial damage, which could lead to activation of oxidative stress pathways [43, 45]. Available evidence supports the benefit of carnitine supplementation in hemodialyzed T2D subjects, but the use of carnitine in DKD deserves more profound studies [40]. The contribution of acylcarnitines to our clinical-metabolomics models to identify diseased individuals with albuminuria or decreased glomerular function, indicates the elevated importance of acylcarnitines as markers of glomerular disease in DKD and its implications in identifying kidney dysfunction in subjects with T2D. Future studies should evaluate the role of metabolomics to evaluate treatment response and prediction of changes in kidney function related to acylcarnitine supplementation in individuals with DKD, as well as the role metabolomics to evaluate the impact of T2D medication in ameliorating kidney dysfunction [46]; proving such approach could be useful as a further clinical application for metabolomics research.

As shown, we were able to replicate findings from previous metabolomics approaches using DBS blotted in filter paper, which demonstrates that this technique is both viable and useful for targeted metabolomics in the study of DKD. Studies of the metabolome require collection and storage of biological samples, which is complex and costly [23]. DBS collection in filter paper is a relevant method to study complex biological samples, particularly in studies that face challenges of large sample size, longitudinal assessment or frequent sampling in which DBS collection in filter paper would reduce storage costs and facilitate sample collection and handling [47]. In general terms, any analyte that can be measured from whole blood, serum or plasma can be measured from DBS on filter paper, with the additional advantage of stabilizing and reducing the degradations of numerous analytes due to buffering by the dried blood-matrix [48, 49]. Our results indicate that the use of this approach could be helpful in studying altered metabolic pathways linked to

Table 5 Logistic regression analyses using decreased GFR, albuminuria and DKD as dependent variables

	Model	Parameters	Parameter	β	OR	95%CI	p Value
Glomerular filtration rate < 60 mL/min	Clinical	$R^2 = 0.344$ $P < 0.001$ $\chi^2 = 6.42$, $p = 0.600$ c-statistic = 0.853 (95%CI 0.776–0.931)	BMI	−0.170	0.844	0.719–0.990	0.037
			Uric acid	0.711	2.036	1.370–3.025	< 0.001
	Clinical + metabolomics	$R^2 = 0.547$ $P < 0.001$ $\chi^2 = 10.31$, $p = 0.244$ c-statistic = 0.924 (95%CI 0.863–0.984)	BMI	−0.197	0.821	0.682–0.989	0.038
			Uric Acid	0.580	1.786	1.149–2.776	0.010
			C10:2	0.063	1.065	1.033–1.098	< 0.001
Albuminuria > 30 mg/day	Clinical	$R^2 = 0.489$ $P < 0.001$ $\chi^2 = 5.69$, $p = 0.682$ c-statistic = 0.891 (95%CI 0.845–0.938)	T2D duration	0.058	1.060	1.006–1.117	0.029
			A1C	0.412	1.510	1.212–1.882	< 0.001
			Uric acid	0.472	1.603	1.138–2.259	0.007
			Creatinine	0.918	2.504	1.054–5.946	0.038
			Protein intake	−1.102	0.332	0.138–0.802	0.014
	Clinical + metabolomics	$R^2 = 0.545$ $P < 0.001$ $\chi^2 = 7.51$, $p = 0.483$ c-statistic = 0.908 (95%CI 0.865–0.951)	T2D duration	0.054	1.051	0.997–1.107	0.062
			A1C	0.464	1.590	1.262–2.004	< 0.001
			Uric acid	0.394	1.482	1.031–2.132	0.034
			Creatinine	0.322	1.380	0.584–3.262	0.463
			Protein intake	−0.861	0.423	0.166–1.077	0.071
			C0	0.053	1.054	0.999–1.112	0.056
			C10:2	0.021	0.099	0.017–0.582	0.010
			C12:1 U	−2.309	1.021	1.000-1.043	0.049
Diabetic kidney disease	Clinical	$R^2 = 0.485$ $P < 0.001$ $\chi^2 = 11.41$, $p = 0.180$ c-statistic = 0.879 (95%CI 0.830–0.929)	BMI	−0.167	0.846	0.743-.963	0.012
			A1C	0.353	1.424	1.153–1.758	0.001
			Uric acid	0.595	1.813	1.299–2.530	< 0.001
			T2D duration	0.071	1.074	1.017–1.134	0.011
	Clinical + metabolomics	$R^2 = 0.589$ $P < 0.001$ $\chi^2 = 4.082$, $p = 0.850$ c-statistic = 0.913 (95% CI 0.874–0.953)	BMI	−0.192	0.825	0.715–0.951	0.008
			A1C	0.520	1.682	1.307–2.165	< 0.001
			Uric acid	0.506	1.659	1.138–2.421	0.009
			Age	0.078	1.081	1.005–1.162	0.037
			C0	0.055	1.056	1.000-1.115	0.050
			C10:2	0.033	1.034	1.012–1.056	0.002
			C8:1	−0.157	0.854	0.747–0.977	0.021
			C12:1 U	−1.751	0.174	0.033–0.919	0.039

Models adjusted for by age, sex, BMI, SBP, DBP, A1C, T2D duration and ACEI/ARB use

BMI body mass index, *SBP* systolic blood pressure, *DBP* dyastolic blood pressure, *A1C* glycosylated hemoglobin, *eGFR* estimated glomerular filtration rate, *OR* Odds ratio, *95% CI* 95% confidence interval, *AUC* area under the curve

DKD and these results could be extrapolated to other disease models.

Our study had some strengths and limitations. First, we were able to replicate previous findings in metabolomics of DKD using a low-cost approach in both training and replication datasets, which could be implemented in other studies to reduce costs associated to sample processing and storage. Second, we could collect both serum and urine samples to estimate differences in metabolite concentrations in a cohort of patients including healthy individuals, which allowed us to construct clinical-metabolomics models to identify kidney dysfunction in DKD using a targeted metabolomics approach. Furthermore, duration of T2D in our cohort had a wide range of disease exposure, with a minimum of 10 years; this reassures that kidney dysfunction is attributable to T2D. Amongst the limitations of our study is the cross-sectional design, which precluded us from estimating the role of metabolites in identifying progression of in kidney dysfunction and the relatively small number of cases with albuminuria > 300 mg/24 h, which did not allow for comparison of overt diabetic nephropathy cases. Furthermore, since variables were controlled in statistical analysis, there exists a possibility of residual confounding.

Table 6 Model parameters for linear logistic regression clinical and clinical-metabolomics models using a training ($N=118$) and validation ($N=82$) datasets

Linear model	Model	Sample	Adjusted r^2	AIC	p value
Albuminuria	Clinical	Training	0.197	133.06	0.004
		Validation	0.278	118.61	< 0.001
	Clinical + metabolomics	Training	0.420	110.61	< 0.001
		Validation	0.411	99.66	< 0.001
eGFR	Clinical	Training	0.445	− 168.32	< 0.001
		Validation	0.354	− 232.52	< 0.001
	Clinical + metabolomics	Training	0.720	− 218.88	< 0.001
		Validation	0.615	− 286.33	< 0.001
		Validation	0.524	4.81	0.903 (0.835–0.970)
Logistic model	Model	Sample	Adj. r^2	χ^2	c-statistic (95%CI)
Albuminuria (> 30 mg/g)	Clinical	Training	0.465	3.34	0.883 (0.819–0.947)
		Validation	0.524	4.81	0.903 (0.835–0.970)
	Clinical + metabolomics	Training	0.584	9.14	0.903 (0.848–0.959)
		Validation	0.578	4.32	0.901 (0.830–0.971)
eGFR (< 60 mL/min)	Clinical	Training	0.323	3.35	0.845 (0.745–0.945)
		Validation	0.487	15.12	0.918 (0.840–0.997)
	Clinical + metabolomics	Training	0.530	3.30	0.918 (0.840–0.997)
		Validation	0.717	16.14	0.931 (0.835-1.000)
DKD	Clinical	Training	0.469	3.880	0.874 (0.808–0.940)
		Validation	0.540	2.948	0.889 (0.815–0.962)
	Clinical + metabolomics	Training	0.673	4.193	0.930 (0.884–0.975)
		Validation	0.596	6.432	0.886 (0.884–0.975)

eGFR estimated glomerular filtration rate, *DKD* diabetic kidney disease, *AIC* Akaike's information criteria, *AUC* area under the curve (Harrel's *c*-statistic)

In conclusion, our study demonstrates the applications of targeted metabolomics in the study of metabolic alterations in DKD using a low-cost approach. The use of metabolomics evaluated in DBS in filter paper as a complementary method for DKD identification offers a practical alternative that could also shed light on the pathophysiology of DKD. Implementation of predictive models grouping clinical variables to identify glomerular dysfunction and albuminuria are improved with the use of recognized altered metabolites. The role of these metabolites as biomarkers of DKD remains to be studied and confirmed in independent longitudinal follow-up and replication cohorts. Targeted metabolomics in the study of DKD, performed simultaneously in blood and urine samples, is feasible and accessible in DBS collected in filter paper, which is a simple recollection device that allows the possibility of massive sampling, storage and analysis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Written informed consent was obtained before the examination from each patient, as well as the approval from our institutional ethics committee.

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Mexican Carriers of the *HNF1A* p.E508K Variant Do Not Experience an Enhanced Response to Sulfonylureas

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OBJECTIVE

To assess if an ethnic-specific variant (p.E508 K) in the maturity-onset diabetes of the young (MODY) gene hepatocyte nuclear factor-1α (*HNF1A*) found in Mexicans is associated with higher sensitivity to sulfonylureas, as documented in patients with MODY3.

RESEARCH DESIGN AND METHODS

We recruited 96 participants (46 variant carriers and 50 age- and sex-matched noncarriers). Response to glipizide (one 2.5–5.0-mg dose), metformin (four 500-mg doses), and an oral glucose challenge was evaluated using a previously validated protocol. Glucose and insulin levels and their areas under the curve (AUCs) were compared between groups.

RESULTS

Carriers of the p.E508 K variant had a lower maximum insulin peak during the glipizide challenge as compared with noncarriers with diabetes ($P < 0.05$). Also, carriers had a lower insulin response after the oral glucose challenge. Following an oral glucose tolerance test in the presence of metformin, carriers of the p.E508 K variant with diabetes had a lower maximum insulin peak and total and incremental insulin AUC value as compared with noncarriers with diabetes ($P < 0.05$). A similar but nonsignificant trend was seen in participants without type 2 diabetes.

CONCLUSIONS

Carriers of variant p.E508 K in *HNF1A* have a reduced insulin response rather than the increased sensitivity to sulfonylureas seen in patients with MODY3.

Type 2 diabetes is the leading cause of death and a major burden for public health of Mexican and Latino populations (1). Ethnic-specific genetic variants have been described in populations with a Native American heritage (such as Mexicans). The Slim Initiative in Genomic Medicine for the Americas (SIGMA) Type 2 Diabetes Consortium has reported associations with a common haplotype in *SLC16A11* (2) through a genome-wide association study in ~9,000 participants and a rare missense variant (c.1522G > A [p.E508 K]) in the gene encoding the hepatocyte nuclear factor-1α (*HNF1A*) through whole-exome sequencing in ~4,000 participants (3), both of which are rare or absent in non–Native American populations. The Mexican population results from a recent admixture of European and Native American populations with similar proportions and a relative low African ancestry (<5%). This admixture is

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known as mestizo population. Therefore, exome-sequencing in Mexican mestizos has resulted in the identification of genetic variants mainly derived from our Native American heritage. In particular, the *HNF1A* variant, located in exon 8, causes a partial defect in the function of the transcription factor (3), which is expressed in liver and pancreas. Though the p.E508 K variant is associated with a fivefold increased risk for developing type 2 diabetes, patients with the variant were clinically undistinguishable in terms of age of onset, adiposity, and glycemia from type 2 diabetes cases (3). It is present in 0.2% of the Mexican participants with type 2 diabetes in the SIGMA exome-sequencing analyses (3). Loss-of-function mutations in *HNF1A* cause maturity-onset diabetes of the young type 3 (MODY3) (4,5). MODY3 is characterized by an early onset of the disease, normal body weight, glycosuria, and high risk for microvascular complications (6–8). In addition, patients with MODY3 have high sensitivity to sulfonylureas; this feature underlies the current indication for sulfonylurea therapy in these patients, over metformin or insulin (8,9), and represents one successful example of precision medicine in diabetes.

Because p.E508 K causes partial loss of function in *HNF1A*, we hypothesized that carriers of this variant might also exhibit heightened sensitivity to sulfonylureas. The aim of our study was to advance precision medicine by evaluating the insulin and glycemic responses to glipizide and metformin in p.E508 K carriers and age- and sex-matched noncarriers. We followed the approach used in the Study to Understand the Genetics of the Acute Response to Metformin and Glipizide in Humans (SUGAR-MGH), a standardized pharmacogenetic protocol (10,11).

RESEARCH DESIGN AND METHODS

The study was supported by the SIGMA Type 2 Diabetes Consortium and performed in accordance with the SUGAR-MGH protocol (10). The study protocol was approved by the Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

Subjects and Setting

Mexican mestizos (defined as a Mexican individual whose father, mother, and grandfathers were born in Mexico, and they do not belong to any other ethnic group [i.e., Jewish, Japanese, etc.]) aged ≥18 years, males or nonpregnant females, healthy control subjects (n = 33), and patients with type 2 diabetes (n = 16) were recruited at the diabetes outpatient clinic of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City, a center attending individuals from the general population. An additional recruitment of carriers and noncarriers of the p.E508 K *HNF1A* variant was done among users of the diabetes outpatient clinic of the Instituto Nacional de Ciencias Médicas y Nutrición or among the first-degree relatives of the E508 K variant carriers. Genotyping of the p.E508 K variant (rs483353044) was performed using a TaqMan probe. For the assay, we included control subjects previously sequenced and selected carrying the three possible genotypes for this single nucleotide polymorphism. Case subjects with type 2 diabetes were eligible if they were being treated with no more than two oral antidiabetic agents and had an A1C ≤7.5% (58 mmol/mol). Noncarriers were matched by age (±5 years), sex, BMI (±2 kg/m^2), and status of diabetes.

Volunteers were excluded from participation if they were pregnant, nursing, or women at risk for becoming pregnant; if they had age of onset of diabetes before 25 years of age, known history of liver or kidney disease, allergy to sulfonamides, history of porphyria, impaired renal function (estimated glomerular filtration rate <60 mL/min/1.73 m^2), established coronary artery disease, or history of bariatric surgery, seizures, or stroke; or if they were taking medications that could affect glycemic parameters; or if they were planning radiologic or angiographic studies requiring contrast within 1 week of completion of the study. All participants read and signed an informed-consent document.

Interventions

The study consisted of two visits (Fig. 1) (10). Prior to visit 1, participants with type 2 diabetes taking an oral antidiabetic agent underwent a 7-day washout period. Blood samples were obtained between 8:00 A.M. and 9:00 A.M. in the morning after an 8–12-h overnight fast. A complete medical and family history as well as anthropometric measurements were obtained. Participants were weighed on calibrated scales, and height was determined with a floor scale stadiometer; BMI was calculated as weight in kilograms divided by the squared product of height in meters. Participants with a fasting glucose ≤80 mg/dL were not eligible to receive the sulfonylurea challenge for safety reasons; those with fasting glucose 80–99 mg/dL received 2.5 mg of glipizide, and those with a fasting glucose ≥100 mg/dL received 5 mg. After glipizide administration, blood samples were collected at 30, 60, 90, 120, 180, and 240 min for glucose and insulin measurements. After the 240-min period, breakfast was given, and patients were

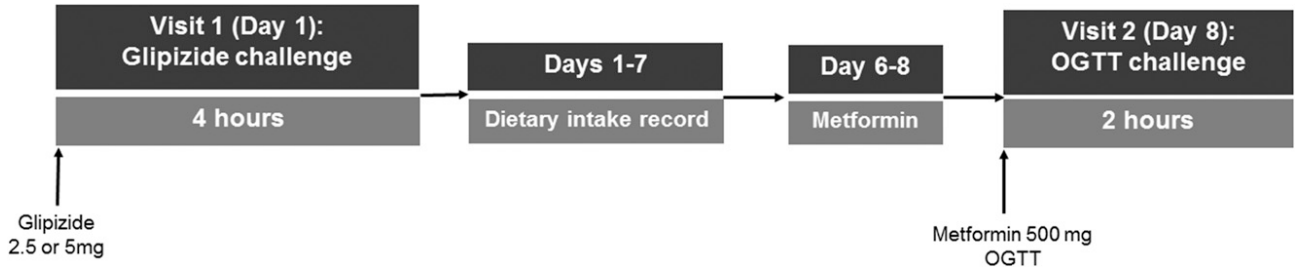

Figure 1—Outline of the study period and intervention. Subjects were divided according to their carrier and noncarrier status and then according to their status of type 2 diabetes into four groups. Subjects with type 2 diabetes had a medication washout period of 7 days before the procedure. Visit 1 consisted of biochemical evaluation and a glipizide challenge that lasted 4 h and had measurements at times 0, 30, 60, 90, 120, 180, and 240 min. From days 1 to 5, the collection of a dietary intake record was instructed for all participants; at day 6, they had to take 500 mg of metformin at night and again before a 2-h OGTT, with blood measurements at times 0, 30, 60, and 120 min.

discharged once their glucose levels were ≥80 mg/dL.

At day 6 after the initial visit and provided they had normal renal function on safety laboratory testing, participants were instructed to take metformin (500 mg) at night. On day 7, they received metformin (500 mg) in the morning and in the evening. On day 8, they returned for their second visit, when they received one last dose of 500 mg metformin 60 min before a 75-g oral glucose tolerance test (OGTT). Blood samples were collected at 5, 10, 15, 30, 60, and 120 min for glucose and insulin measurements. Once visit 2 was completed, participants with treated type 2 diabetes were allowed to restart their routine antidiabetic therapy.

Glucose and insulin levels and their areas over the curve (AOCs) and areas under the curve (AUCs), respectively, after the glipizide dose were used to assess the sulfonylurea response. AUCs for insulin and glucose were also used to evaluate the response to oral glucose challenge. Metformin response was evaluated by comparing fasting glucose after the metformin challenge and HOMA of insulin resistance (HOMA-IR) scores between visits 1 and 2.

Serum insulin concentration was measured using a chemiluminescent immunoassay (Access 2; Beckman Coulter). HbA_{1c} levels were estimated using high-performance liquid chromatography (Variant II Turbo; Bio-Rad). Plasma glucose and lipid concentrations (cholesterol, triglycerides, and HDL cholesterol), uric acid, creatinine, and hepatic enzymes were measured using commercially available assays (UniCel DxC 600 Synchron Clinical System; Beckman Coulter). LDL cholesterol was calculated with the Friedewald equation when triglycerides were <250 mg/dL. Plasma apolipoproteins B and AI were measured using immunonephelometry (Beckman Coulter).

Statistical Analyses

To evaluate differences between groups in clinical, sociodemographic, and biochemical measures, we performed a Student *t* test and Mann-Whitney *U* for parametric and nonparametric quantitative variables, respectively. Frequency distribution of the categorical variables in the four groups was compared using χ^2 tests. Logarithmic transformations were applied to approximate normality in those variables showing a nonparametric distribution using the Kolmogorov-Smirnov test.

The primary outcome measure was insulin peak concentration during the glipizide challenge and during the OGTT after metformin exposure. As complementary analyses to evaluate differences in insulin secretion, action, and response in carriers and noncarriers, we calculated AUCs of insulin concentrations across time after the glipizide challenge and insulin and glucose concentration after the metformin challenge using the trapezoidal method adjusted for baseline concentrations; and the AOC, to evaluate changes in glucose concentrations during the glipizide challenge, was calculated using the formula $AOC = FG_0 \times 4 \times AUC$, in which FG_0 was fasting glucose before glipizide administration and the estimated AUC for glucose during the challenge using the trapezoidal method. The Δ values were also calculated to assess changes in glucose and insulin over time, comparing maximum and minimum concentration peaks and fasting and baseline levels of both parameters. Data are presented as mean ± SD or median and interquartile range, where appropriate. Categorical variables are reported as frequencies and percentages.

RESULTS

Study Participants

We screened 2,981 individuals; of them, 79 were p.E508 K carriers. Forty-six carriers fulfilled the inclusion criteria and accepted to participate. Fifty healthy noncarriers free of diabetes were included to match the characteristics of the carriers (by age, sex, BMI, and status of type 2 diabetes) (Supplementary Fig. 1). Among carriers, 14 had type 2 diabetes, and 32 were normoglycemic. In the noncarrier group, 16 participants with type 2 diabetes and 33 without diabetes were included (Table 1). The matching process was successful; no differences in age, sex, BMI, HbA_{1c}, glipizide dosage, and time of exposure to type 2 diabetes were found between groups. All participants filled in a dietary intake record to verify dietary adherence and metformin intake as indicated.

Insulin and Glucose Response During the Glipizide Challenge

The primary outcome measure was to evaluate if carriers of the *HNF1A* p.E508 K variant have an enhanced response to sulfonylureas by comparing the peak insulin concentration between groups. After the glipizide challenge, the insulin peak was lower in p.E508 K carriers with type 2 diabetes compared with their noncarrier peers ($P = 0.03$) (Fig. 2*A*). The difference remained significant when the baseline insulin concentration was considered (Supplementary Table 1). The same trend was observed for the normoglycemic group, but the differences did not attain statistical significance (Fig. 2*B*). No significant difference was seen in the total AUC and the incremental AUC insulin concentrations between carrier and noncarrier subjects, regardless of their status of type 2 diabetes, following the glipizide challenge (Supplementary Fig. 1).

Most measures of glucose response during the glipizide challenge did not differ between the p.E508 K genotype groups (Fig. 2*C* and *D*). However, the AOC for glucose response was significantly lower in carrier individuals with type 2 diabetes compared with noncarriers ($P = 0.046$) (Supplementary Table 1). The frequency of plasma glucose levels <50 mg/dL trend was lower in carriers of the p.E508 K variant compared with noncarriers, but the difference did not reach statistical significance (22.9 vs. 10.9%; $P = 0.12$). Only three participants with type 2 diabetes had hypoglycemia during the glipizide challenge: two from the noncarrier group and one from the carrier group.

Insulin and Glucose Response During the OGTT Challenge

During the OGTT challenge (under metformin), the peak insulin level was lower for the p.E508 K carriers with type 2 diabetes as compared with noncarriers with type 2 diabetes (Fig. 3). Fasting glucose concentrations after the OGTT challenge under metformin, our primary outcome of interest, were significantly lower for carrier individuals without type 2 diabetes compared with noncarriers ($P = 0.01$) (Supplementary Table 2). Among participants with type 2 diabetes, significantly lower insulin peak concentration and total AUC insulin level were observed for carriers compared with the noncarriers. These comparisons did not achieve statistical significance in the normoglycemic group, but a similar trend was observed (Supplementary Table 3). There were no significant differences in glucose concentrations during the 2-h OGTT for the p.E508 K carriers compared with noncarriers (Fig. 3). Insulin action was not different between genotype groups (Supplementary Table 3).

As expected, HOMA-IR scores were higher in participants with type 2 diabetes,

Table 1—Biochemical and demographic characteristics of the studied population

	Without type 2 diabetes			With type 2 diabetes		
	p.E508 K(+) (n = 32)	p.E508(−) (n = 33)	P value	p.E508 K(+) (n = 14)	p.E508(−) (n = 16)	P value
Age (years)	38.41 ± 15.71	41.55 ± 13.10	0.38	55.64 ± 13.90	53.44 ± 8.47	0.60
BMI (kg/m^2)	28.51 ± 5.28	27.96 ± 5.66	0.63	27.14 ± 4.45	29.94 ± 5.82	0.15
Age at diagnosis of type 2 diabetes (years)	—	—	—	52.93 ± 13.94	49.94 ± 8.79	0.48
Duration of type 2 diabetes	—	—	—	2.0 (1.0–6.5)	1.0 (1.0–5.0)	0.75
Glucose (mg/dL)	95.75 ± 10.65	99.19 ± 10.65	0.23	127.36 ± 30.61	135.60 ± 38.88	0.55
Insulin (mU/mL)	7.4 (4.5–12.4)	4.9 (3.0–12.1)	0.69	6.8 (3.5–8.7)	10.35 (5.27–17.2)	0.21
HOMA-IR	1.82 (1.3–3.0)	1.68 (0.96–2.3)	0.40	—	—	—
HbA_{1c}, % (mmol/mol)	5.39 ± 0.47 (37.62 ± 4.92)	5.59 ± 0.46 (35.40 ± 5.25)	0.09	6.37 ± 0.86 (45.75 ± 7.65)	6.34 ± 0.70 (46.14 ± 9.35)	0.91
Total cholesterol (mg/dL)	180.41 ± 25.09	186.19 ± 41.44	0.50	184.94 ± 34.56	177.36 ± 35.16	0.56
Triglycerides (mg/dL)	140.0 (118.0–179.0)	124.0 (97.0–192.0)	0.59	110.0 (95.0–164.0)	147.5 (118.2–204.0)	0.27
HDL cholesterol (mg/dL)	41.12 ± 7.04	42.50 ± 10.54	0.54	45.28 ± 12.92	44.44 ± 9.15	0.84
LDL cholesterol (mg/dL)	108 (87.5–121.9)	103 (83.0–135.8)	0.35	106.0 (92.2–126.6)	103.7 (93.7–126.8)	0.47
Apolipoprotein B (mg/dL)	95.65 ± 18.68	100.88 ± 23.89	0.34	97.81 ± 20.47	103.62 ± 23.73	0.87
ALT (IU/L)	19.0 (16.0–31.0)	26.0 (15.0–32.0)	0.63	24.0 (20.0–29.0)	33.0 (18.7–64.2)	0.33
AST (IU/L)	22.0 (19.0–26.5)	26.0 (21.0–32.0)	0.10	25.0 (23.0–31.0)	28.0 (21.7–37.2)	0.47
GGT (IU/L)	15.0 (12.0–22.5)	21.0 (15.0–32.0)	0.15	17.0 (13.0–33.0)	25.0 (16.2–37.5)	0.38

Comparison of individuals with and without type 2 diabetes and with and without the p.E508 K variant in the *HNF1A* gene. Data are mean ± SD or median (interquartile range) unless otherwise noted. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase.

regardless their genotype status. A trend for lower HOMA-IR scores was observed after metformin therapy in carriers without type 2 diabetes. No statistical difference was found in the delta HOMA-IR score between genotype groups (Supplementary Table 3), suggesting that the p.E508 K variant did not modify the metformin response.

CONCLUSIONS

HNF1A mutations cause decreased insulin secretion and a set of clinical features that characterize the MODY3 phenotype. Among them, an enhanced response to sulfonylureas enables the transition from insulin to oral drugs for a large percentage of MODY3 cases (12). In Mexicans, 2% of the cases of type 2 diabetes who

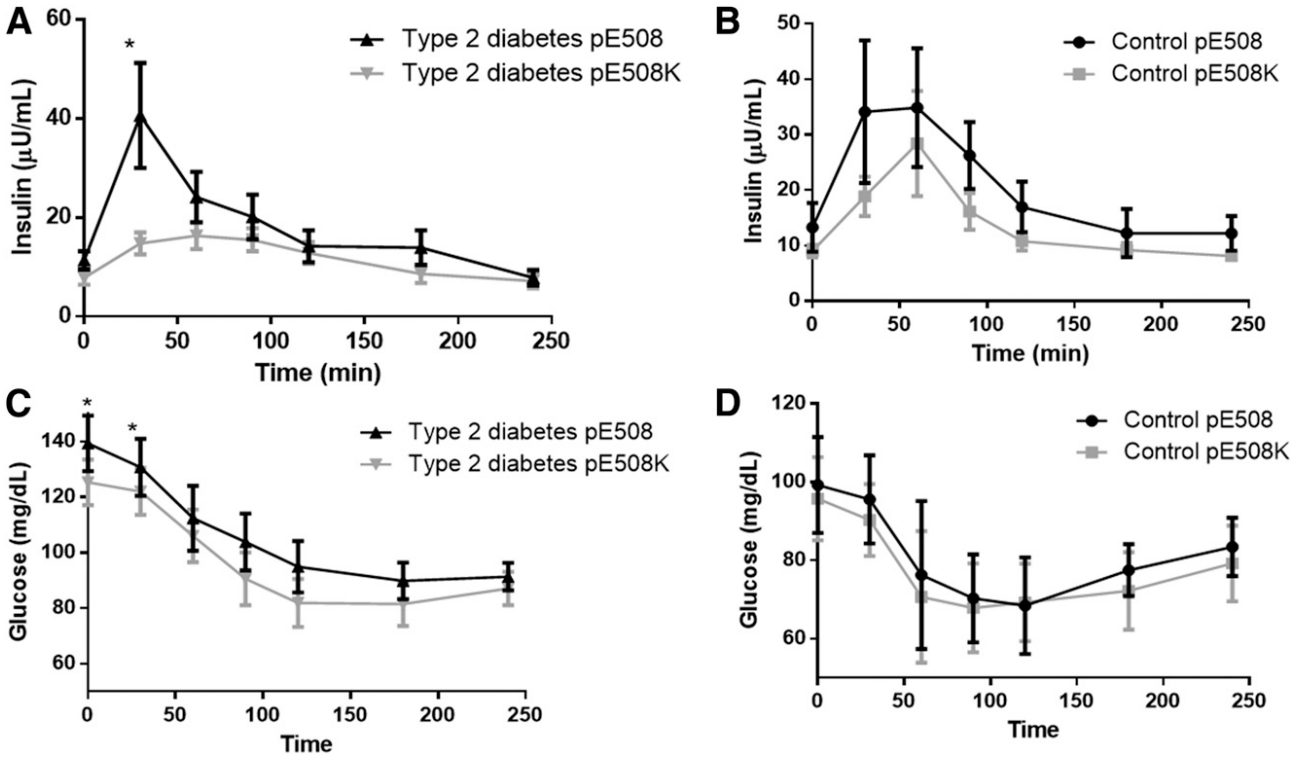

Figure 2—Comparison of insulin (*A* and *B*) and glucose (*C* and *D*) response curves after the administration of 2.5 or 5 mg of oral glipizide in carriers and noncarriers of the *HNF1A* p.E508 K variant with (*A* and *C*) and without type 2 diabetes (*B* and *D*). *$P < 0.05$.

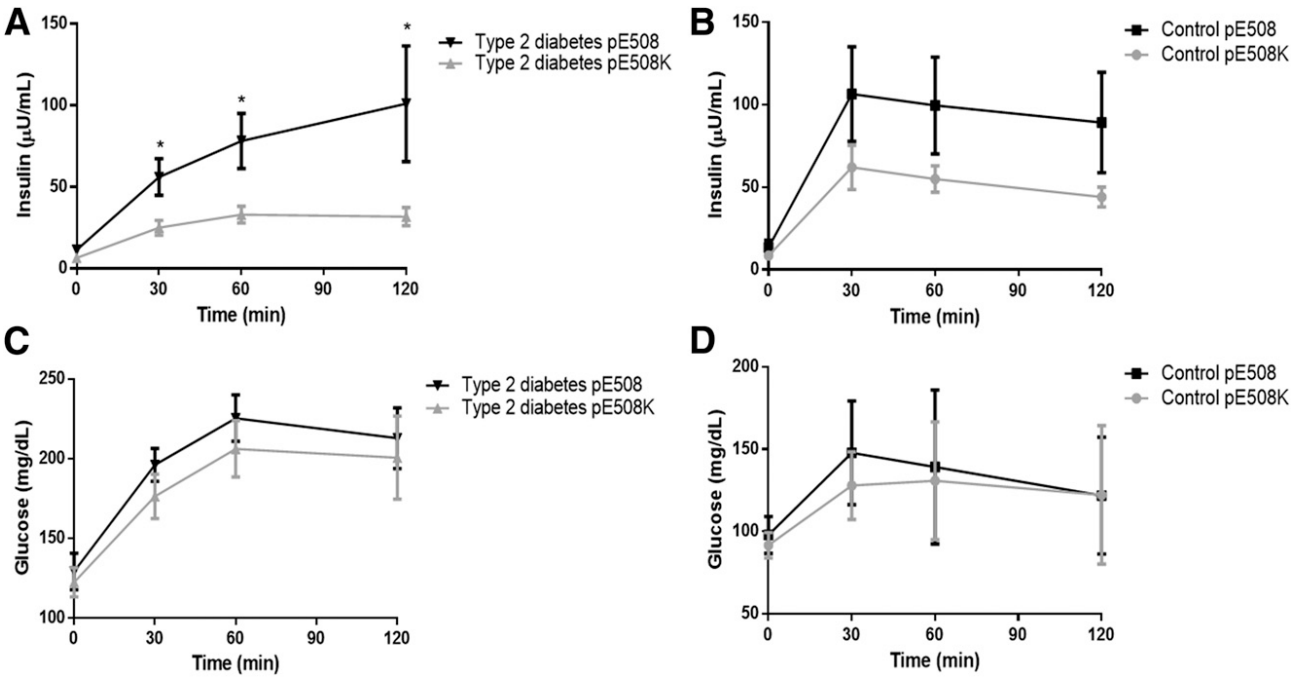

Figure 3—Comparison of insulin (*A* and *B*) and glucose (*C* and *D*) response curves in an OGTT after the administration of metformin 500 mg in carriers and noncarriers of the *HNF1A* p.E508 K variant with (*A* and *C*) and without type 2 diabetes (*B* and *D*). *$P < 0.05$.

participated in the exome-sequencing component of the SIGMA Type 2 Diabetes study carried an ethnic-specific *HNF1A* variant (p.E508 K). This variant is associated with a lower transcriptional activity (40%) on the *HNF1A* responsive promoters, a lower nuclear localization (15%), and lower protein expression (50%). However, the decreased transactivation was not as severe as that seen in three MODY-causing *HNF1A* mutations. Also, some clinical features of the p.E508 K carriers were not in accordance with the MODY3 profile (i.e., age of onset). Based on the partial loss of transcriptional activity conferred by the Mexican-specific *HNF1A* p.E508 K variant, we hypothesized that carriers of this variant might also exhibit heightened sensitivity to sulfonylureas. If confirmed, this finding would have had substantial pharmacogenetic implications in regards to the diagnosis and treatment of this form of diabetes in Mexico. In this study, we do show that carriers of the risk variant (particularly those with type 2 diabetes) have a remarkably lower insulin response during an OGTT. However, in contrast to our expectations, carriers with type 2 diabetes had a less vigorous insulin response when challenged with the sulfonylurea glipizide compared with noncarriers. Following an OGTT in the presence of metformin, carriers with type 2 diabetes also demonstrated a reduced insulin response as compared with noncarriers with type 2 diabetes. In participants free of diabetes, there was no evidence that carriers respond better to sulfonylureas than noncarriers. In addition, they were no more likely to develop hypoglycemia during sulfonylurea administration. Thus, it appears that identification of this variant will not affect treatment selection.

The unexpected reduced insulin response to glipizide or glucose in carriers with diabetes compared with noncarriers may be due to a more aggressive form of the disease that accelerates β-cell failure when compared with common type 2 diabetes, rendering sulfonylureas less effective in variant carriers. Though no significant differences in age of onset were noted between carriers and noncarriers in the original SIGMA study (3), it is possible that once diabetes sets in, β-cell failure proceeds more rapidly. This is not consistent with what is seen in MODY3 and may be specific to the Mexican context or to this variant. Longitudinal studies are needed to establish whether variant carriers with diabetes progress to insulin therapy faster than noncarriers.

Pearson et al. (9) proposed that neither the type of *HNF1A* mutation nor the mutation site influence the sulfonylurea response in patients with MODY3. Interestingly, carriers of some variants did not exhibit the enhanced sulfonylurea response, such as those with the p.W206X stop mutation, whose severe phenotype is due to the loss of the DNA-binding domain (13). The *HNF1A* p.E508 K variant is located in the middle of the transactivation domain. Additional studies with a large enough sample of cases with different types of variants (including frameshift, missense, nonsense, or splice site mutations) and in a longer term are needed to understand why some, but not all, of *HNF1A* variants are associated with the enhanced sulfonylurea response (14–16).

HNF1A variants modify the response to other glucose-lowering agents, besides sulfonylureas. Recently, an enhanced glycosuric response to a sodium–glucose cotransporter 2 inhibitor in a small group of MODY3 cases has been reported (17). This finding was unexpected because sodium–glucose cotransporter 2 expression is downregulated in MODY3 cases. Thus, the large number of genes regulated by *HNF1A* opens the possibility of additional pharmacogenetic effects to be identified in patients with MODY3 and carriers of other *HNF1A* variants.

The clinical spectrum of the *HNF1A* variants has been expanded with the evidence provided by the exome-sequencing

collaborative studies. Two ethnic-specific variants (G319S for Oji-Cree [18] and p.E508 K for Mexicans) have moderate functional consequences, and clinical expression is similar to type 2 diabetes. Recently, Najmi et al. (19) reported the *HNF1A* variants found in the Framingham Heart Study Offspring cohort, the Jackson Heart Study, and the Extreme cohort with type 2 diabetes. They found 27 nonsynonymous variants in 4,115 participants (prevalence 0.6%); 9 of them were novel. Some, but not all, were associated with type 2 diabetes, with the p.E508 K variant having the highest odds ratio. Because current bioinformatic programs do not establish pathogenicity conclusively, the authors used functional assays to classify variants as likely or unlikely pathogenic. They proposed that those variants with a transcriptional activity of $<$60% are likely to be pathogenic (odds ratio 5.04 [95% CI 1.99–12.8]; P = 0.0007).

Our study has its own strengths and limitations. This study was performed using a previously validated pharmacogenetic study protocol applicable both in healthy subjects and case subjects with type 2 diabetes. Pharmacogenomic evaluations are relevant for the development of precision medicine, as well as for understanding the physiological responses in carriers of genetic variants of interest. We recognize that we do not have a large number of study subjects. However, it is enough to test the hypothesis. In the sample of individuals with type 2 diabetes, considering an α of 0.05 and log-transformed maximum insulin peak concentrations, we have a power to detect differences between carriers and noncarriers of 87.75% (β = 0.12). Overall, for the maximum insulin peak concentrations, considering an α of 0.05, the sample size has 80.73% power to detect differences in log-transformed insulin concentrations between carriers and noncarriers (β = 0.19).

In conclusion, this report extends the clinical characterization of the *HNF1A* p.E508 K variant. It is associated with lower insulin response to glucose and the lack of an enhanced response to glipizide. Further research must be performed in order to understand the penetrance of this variant and the spectrum of its contribution to different clinical manifestations of diabetes.

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Performance of LDL-C calculated with Martin's formula compared to the Friedewald equation in familial combined hyperlipidemia

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ABSTRACT

Background and aims: A novel method to estimate low density lipoprotein cholesterol (LDL-C) has been proposed by Martin et al. This may permit a more accurate estimation of cardiovascular risk, however, external validation is needed. Here, the performance of LDL-C using this new method (LDL-N) is compared with LDL-C estimated with Friedewald equation (LDL-F) in familial combined hyperlipidemia (FCHL), a common primary dyslipidemia in which apolipoprotein B containing particle composition is abnormal and interferes with LDL-C estimation.

Methods: A total of 410 FCHL subjects were included. LDL-C was estimated with both the Friedewald equation (LDL-F) and the novel formula (LDL-N). Apolipoprotein B levels and non- HDL-C were recorded. The correlation and concordance between LDL-F and LDL-N and both Apolipoprotein B and non-HDL-C levels were calculated. Analysis stratifying for triglyceride tertiles and FCHL lipid phenotypes was also carried out.

Results: The correlations between LDL-N and Apo B and non-HDL-C were $\rho = 0.777$ (95%CI 0.718–0.825) and $\rho = 0.735$ (95%CI 0.648–0.816), respectively. The corresponding correlations for LDL-F were $\rho = 0.551$(95%CI 0.454–0.637) and $\rho = 0.394$ (95%CI 0.253–0.537), respectively. In mixed dyslipidemia or isolated hypertriglyceridemia, these correlations were significantly better using LDL-N. With respect to concordance, LDL-N performed significantly better than LDL-F when considering apoB <90 mg/dL (κLDL-N = 0.495 *vs.* κLDL-F = 0.165) and non-HDL-C <130 (κLDL-N = 0.724 *vs.* κLDL-F = 0.253).

Conclusions: In FCHL, LDL-C estimation using Martin's formula showed greater correlation and concordance with non-HDL-C and Apo B compared with the Friedewald equation.

1. Introduction

Low density lipoprotein cholesterol (LDL-C) remains the principle goal of therapy in the management of dyslipidemia [1–4]. However, many people who achieve LDL-C goals still develop atherosclerotic disease due to residual risk [5]. In certain patients there is a mismatch between the concentration of LDL-C and the number of atherogenic particles, expressed as the number of lipoproteins containing apolipoprotein B. Low density lipoprotein (LDL) particles are heterogeneous with respect to the amount of cholesterol they carry [6]. One person may have large LDLs, rich in cholesterol, while a second person can have small LDLs, which contain only a small amount of cholesterol. Therefore, at the same concentration of LDL-C, the second person will have a greater number of atherogenic particles (LDLs), and consequently increased cardiovascular risk [6]. As a consequence of this discrepancy, several expert panels suggest the use of other parameters to improve the evaluation of cardiovascular risk and determine intensity of therapy. These include apolipoprotein B (ApoB) and non-high density cholesterol (non-HDL-C); both parameters are useful but not equivalent.

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LDL-C represents the mass of cholesterol within LDL particles, whereas the ApoB concentration represents the total number of circulating atherogenic particles [7]. The measurement of this parameter is standardized among laboratories and does not require fasting but it represents a significant additional cost to the patient. Non-HDL-C is calculated by subtracting the concentration of HDL-C from total cholesterol and represents the cholesterol contents of all the atherogenic lipoproteins. It is considered a good therapeutic goal because its value does not change regardless of lipid exchange between VLDL-C and LDL [8]. In summary, non-HDL-C represents the cholesterol content of atherogenic lipoproteins (VLDL, IDL, LDL and Lp(a)), whereas apolipoprotein B measures the total number of atherogenic particles. When the content of cholesterol in the LDL-C particles is normal, both parameters are consistent. This means that they are equal for reporting cardiovascular risk. However, when the cholesterol content in the LDL-C particles is higher or lower than normal, the two parameters are discordant and predict differing risks.

The superiority of ApoB and non-HDL cholesterol for the prediction of cardiovascular risk compared with LDL-C has been shown in several studies [9–14]. The assessment of ApoB and non-HDL cholesterol may be even more relevant in persons with atherogenic dyslipidemias characterized by triglyceride-rich lipoproteins, low levels of HDL-C and increased levels of small dense LDL-C particles, including type 2 diabetes, metabolic syndrome and certain primary dyslipidemias such as familial combined hyperlipidemia (FCHL). In these cases, the total number of LDL-C particles may be higher than the calculated LDL-C level. Thus, using the LDL-C goal alone may not be enough.

FCHL is the most common primary atherogenic dyslipidemia in Mexico, being present in approximately 14% of patients with premature coronary heart disease [15,16]. It is associated with other metabolic abnormalities including obesity, insulin resistance, diabetes and metabolic syndrome [17]. FCHL is characterized by hypercholesterolemia and/or hypertriglyceridemia and elevated apolipoprotein B levels, a fluctuating lipid profile and variable expression within the same kindred. LDL-C may not be the best treatment target in this population, given the frequent presence of hypertriglyceridemia, other lipid targets including non-HDL-C and ApoB levels are probably more relevant in FCHL.

Conventionally, LDL-C is calculated by the Friedewald equation, avoiding the need for an ultracentrifuge [18]. This equation estimates LDL-C as (total cholesterol) − (high-density lipoprotein cholesterol [HDL-C]) − (triglycerides/5) in mg/dL. The final term assumes a fixed ratio of triglyceride levels to very low-density lipoprotein cholesterol (TG:VLDL-C) of 5:1.This estimate is unreliable in patients with triglycerides ≥150 mg/dL due to this fixed triglyceride to VLDL-C ratio, and does not consider the variance of this ratio across different concentrations of triglycerides and non-HDL-C [18]. Martin et al. have developed a novel method for estimating LDL-C using an adjustable factor for the TG: VLDL-C ratio (using triglyceride and non-HDL-C concentrations), which offers a greater concordance with measurement of LDL-C by ultracentrifugation [19]. This novel method has not been validated in populations that are characterized by abnormal apolipoprotein B containing particle composition, such as in FCHL; this method might be particularly helpful in such population. The objective of this study is to evaluate the correlation and the concordance of LDL-C, as calculated with the Friedewald equation (LDL-F) and Martin's formula (LDL-N), with non-HDL-C and ApoB targets in patients with FCHL. The results will determine the usefulness of this new method of LDL-C estimation in patients with atherogenic dyslipidemia.

2. Materials and methods

2.1. Study population

Subjects with a previous diagnosis of familial combined hyperlipidemia (FCHL) attending the lipid Clinic at the Instituto Nacional de Ciencias Medicas y Nutricion, Salvador Zubirán (INCMNSZ) in Mexico City were included. All participants gave informed consent. The Human Research Ethics Committee of the INCMNSZ approved the study. All procedures were done in accordance with the Declaration of Helsinki.

2.2. Clinical evaluations

All participants completed a questionnaire which included demographic data, medical history, and lifestyle factors. Patients arrived with the results of a routine lipid profile taken a week before their clinic visit. Diagnostic criteria considered for FCHL were the presence of hypercholesterolemia (total cholesterol >200 mg/dL) or hypertriglyceridemia (triglycerides >150 mg/dL) along with the demonstration of hypercholesterolemia, hypertriglyceridemia and mixed hyperlipidemia in three different first degree relatives and apolipoprotein B level >90th percentile for the Mexican population (>108 mg/dL for men and >99 mg/dL for women). Exclusion criteria included history of an acute illness within the previous six weeks, pregnancy and the presence of any disease or medication known to significantly influence lipid parameters. A complete medical and family history, including use of medications was obtained from all subjects. Subjects were weighed on calibrated scales and height was determined with a floor scale stadiometer. Body mass index (BMI) was calculated as weight in kg divided by the squared product of height in meters.

2.3. Laboratory measurements

Blood samples were obtained after an 8–12 h fast. Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments Co.), serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2). Lipid concentrations (cholesterol, triglycerides, and HDL cholesterol) and apo B measurements were performed using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). LDL-cholesterol was calculated with the Friedewald equation and the calculation proposed by Martin et al. [18].

2.4. Statistical analyses

Data are presented as mean ± SD or as median and interquartile range. Proportions and medians were compared between groups using the chi-square test and Mann Whitney-U tests. Variables with a parametric distribution were evaluated using Student's t-test. Spearman correlations were performed to evaluate the degree of linear association between LDL-C, LDL-N, apolipoprotein B and non-HDL cholesterol. Linear regression analyses were also performed using logarithmic transformation. Concordance between LDL-C, LDL-N, non-HDL cholesterol and apolipoprotein B targets was assessed using the kappa coefficient in the total population and in subpopulations. We also evaluated correlations and concordance across tertiles of triglyceride levels and according to the differing phenotypes of FCHL, namely isolated hypertriglyceridemia (IHTG), mixed dyslipidemia (MDLP) and isolated hypercholesterolemia (IHCT). Performance of the index was evaluated using areas under

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the receiving operating characteristic curve (Harrell's *c-statistic*) and 95% confidence intervals were estimated using bootstrap sampling drawing 2000 stratified random samples. To estimate differences between the AUC of the ROC curves, we performed non-parametric ROC tests using a stratified bootstrap sampling method using the *pROC* package from R version 3.4.3. Finally, we estimated thresholds for LDL-N and LDL-F using the Youden index in the *OptimalCutpoints* package in R. A two-tailed *p*-value <0.05 was considered significant as statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 21.0), R software (Version 3.4.4) and GraphPad Prism version 6.0.

3. Results

3.1. Study subjects

A total of 410 persons with a diagnosis of FCHL were included in the study. The mean age of participants was 49.5 ± 15.0 years, the mean BMI was 27.72 ± 4.28 kg/m^2 and 55.4% were women. Table 1 shows the laboratory characteristics of all study participants. Overall, 23.4% had a diagnosis of arterial hypertension and 25.1% had type 2 diabetes mellitus. Previous coronary heart disease was present in 2.7% of subjects and 2.2% had a history of stroke. In terms of lipid lowering treatment, 34.9% were on statins, 26.1% on fibrates and 4.4% on ezetimibe. Monotherapy was reported in 18.3%, combination therapy in 22.9% and dietary management alone in 58.8%. The number of patients achieving non-HDL-C and Apo B targets was recorded. One-hundred and fourteen (27.8%) patients had non-HDL-C <130 mg/dL, whilst only 18.8% had an ApoB level <90 mg/dL.

3.2. Differences across FCHL phenotypes

On comparing differences across the three FCHL phenotypes (namely isolated hypercholesterolemia, isolated hypertriglyceridemia and mixed dyslipidemia), there was no significant difference with respect to gender (p=0.128), family history of cardiovascular disease (p=0.614), hypertension (p=0.302), type 2 diabetes (T2D) (p=0.144), obesity (p=0.657) or previous myocardial infarction (p=0.275). We did not observe significant differences in biochemical parameters aside from the expected differences in lipid profiles.

Table 1
Biochemical characteristics of patients with diagnosed FCHL included in the study.

Parameter	Mean ± SD or median (IQR) N = 410
Age (years)	49.54 ± 15.01
Female sex (%)	227 (55.4%)
BMI (kg/m2)	27.72 ± 4.28
Systolic blood pressure (mmHg)	119.66 ± 14.97
Diastolic blood pressure (mmHg)	76.96 ± 9.00
Triglycerides (mg/dL)[a]	235.5 (160.8–381.0)
Total colesterol (mg/dL)[b]	198.70 ± 42.25
HDL-c (mg/dL)	42.32 ± 10.68
Non-HDL-c (mg/dL)	156.38 ± 42.58
LDL-F (mg/dL)	95.58 ± 34.06
LDL-N (mg/dL)	111.43 ± 28.62
Glucose (mg/dL)	104.96 ± 41.79
Insulin (mU/L)	12.80 (8.95–24.65)
Apolipoprotein B	111.26 ± 24.86

[a] Conversion factor for LDL-C, HDL-C and total cholesterol from mg/dL to mmol/L = 0.02585983966.
[b] Conversion factor for triglycerides from mg/dL to mmol/L = 0.01129050468.

3.3. Correlation of LDL-N and LDL-F with ApoB and non-HDL-C levels

There was a significant correlation between non-HDL-C and apolipoprotein B levels adjusted for age, sex, BMI, treatment modality and presence of T2D (ρ = 0.794, 95%CI 0.730–0.849). This correlation was higher for individuals with triglycerides <400 mg/dL (ρ = 0.861, 95%CI 0.818–0.898) and lower for subjects with triglycerides ≥400 mg/dL (ρ = 0.326, 95%CI 0.051–0.611). When this analysis was conducted according to FCHL lipid phenotype, we observed an improvement in correlation in isolated hypercholesterolemia (ρ = 0.838, 95%CI 0.776–0.903), followed by isolated hypertriglyceridemia (ρ = 0.787, 95%CI 0.640–0.877) and mixed dyslipidemia (ρ = 0.729, 95%CI 0.611–0.818).

There was a greater correlation between LDL-N and non-HDL-C (ρ = 0.735, 95%CI 0.648–0.816) compared with LDL-F (ρ = 0.394, 95%CI 0.253–0.537) (Fig. 1A). For individuals with triglyceride concentrations <400 mg/dL the adjusted correlation was still better for LDL-N (ρ = 0.959, 95%CI 0.946–0.968) compared to LDL-F (ρ = 0.870, 95%CI 0.833–0.900) (Fig. 1B). In the case of individuals with triglyceride concentrations ≥400 mg/dL the correlations for LDL-N (ρ = 0.061, 95%CI -0.145-0.280) and LDL-F (ρ = −0.116, 95%CI -0.335-0.138) were both much lower and lost statistical significance. When evaluating these correlations according to FCHL phenotype, we observed a good correlation in isolated hypercholesterolemia for both LDL-N (ρ = 0.990, 95%CI 983–0.994) and LDL-F (ρ = 0.977 95%CI 0.958–0.987). In isolated hypertriglyceridemia, the correlation was markedly better with LDL-N (ρ = 0.907, 95%CI 0.829–0.948) compared to LDL-F (ρ = 0.637, 95%CI 0.447–0.772). Finally, in mixed dyslipidemia, both correlations decreased significantly but the result was much better with LDL-N (ρ = 0.676, 95%CI 0.559–777) compared to LDL-F (ρ = 0.339, 95%CI 0.164–0.502).

With respect to apoB, there was a greater correlation with LDL-N (ρ = 0.777, 95%CI 0.718–0.825) compared to LDL-F (ρ = 0.551, 95%CI 0.454–0.637) (Fig. 1C). When comparing this correlation in individuals ≥400 mg/dL there was a better adjusted correlation for LDL-N compared to LDL-F (Fig. 1D). When the analysis was conducted according to lipid phenotype, LDL-N and LDL-F showed similar correlations with apoB in isolated hypercholesterolemia (ρ = 0.825, 95%CI 0.760–0.898 and ρ = 0.814, 95%CI 0.735–0.892, respectively). In isolated hypertriglyceridemia LDL-N showed a better correlation (ρ = 0.723, 95%CI 0.571–0.832) compared to LDL-F (ρ = 0.529, 95%CI 0.347–0.677). Finally, in mixed dyslipidemia, the correlation coefficient was also significantly better with LDL-N than with LDL-F (ρ = 0.769, 95%CI 0.694–0.831 vs. ρ = 0.562, 95%CI 0.454–0.664 respectively).

3.4. Concordance with respect to treatment goals comparing LDL-N vs. LDL-F

Given the importance of LDL-C treatment goals in patients with FCHL, we evaluated the concordance of the LDL-C targets in relation to apoB and non-HDL-C goals (Fig. 2A–D). When comparing an LDL-C goal <100 mg/dL with non-HDL-C <130 mg/dL, we observed a higher concordance for Martin's over Friedewald formula ($\kappa_{LDL\text{-}N} = 0.724$ *vs.* $\kappa_{LDL\text{-}F} = 0.253$); if the goal was set to a lower threshold (LDL-C <70 mg/dL, non-HDL-C <100 mg/dL), we observed a similar trend but with a reduced concordance ($\kappa_{LDL\text{-}N} = 0.674$ *vs.* $\kappa_{LDL\text{-}F} = 0.295$). When evaluating the goals based on ApoB, we observed a higher concordance for Martin's equation in both LDL-C <100 mg/dL and ApoB <90 mg/dL ($\kappa_{LDL\text{-}N}$=0.495 *vs.* $\kappa_{LDL\text{-}F}$=0.165) and with LDL-C <70 mg/dL and ApoB <80 mg/dL ($\kappa_{LDL\text{-}N} = 0.463$*vs.* $\kappa_{LDL\text{-}F} = 0.194$).

When we evaluated concordance based on triglyceride tertiles for the whole population, we observed a consistent decrease in

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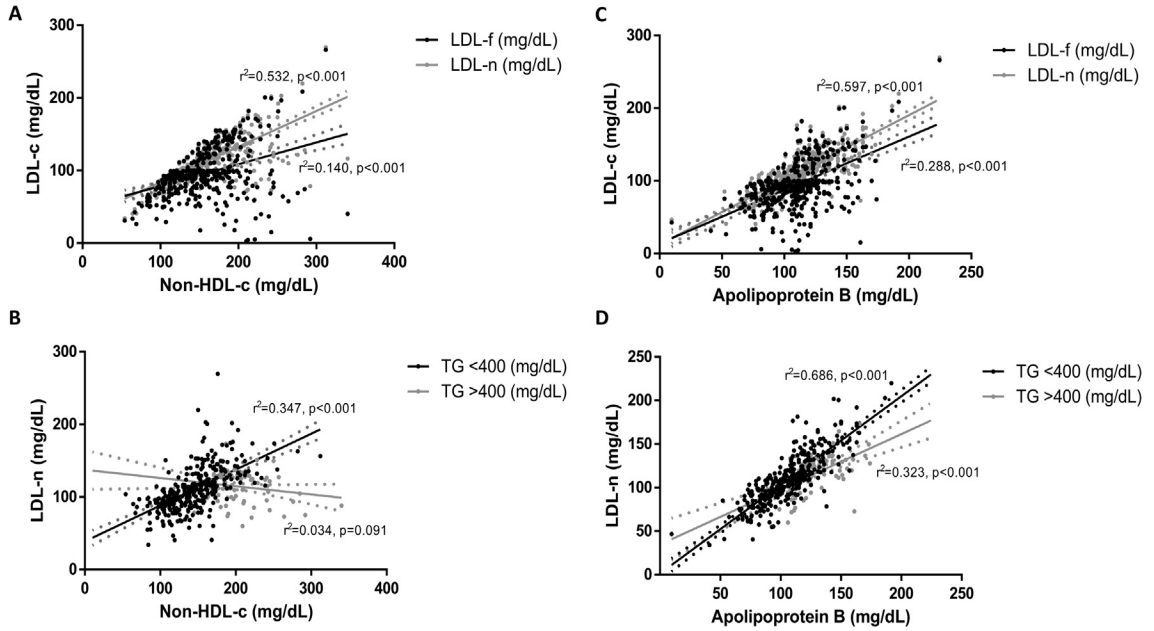

Fig. 1. Correlation between LDL-C estimated by the Friedewald equation and Martin's formula with ApoB and non-HDL-C in FCHL.
We observed a significant correlation between LDL-C estimated by the Friedewald formula (LDL-f) and Martin's formula (LDL-n) with non-HDL-C (A) and apolipoprotein B (C) that is higher for LDL-N and it remains higher even when further stratified by triglyceride levels (B and D).

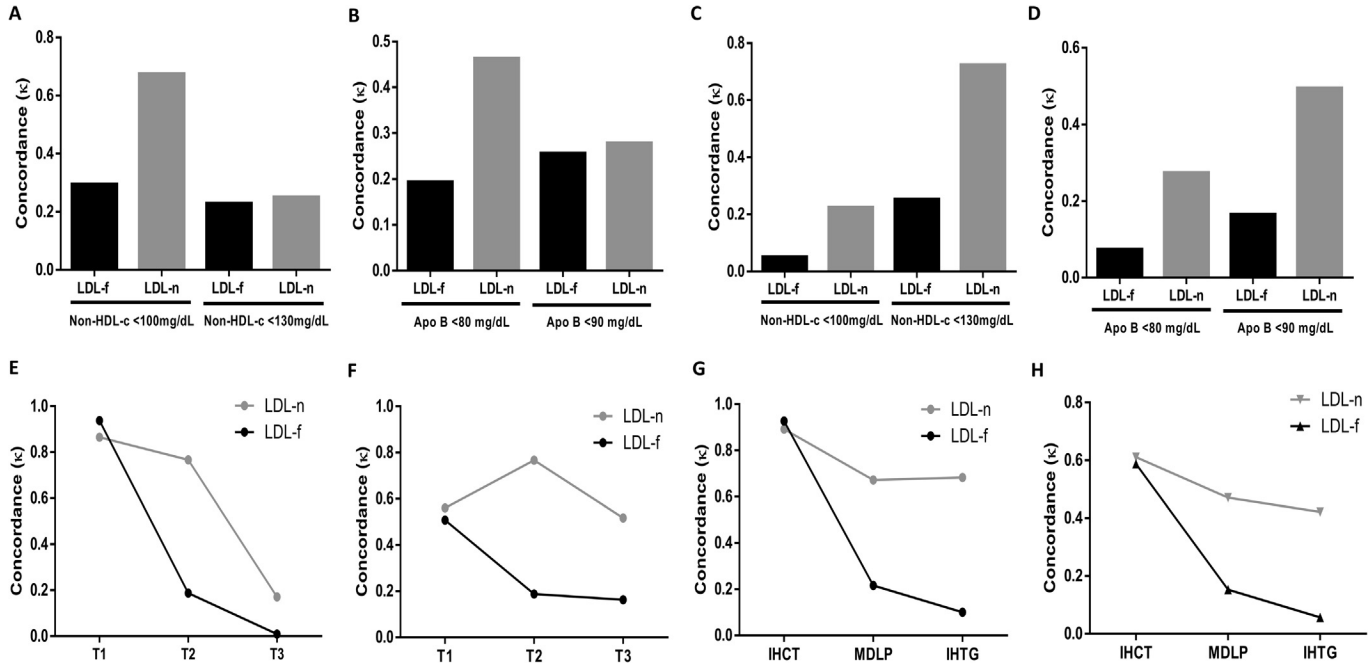

Fig. 2. Concordance (κ) between the Friedewald equation (LDL-F) and Martin's formula's (LDL-N) goals according to ApoB and non-HDL-C targets.
Concordance (κ) between LDL-f and LDL-n with targets of therapy for FCHL against LDL-c <70 (A and B) and against LDL-c <100 (C and D). The figure also shows concordance (κ) between LDL-f and LDL-n across terciles of triglyceride concentrations in FCHL against non-HDL-c <130 mg/dL (E) and apolipoprotein B < 90 mg/dL (F). Finally, we showed how both equations performed in different syndromes of FCHL including isolated hypercholesterolemia (IHCT), mixed dyslipidemia (MDLP) and isolated hypertriglyceridemia against non-HDL-c <130 mg/dL (G) and apolipoprotein B < 90 mg/dL (H).

concordance across tertiles (Fig. 2E–F) for both non-HDL-C and ApoB; however, concordance was maintained at higher levels for LDL-C estimated using Martin's formula (LDL-N) compared to the Friedewald equation (LDL-F). Finally, we evaluated concordance according to FCHL lipid phenotypes. We observed that concordance was nearly the same for patients with isolated hypercholesterolemia, but Martin's formula showed better concordance for patients with mixed dyslipidemia and isolated hypertriglyceridemia compared to the Friedewald equation for both non-HDL-C and ApoB targets (Fig. 2G–H) (see Fig. 3).

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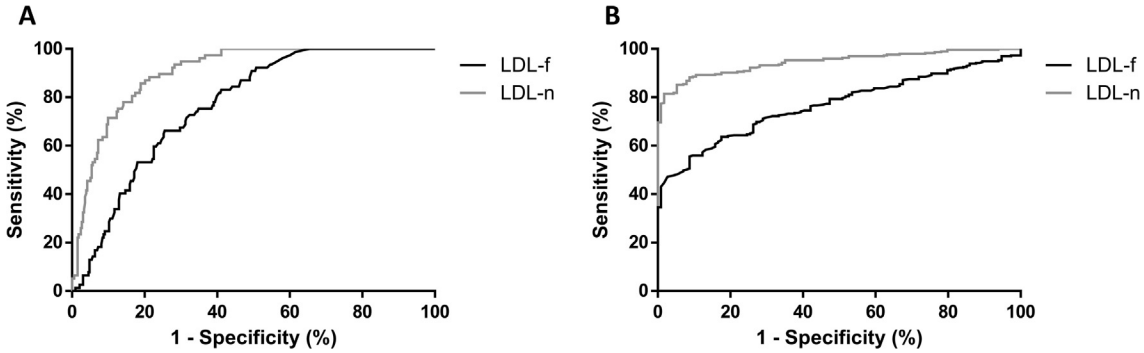

Fig. 3. Performance of LDL estimation comparing Martin's formula with the Friedewald equation to detect goals based on ApoB and non-HDL-C.
Here, we observe that the performance of LDL estimation is superior for Martin's formula (LDL-n) compared to the Friedewald equation (LDL-f) in FCHL using receiver operating characteristic (ROC) curves against apolipoprotein B < 90 mg/dL (A) and non-high density lipoprotein cholesterol <130 mg/dL (B).

3.5. Performance of LDL-N and LDL-F compared to Apo B and non-HDL-C

Finally, the area under the receiving operating characteristic curve (AUC of ROC) was estimated, to evaluate the performance of the LDL-C calculation using Martin's formula and the Friedewald equation. First, the accuracy of both estimations to detect non-HDL-C <130 mg/dL was evaluated; a significantly higher AUC for LDL-N (AUC 0.945 95%CI 0.925–0.966) compared to LDL-F (AUC 0.769 95%CI 0.727–0.813) was found ($p < 0.001$). A similar result was observed when comparing the AUC to detect ApoB <90 mg/dL; here, LDL-N also had a higher AUC (0.905 95%CI 0.874–0.934) compared to LDL-F (AUC 0.767 95%CI 0.716–0.814), reaching statistical significance ($p < 0.001$).

Subsequently, the Youden index was used to calculate the best LDL-N and LDL-F thresholds for the detection of target ApoB levels. A threshold of 99.2 mg/dL for LDL-N consistently detected ApoB levels <90 mg/dL (78.7% sensitivity, 88.3% specificity). In persons with TG < 400 mg/dL a similar threshold was identified, 99.2 mg/dL (~100 mg/dL) (79.2% sensitivity, 88.1% specificity). However, in individuals with TG > 400 mg/dL, a lower threshold of 81.8 (~80 mg/dL) was found to detect ApoB<90 mg/dL (92.8% sensitivity, 97.3% specificity). In the case of LDL-F, a threshold of 93.8 mg/dL detected ApoB levels <90 mg/dL; this had a lower sensitivity and specificity compared to the LDL-N threshold (58.8% sensitivity, 83.1% specificity). In persons with TG < 400 mg/dL, the corresponding LDL-F threshold was 93.6 mg/dL (68.0% sensitivity, 82.9% specificity). In contrast, in individuals with TG ≥ 400 mg/dL, the LDL-F threshold was significantly lower (15.2 mg/dL).

Finally, the best LDL-N and LDL-F thresholds for the detection of target non-HDL-C levels was estimated. A threshold of 101.2 mg/dL for LDL-N consistently detected non-HDL-C levels <130 mg/dL (85.2% sensitivity, 95.6% specificity). For LDL-F, a threshold of 97.2 mg/dL (55.8% sensitivity, 92.03% specificity) was identified. We were not able to carry out an analysis with triglyceride levels above and below 400 mg/dL, since no patient had a non HDL-C <130 mg/dL and TG ≥ 400 mg/dL. Instead we carried out this analysis using a threshold of 300 mg/dL. When considering a non-HDL-C target <130 mg/dL, in individuals with TG ≥ 300 mg/dL and <300 mg/dL, the thresholds for LDL-N were 85.2 mg/dL (94.0% sensitivity, 100.0% specificity) and 104.2 mg/dL (~100 mg/dL) (90.5% sensitivity, 99.1% specificity), respectively. The corresponding thresholds for LDL-F were 54.4 mg/dL (86.0% sensitivity, 100.0% specificity) and 97.6 mg/dL (81.6% sensitivity, 91.7% specificity) respectively.

4. Discussion

Familial combined hyperlipidemia is characterized by an overproduction of very low density lipoprotein particles and an innate variability in lipoprotein composition. Typically there is an atherogenic lipid profile, namely hypertriglyceridemia, hypoalphalipoproteinemia and the production of small dense LDL-C particles. The performance of the Friedewald equation in estimating LDL-C in these circumstances is not adequate (due to the moderate to severe hypertriglyceridemia). The possibility of an alternative formula which provides a superior estimation of LDL-C in this situation, is particularly appropriate in FCHL. In order to be considered an improvement, LDL-C estimation by Martin's formula would have to better reflect cardiovascular risk. An indirect measure of this is the degree of correlation and concordance with lipoprotein parameters known to be relevant in FCHL, namely non-HDL-C and apoB levels. Our results demonstrate that LDL-C estimated using Martin's formula (LDL-N) is an improvement over the traditional formula, showing a significantly greater correlation and concordance with both apoB and non-HDL-C targets in subjects with FCHL. Furthermore, in the setting of hypertriglyceridemia, even though the correlation and concordance with apoB and non-HDL-C becomes lower, LDL-N is still significantly better than the LDL-C estimated using the Friedewald equation (LDL-F). On analyzing FCHL lipid phenotypes, LDL-N and LDL-F perform similarly in the setting of isolated hypercholesterolemia. However, LDL-N is superior in the setting of mixed dyslipidemia and isolated hypertriglyceridemia in FCHL patients. The observation of decreased performance of both LDL-N and LDL-F in the setting of severe hypertriglyceridemia this is not entirely unexpected due to the presence of chylomicrons and highlights the problems associated with utilizing calculated LDL-C. With respect to FCHL phenotypes, the concordance with non-HDL-C and apoB was generally adequate, being only marginally lower in phenotypes with increased TG concentrations. In relation to sex, we observed differences in correlation and concordance for all evaluated parameters with consistent superiority for LDL-N over LDL-F; however, this could be attributable to significantly higher triglyceride values in male compared to female participants (Online Supplement).

In their original publication Martin et al. evaluated the concordance between LDL-N and directly measured LDL-C; they did not compare concordance with secondary measures of cardiovascular risk, specifically apoB and non-HDL-C. Martin et al. observed an improved concordance between LDL-N and ultracentrifugation

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measured LDL-C compared to LDL-F. In accordance with our findings, the authors stated that LDL-N performed best in the classification of LDL-C concentrations lower than 70 mg/dL, especially in patients with elevated triglyceride concentrations.

In FCHL, there exists a moderate correlation between non-HDL-C and apoB. This correlation improves with TG < 400 mg/dL but weakens in the presence of TG ≥ 400 mg/dL. In the presence of hypertriglyceridemia, ATP-III guidelines recommend the use of non-HDL-C as a secondary treatment goal once the LDL-C target is reached [20]. Up until now, it is unknown whether non-HDL-C and apoB are equivalent markers of cardiovascular risk. Sniderman et al. compared subjects with and without myocardial infarction when both parameters were discordant. When apoB > non-HDL-C, (when the apoB particles are poor in cholesterol), cardiovascular risk is increased. In contrast, when the non-HDL-C > apoB, (when apoB particles are rich in cholesterol), risk is lower than the reference concordant group. Therefore, these investigators concluded that when non-HDL-C and apoB are discordant, apoB was a more accurate marker of cardiovascular risk than non-HDL-C. This suggests that the atherogenic particle number is a more important determinant than the mass of cholesterol within LDL-C particles [21,22]. Therefore, when all three parameters are concordant, the clinical utility of these variables is similar. The moment they are discordant, cardiovascular risk can be under or overestimated if only LDL-C is considered [23–25].

Residual cardiovascular risk in FCHL may be indicated by discordance of LDL-C with apoB and non-HDL-C. In fact, both non-HDL-C and apoB predict overall cardiovascular risk better than LDL-C [21]. Otvos et al. reported that when there is discordance between apoB levels and LDL-C, only the number of particles is significantly associated with the incidence of cardiovascular events and the thickness of the carotid intima-media [6]. They concluded that when such a discrepancy exists, the risk attributable to LDL is best established by apoB levels. Patients with LDLs poor in cholesterol may have residual risk and, despite reaching LDL-C targets, they might continue to have high numbers of LDL particles. Discordance between LDL-C and non-HDL-C has also been reported. Masana et al. evaluated individuals, who having achieved LDL-C targets, continued to have uncontrolled non-HDL cholesterol levels [22]. They reported that 90% of patients with hypertriglyceridemia ≥400 mg/dL, showed LDL-C at target, but non-HDL-C was ≥130 mg/dL. Furthermore, 2 of every 5 patients with triglycerides ≥150 mg/dL and normal LDL-C levels had elevated levels of non-HDL-C. A recent study showed that approximately 20% of patients with Friedewald LDL-C <70 mg/dL have a LDL-C by Martin's formula of ≥70 mg/dL, and these individuals also have higher non-HDL-C and apoB concentrations [26]. Therefore, addressing accuracy of LDL-C estimation also addresses non-HDL-C and apoB discordance to an extent. Indeed, as shown by Sathiyakumar et al., when LDL-C is better estimated by Martin's formula and the LDL-C goal is achieved, then guideline non-HDL-C and apoB targets are also achieved in 98% or more of individuals and therefore are of modest additional utility in clinical management for individuals with elevated cardiovascular risk such as FCHL [27].

Our study has strengths and limitations. Firstly, this is a cross-sectional evaluation, which limits the possibility of establishing causality. Prospective studies with long-term follow-up to assess cardiovascular endpoints would aid in evaluating the relevance of discordant targets and evaluation of all three lipid parameters. Secondly, to evaluate the confounding effect of age, gender and lipid lowering treatment in correlations we adjusted for these variables; however, there exists the possibility of residual confounding. To the best of our knowledge, this is the first study validating LDL-N in FCHL, a high risk cardiovascular population in which the use of LDL-C estimation is problematic. In addition, it is noteworthy that validation in a cohort of FCHL patients is skewed towards subjects with a greater alteration in lipid profiles, as opposed to the general population. However, our results demonstrate that LDL-C estimation using Martin's formula is more useful than traditional methods in an atherogenic dyslipidemia with comorbid hypertriglyceridemia.

In conclusion, in FCHL, LDL-N offers improved correlation and concordance with apoB and non-HDL-C compared to LDL-F. LDL-N and LDL-F perform similarly when the lipid phenotype is restricted to isolated hypercholesterolemia. In FCHL, in the setting of elevated triglycerides, LDL-N outperforms LDL-F. An LDL-N threshold of 81.8 mg/dL could be used to identify patients at target without the need to measure ApoB levels in the setting of elevated TG.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

Research idea and study design: RM, ERR, CAAS, ICB; data acquisition: RM, ICB, ERR, ACGD; data analysis/interpretation: OYBC, RM, ERR; statistical analysis: OYBC, RM, AVV; manuscript drafting: OYBC, RM, CAAS, ICB, AVV; supervision or mentorship: CAAS, ICB, RM. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.atherosclerosis.2018.06.868.

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BMC Endocrine Disorders

RESEARCH ARTICLE Open Access

Development and validation of a predictive model for incident type 2 diabetes in middle-aged Mexican adults: the metabolic syndrome cohort

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Abstract

Background: Type 2 diabetes mellitus (T2D) is a leading cause of morbidity and mortality in Mexico. Here, we aimed to report incidence rates (IR) of type 2 diabetes in middle-aged apparently-healthy Mexican adults, identify risk factors associated to ID and develop a predictive model for ID in a high-risk population.

Methods: Prospective 3-year observational cohort, comprised of apparently-healthy adults from urban settings of central Mexico in whom demographic, anthropometric and biochemical data was collected. We evaluated risk factors for ID using Cox proportional hazard regression and developed predictive models for ID.

Results: We included 7636 participants of whom 6144 completed follow-up. We observed 331 ID cases (IR: 21.9 per 1000 person-years, 95%CI 21.37–22.47). Risk factors for ID included family history of diabetes, age, abdominal obesity, waist-height ratio, impaired fasting glucose (IFG), HOMA2-IR and metabolic syndrome. Early-onset ID was also high (IR 14.77 per 1000 person-years, 95%CI 14.21–15.35), and risk factors included HOMA-IR and IFG. Our ID predictive model included age, hypertriglyceridemia, IFG, hypertension and abdominal obesity as predictors (D_{xy} = 0.487, *c-statistic* = 0.741) and had higher predictive accuracy compared to FINDRISC and Cambridge risk scores.

Conclusions: ID in apparently healthy middle-aged Mexican adults is currently at an alarming rate. The constructed models can be implemented to predict diabetes risk and represent the largest prospective effort for the study metabolic diseases in Latin-American population.

Keywords: México, Incidence, Obesity, Latinos, Urbanization, Diabetes prediction

Background

Type 2 diabetes (T2D)-related burden of disease in Mexico is among the biggest worldwide as there are currently over 9 million Mexicans living with diabetes [1]. T2D is among the top causes of morbidity, disability and mortality in Mexico [2]. Furthermore, Mexican-derived populations living in the US are among the ethnic groups with the highest risk of T2D and its complications [3]. Increased susceptibility for T2D is mainly explained by the interaction between genetic factors including Amerindian-specific risk alleles and chronic exposure to a positive caloric balance [4]. Thus, T2D prevention programs are an urgent need for the healthcare system in Mexico. Nevertheless, evidence of population-specific

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statistics is required for the design and implementation of such actions.

Prevalence data has been consistently collected in the National Health Surveys every 6 years since 1994 and the surveys have shown significant growth in T2D prevalence over the years [2, 5–8]. The 2006 National Health and Nutrition Survey (ENSANut 2006), reported a T2D prevalence of 14.4%, among which 7.1% were previously undiagnosed. The prevalence of T2D, based on the number of diagnosed cases, increased to 9.2% in 2012 and 9.4% in 2016 [8]. However, information about incident diabetes is scarce [9]. Diabetes prevention depends on the prompt identification and treatment of at-risk individuals [1–3], who are often detected through risk factor assessment [10]. Several risk factors have been previously reported in Mexicans; a previous report from the Mexico City study links increased body-mass index (BMI), abdominal obesity, impaired fasting glucose, advanced age and hypertension with increased risk of incident T2D [11–13]. The aim of this report is to inform the incidence rates of T2D and impaired fasting glucose (IFG) found in middle-aged apparently-healthy Mexican adults living in urban centers during a three-year follow-up period, in order to identify risk factors associated to T2D incidence and develop a predictive model for T2D in a high-risk population. Before the present study, longitudinal data to evaluate and predict T2D risk had been lacking, which posed limitations on risk factor prediction, estimation of the impact of prevention programs and generation of pharmaco-economic models. To the best of our knowledge, this is the first prospective study with large-enough sample to validate risk factors definitions for T2D prediction adjusted to our population.

Research design and methods

Study sample and research design

We performed a prospective observational cohort study including Mexican adults living in large urban settings of central Mexico including Mexico City, Cuernavaca, Leon, Toluca and Aguascalientes to evaluate incidence of T2D, arterial hypertension and cardiovascular disease, We aimed to identify risk factors associated to ID in order to develop a predictive model for ID in our population. The study sample was composed by apparently-healthy adults ≥20 years, with BMI ≥20 kg/m^2, who resided for > 6 months in the evaluated city, and without plans to move to other city in the short term, whose grandparents and parents were born in Mexico. We excluded individuals with previously diagnosed diabetes, cardiovascular disease, cerebral vascular disease, incapacitated to lift themselves out of their home, pregnancy, alcoholism (≥10 servings of alcohol per week), acute stress event or any condition that could potentially endanger her life in the three following years. Participants were identified and evaluated at their workplaces (offices of the federal government or private companies) (n = 3246), homes (n = 189) or during a visit of a relative to a medical unit (n = 2709). The home-based component of the study sample was part of the "Mexican Study of Nutritional and Psychosocial Markers of Frailty", a population-based cohort study designed to assess the nutritional and psychosocial determinants of frailty and its consequences on health of Mexican older adults living in Coyoacán in Mexico City [14].

All assessments were performed at morning, after a 9-12 h fasting period. The evaluation consisted in a clinical examination using standardized questionnaires, anthropometric measurements and a blood draw. Demographic information and a medical history, including personal and family history of the most common chronic diseases, were obtained. The evaluation included a 24-h diet recall, 7-day food frequency questionnaire, the three-factor eating questionnaire [15], the short version of the International physical activity questionnaire (IPAQ) [16] and for adults ≥50 years, an assessment of their functionality and depression. Participants were informed about their results and were advised to visit a primary care physician to seek for treatment if required. They were contacted after a three-year period (±6 months) and invited to repeat the evaluation using the same tools and methods. Multiple approaches were applied to cases that were not reachable at the place in which they were originally invited to participate, including phone calls, e-mail messages, telegrams, invitations through friends or relatives, and visits to the workplace. The response rate was 80.7% (n = 6166). The study was approved by the Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición and all participants signed an informed consent form.

Laboratory measurements

All serum samples were kept frozen until processed in a central laboratory certified by the External Comparative Evaluation of Laboratories Program of the College of American Pathologists (Departamento de Endocrinología y Metabolismo, Instituto Nacional de Ciencias Médicas y Nutrición, México City). Clinical chemistry parameters and the lipid profile were measured using commercially available reagents (Synchron CX5 delta, Beckman Coulter). Immunonephelometric methods were applied for the measurement of apolipoprotein B (IMMAGE, Beckman Coulter) and C reactive protein (BN ProSpec, Siemens). Insulin concentrations were measured using an ELISA method (AxSYM, Abbott).

Outcomes and variable definitions

Incident diabetes (ID) was defined if a previously healthy subject (fasting plasma glucose (FPG) < 126 mg/dL) at baseline had a medical diagnosis of T2D or started treatment with a glucose-lowering drug after follow-up and/or had a fasting glycemia ≥126 mg/dL in the second visit. Incident impaired fasting glucose (IFG) was defined by FPG in the range 100-125 mg/dL in the final visit for individuals that had the same variable < 100 mg/dL at baseline. Early-onset

T2D was defined as T2D diagnosed < 40 years, as previously described [17]. Arterial hypertension was diagnosed according to the AHA guidelines [18]. Hypercholesterolemia was defined by the presence of a total cholesterol concentration > 200 mg/dL or being under statin therapy. Metabolic syndrome and its components were defined according to IDF and ATP-III recommendations [19].

Statistical analyses

To evaluate inter-group differences, we used Student's t and Mann-Whitney U tests, where appropriate. Frequency distribution of categorical variables were reported as frequencies and percentages and compared using chi-squared tests. For follow-up evaluations we used Student's paired t and Wilcoxon's rank-sign tests, where appropriate. Logarithmic transformations were applied to approximate normality in variables showing a non-parametric distribution. Missing values were imputed using Multiple Imputation by Chained Equations (MICE) and variables with > 5% of missing values were not included in the analyses. Data are presented as mean ± SD or as median and interquartile range.

Person-years for diabetes were calculated from baseline examination until the event or death occurred or until the last follow-up, whichever came first. Incidence of diabetes with 95%CI was calculated per 1000 person-years and risk factors were evaluated using unadjusted Cox proportional hazard regression models. To develop a risk score to predict ID in Mexican population, we fitted Cox proportional hazard regression models stratified by sex in two models: a first model comprising only demographic and anthropometric data and a model which also included biochemical measurements. β-coefficients from Cox regression models were used to develop a point-score for ID prediction, which was later validated using k-fold and bootstrap cross-validation to correct for over-optimism. Predictive performance of these models was evaluated using Harrerl's *c-statistic* and Sommer's D_{xy}: the performance of our score was compared with FINDRISC and the Cambridge risk using non-parametric ROC tests. A two-tailed *p*-value< 0.05 was considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS, version 21.0), R software (Version 3.4.4) and GraphPad Prism version 6.0.

Results

Study population

Clinical data and blood samples were obtained from 10,052 individuals at baseline from 2007 to 2011. Among them, 2416 individuals had either undiagnosed T2D (*n* = 429) or declined permission to be included in the follow-up (*n* = 1987). Consequently, our study sample considered for the primary end-point of this report 7636 participants. The follow-up visit was performed 29.5 ± 9.7 months later (2010–2013); 6166 patients were reached for the second evaluation. Twenty-two deaths were recorded among participants. Therefore, 6144 subjects completed the second evaluation, comprising 15,501 person-years of follow-up (Fig. 1). Mexico City had the highest participation rate (*n* = 2493, 40.6%), followed by Aguascalientes (*n* = 1589, 25.9%), León (*n* = 997, 16.2%), Toluca (*n* = 864, 14.0%) and Cuernavaca (*n* = 201, 3.3%). The population is composed by middle-aged adults (42.6 ± 11.0 years), predominantly women (*n* = 4092, 66.6%), who had 12.1 ± 6.7 years of education. No significant differences were found in any of the socio-demographic or clinical parameters evaluated between study participants who completed or missed the follow-up visit. Our data confirmed a high prevalence of several metabolic abnormalities found in Mexicans. Abdominal obesity was found in 78.1%. The prevalence IDF-defined

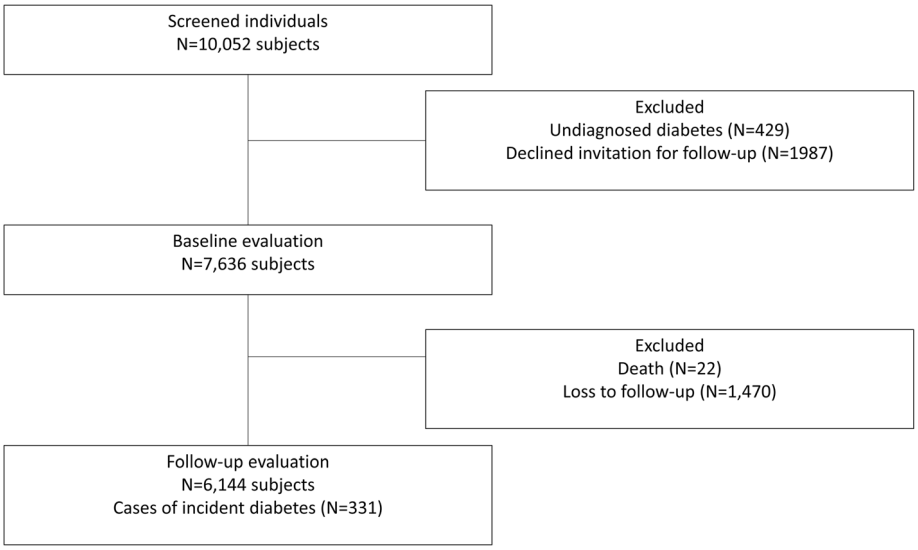

Fig. 1 Flowchart of study participants at baseline and follow-up, outlining reasons for exclusion

metabolic syndrome was 43.9%. Impaired fasting glucose was observed in 682 subjects (11.1%). The most common lipid abnormality was hypoalphalipoproteinemia, defined as HDL cholesterol< 40 mg/dL (59.8%).

Diabetes incidence across subgroups

ID occurred in 331 cases (5.3%). In general, ID cases were older, had higher blood pressure, higher FPG, insulin, lipids, apolipoprotein B and C-reactive protein both at baseline and follow-up (Table 1). The incidence rate (IR) in the whole population was 21.9 cases per 1000 persons/year (95%CI 21.37–22.47) with a higher rate observed in men (IR 22.4, 95%CI 21.5–23.4) compared to women (IR 21.6 95%CI 21.0–22.3). At baseline, IFG was present in 682 cases at baseline (11.1%). Of them, 288 (42.2%) remained in the IFG category, 150 (22%) progressed to diabetes and 244 (35.8%) had an FPG < 100 mg/dL at the end of the follow-up. ID ranged from 13.6 cases per 1000 persons/year (95%CI 13.2–13.9) with glucose < 100 mg/dL to 162.74 cases per 1000 persons/year (95%CI 137.2–193.0) in the population with fasting blood glucose between 110 and 125 mg/dL. Among individuals with IFG at baseline (100–125.9 mg/dL), the incidence was 84.8 cases per 1000 persons/year (95%CI 78.7–91.4), a rate seven-fold higher compared to the rest of the population. ID was also proportional to BMI and we observed a four- fold difference in ID rates between lean persons and subjects with BMI > 35 kg/m^2. Incidence rates were also higher in older subjects (≥55 years, 38.4 per 1000 persons/year, 95%CI 35.8–41.2) or with triglycerides > 150 mg/dL (29.0 per 1000 persons/year, 95%CI 28.0–29.9). Incidence rates observed in subsets of cases defined by age, gender, FPG and BMI are shown in Table 2.

The rates of incident IFG were greater compared to ID. Incident IFG occurred in 450 cases (8.1% of the normoglycemic population at baseline). The incidence rate in the whole population was 25.59 cases per 1000 persons/year. Higher rates were observed in men (27.4 vs 24.7 per 1000/year) and in subjects older than age 55, BMI ≥ 35 kg/m^2 or triglycerides > 150 mg/dL. The highest IFG incidence rates were observed in subjects with FPG ≥90 mg/dL. Young obese subjects had similar IFG incident rates than those observed in lean individuals older than age 55.

Anthropometric and sociodemographic risk factors for ID

We observed a higher risk of ID in first-degree relatives of T2D cases. Furthermore, we observed higher ID risk for individuals ages 45–60 (HR 1.89 95%CI 1.25–2.84) and > 60 years (HR 2.20 95%CI 1.33–3.64) compared to the reference group (Table 3). In addition, we identified significantly higher risk of ID with abdominal obesity by IDF criteria, which was higher in men (HR 2.45 95%CI 1.37–4.37) compared to women (HR 1.98 95%CI 1.30–3.03). Abdominal obesity by ATP-III criteria was also associated, though the risk was lower. Overweight and obese BMI categories were also associated with higher ID risk in comparison to normal BMI group. When evaluating other anthropometric measures, we observed an increased risk for waist-hip (WH) ratios > 0.85 in females and > 0.90 in males and the waist-height ratio (WHtr) > 0.5; using ROC curves,

Table 1 Baseline and follow-up biochemical and anthropometric characteristics comparing individuals who did and did not develop incidence diabetes after follow-up

Parameter	No diabetes (*N* = 5813)		*p*-value	Incident diabetes (*n* = 331)		*p*-value
	Baseline	Follow-up		Baseline	Follow-up	
Metabolic syndrome IDF (%)	2455 (42.2%)	2673 (46.0%)	<0.001	244 (73.7%)	271 (81.9%)	<0.001
Metabolic syndrome ATP-III (%)	1821 (31.3%)	2040 (35.1%)	<0.001	217 (65.6%)	276 (83.1%)	<0.001
Fasting glucose (mg/dL)	85.35 ± 10.45	85.53 ± 11.89	0.227	97.47 ± 13.42	112.69 ± 38.41	<0.001
BMI (kg/m^2)	28.65 ± 4.58	28.74 ± 4.66	0.001	31.01 ± 5.19	30.79 ± 5.18	0.061
Waist circumference (cm)	92.72 ± 11.44	93.57 ± 11.47	<0.001	98.14 ± 12.40	98.92 ± 12.70	0.064
Waist-hip ratio	0.88 (0.83–0.94)	0.89 (0.84–0.94)	<0.001	0.91 (0.86–0.98)	0.91 (0.86–0.96)	0.482
Waist-height ratio	0.57 (0.53–0.62)	0.58 (0.54–0.62)	<0.001	0.60 (0.57–0.65)	0.61 (0.57–0.67)	0.086
Fasting triglycerides (mg/dL	187.90 ± 141.26	174.20 ± 112.42	<0.001	226.93 ± 154.87	207.59 ± 121.16	0.020
Fasting insulin (μl/mL)	11.75 ± 7.64	12.11 ± 10.14	0.007	15.82 ± 10.30	17.26 ± 15.37	0.083
Total cholesterol (mg/dL)	205.76 ± 40.94	197.78 ± 40.07	<0.001	211.17 ± 40.69	202.69 ± 39.33	<0.001
HDL-c (mg/dL)	44.81 ± 11.69	41.71 ± 12.11	<0.001	42.41 ± 10.97	40.20 ± 10.88	<0.001
LDL-c (mg/dL)	125.48 ± 32.09	122.64 ± 31.34	<0.001	128.91 ± 32.61	126.18 ± 30.42	0.166
Non-HDL-c (mg/dL)	160.94 ± 39.31	156.06 ± 37.87	<0.001	168.76 ± 37.86	162.48 ± 36.50	<0.001
Apolipoprotein B (mg/dL)	108.27 ± 26.95	103.05 ± 26.11	<0.001	114.92 ± 26.00	108.42 ± 27.60	<0.001
C-reactive protein	1.88 (0.97–3.91)	1.82 (0.86–3.81)	0.057	2.97 (1.45–5.32)	3.04 (1.46–6.10)	0.689

P-values for paired comparisons in each group

Table 2 Diabetes incidence rates (cases/1000 persons per year) in the study sample stratified by gender, age and impaired fasting glucose

Sex	Category	< 35	35–44.9	45–54.9	≥55
Males	BMI < 25 kg/m^2	12.66	9.61	8.85	13.60
	BMI 25–29.9 kg/m^2	6.45	14.06	10.60	43.26
	BMI ≥30 kg/m^2	12.22	26.86	43.66	44.44
	BMI < 25 kg/m^2 + FPG < 100 mg/dl	12.86	6.97	4.93	–
	BMI < 25 kg/m^2 + FPG ≥100 mg/dl	–	40.00	43.48	74.07
	BMI 25–29.9 kg/m^2 + FPG < 100 mg/dl	4.09	7.80	7.21	23.03
	BMI 25–29.9 kg/m^2 + FPG ≥100 mg/dl	47.62	71.43	36.70	112.36
	BMI ≥30 kg/m^2 + FPG < 100 mg/dl	13.23	8.93	29.89	29.41
	BMI ≥30 kg/m^2 + FPG ≥100 mg/dl	–	118.18	92.59	90.91
Females	BMI < 25 kg/m^2	6.68	4.30	13.77	18.25
	BMI 25–29.9 kg/m^2	15.68	10.24	16.74	33.97
	BMI ≥30 kg/m^2	18.61	21.47	36.27	28.71
	BMI < 25 kg/m^2 + FPG < 100 mg/dl	5.42	4.46	10.83	4.27
	BMI < 25 kg/m^2 + FPG ≥100 mg/dl	100.00	–	74.07	100.00
	BMI 25–29.9 kg/m^2 + FPG < 100 mg/dl	13.66	8.32	13.87	11.56
	BMI 25–29.9 kg/m^2 + FPG ≥100 mg/dl	60.00	39.06	40.54	107.59
	BMI ≥30 kg/m^2 + FPG < 100 mg/dl	11.09	11.44	23.15	20.64
	BMI ≥30 kg/m^2 + FPG ≥100 mg/dl	82.35	81.90	86.06	47.12

Abbreviations: *FPG* Fasting plasma glucose, *BMI* Body-mass index

Table 3 Assessment of anthropometric, demographic and biochemical risk factors for incident diabetes obtained through unadjusted Cox-proportional hazard regression analyses in Mexican population according to predefined cut-off values

Parameter	β	HR	95%CI	*P*-value
Age ≥ 40 years	0.537	1.711	1.345–2.175	<0.001
Family history of T2D	0.287	1.332	1.069–1.660	0.011
Waist circumference (IDF)	0.918	2.505	1.677–3.744	<0.001
Waist circumference (ATP-III)	0.660	1.934	1.539–2.430	<0.001
Overweight 25–29.99 kg/m^2	0.453	1.572	1.068–2.316	0.022
Obesity (≥30 kg/m2)	0.902	2.464	1.685–3.605	<0.001
High Waist-hip ratio[a]	0.542	1.720	1.346–2.198	<0.001
Waist-height index > 0.5	0.983	2.673	1.499–4.767	<0.001
Blood pressure > 140/90 mmHg	0.672	1.958	1.566–2.447	<0.001
Fasting glucose 100–110 mg/dL	1.470	4.347	3.378–5.594	<0.001
Fasting glucose 111–125 mg/dL	2.353	10.512	7.792–14.182	<0.001
Fasting insulin ≥15uUI/L	0.634	1.886	1.501–2.368	<0.001
HOMA2-IR > 2.5	0.871	2.389	1.877–3.042	<0.001
Fasting triglycerides > 150 mg/dL	0.824	2.280	1.767–2.943	<0.001
Total colesterol > 200 mg/dL	0.391	1.478	1.174–1.861	0.001
Low HDL-C	−0.225	0.798	0.636–1.003	0.053
Non-HDL-C > 130 mg/dL	0.599	1.820	1.325–2.500	<0.001
Apolipoprotein B (>90th percentile)	0.415	1.515	1.205–1.903	<0.001
CPR ≥2.3	0.470	1.600	1.278–2.003	<0.001

[a]Waist hip ratio > 0.85 females, > 0.9 males

we observed the highest AUC for the WHtr, which also had the highest risk amongst all other anthropometric indexes (Additional file 1: Table S1). When assessed the role of gender-based differences in ID risk, we observed no significant differences between men and women ($p = 0.418$), but we did observe a significant interaction of sex with increasing age. Compared to women < 40 years, men aged 55–70 (HR 1.94 95%CI 1.20–3.16) and men > 70 years (HR 3.20 95%CI 1.31–7.83) had higher rates if ID adjusted for WC, family history of T2D, physical activity and smoking. Among women, the ID risk was higher in post-menopausal women (HR 1.39 95%CI 1.02–2.24) adjusted for hypertension, family history of T2D, WC, physical activity and smoking.

Metabolic risk factors for ID

Among biochemical variables, the strongest predictor of ID was FPG. Subjects with FPG 100-110 mg/dL had four-fold higher risk of ID compared to subjects with FPG < 100 mg/dL, with the highest risk attributable to FPG 111–125 mg/dL. Overall, subjects with IFG had five-fold higher risk of ID compared to normoglycaemic subjects. We also observed a significant interaction between age and fasting glycaemia to predict ID cases ($p < 0.001$). Subjects with IFG aged 30–45 years had higher risk of ID compared to individuals < 30 years (HR 5.40 95%CI 4.0–7.29), an observation that was also confirmed in the 46–60 (HR 5.71 95%CI 4.31–7.56) and > 60 year-groups (HR 7.09 95%CI 4.46–11.26). The predictive capacity of each biochemical measure according to pre-defined cut-offs showed the highest ID risk for HOMA2-IR > 2.5 and triglycerides > 150 mg/dL (Table 3).

Metabolic syndrome and ID

We observed a three-fold higher ID risk in subjects who had metabolic syndrome by IDF criteria (MS-IDF) at baseline (HR 3.42, 95%CI 2.68–4.37) compared to those who did not. ID risk was higher using the ATP-III criteria MS definition (MS-ATP-III, HR 1.81 95%CI 1.72–2.13). In relation to MS-IDF criteria, we observed significantly higher risk with ≥2 components. We observed a higher risk with 2 components (HR 3.84 95%CI 2.21–6.68), 3 components (HR 6.76 95%CI 3.86–11.85) and the highest with 4 components (HR 11.59 95%CI 6.29–21.37). Using MS-ATP-III the risk increased with 2 components (HR 2.15 95%CI 1.17–3.97), 3 components (HR 4.52 95%CI 2.49–8.21), 4 components (HR 6.84 95%CI 3.72–12.59) and 5 components (HR 10.43 95%CI 5.32–20.45), which was lower compared to MS-IDF (Fig. 2).

Risk factors for early-onset incident diabetes

We observed 93 cases of early onset ID over 6298-person years, yielding an incidence rate of 14.77 cases per 1000 person-years (95%CI 14.21–15.35), which was lower to that observed in individuals with ID onset > 40 years (IR 27.02 95%CI 26.14–27.92). At baseline, subjects with early-onset ID had higher HOMA-IR, fasting insulin, triglycerides compared to subjects with ID ≥40 years. Furthermore, subjects with early-onset ID had lower FPG, BMI, waist circumference, systolic and diastolic blood pressure, total cholesterol, HDL-C and apoB levels, adjusted for age and sex. Using multivariate Cox regression, we observed that HOMA-IR > 2.5 (HR 1.82 95%CI 1.13–2.93) and FPG > 100 mg/dL (HR 2.26 95%CI 1.63–3.14) were risk factors for early onset ID, whilst physical activity was a protective factor (HR 0.55 95%CI 0.36–0.83), adjusted for age, sex, first-degree family history of diabetes, WHtr > 0.5, smoking and hypertension. Finally, we observed a statistically significant interaction between HOMA-IR > 2.5 and first-degree family history of T2D (HR 1.79 95%CI 1.05–3.04) only in individuals with early onset ID. For ID in individuals ≥40 years, risk factors included hypertension (HR 1.47 95%CI 1.11–1.94), WHtr > 0.5 (HR 1.82 95%CI 1.27–2.61) and FPG > 100 mg/dL (HR 3.17 95%CI 2.66–3.79). Physical activity and insulin resistance estimated using HOMA-IR were not associated with ID in individuals > 40 years.

Development of a predictive model for diabetes incidence

We developed two main models for prediction of ID in Mexican population, an office-based model, which does not rely on fasting laboratory measurements, and a clinical biochemical method. For the office-based model, we identified as potential predictors age > 40 years, first-degree family history of T2D, WHtr > 0.5, arterial hypertension and BMI ≥ 30 kg/m^2 (Table 4); the model was validated using k-fold cross-validation (k = 10) and bootstrap validation ($D_{xy} = 0.287$, *c-statistic* = 0.656). We constructed a point-based model using β-coefficients assigning a score = 1.0 to β-coefficients < 0.35, 2 to β-coefficients 0.35–0.7 and 3 to coefficients > 0.7. Using Cox regression, we evaluated the predictive capacity of threshold scores for ID. Using as reference level scores 1–3, scores between 4 and 6 had nearly two-fold higher risk for ID (HR 1.87 95%CI 1.18–2.98), followed by scores 7–8 (HR 3.36 95%CI 2.11–5.37) and the highest risk for scores 9–10 (HR 5.43 95%CI 3.31–8.91). Accumulated incidence was different between score categories (log-rank $p < 0.001$).

For the biochemical model, we identified as potential predictors age > 40 years, fasting triglycerides > 150 mg/dL, FPG 100–110 mg/dL, FPG 111–125 md/dL, arterial hypertension and abdominal obesity as diagnosed by IDF criteria, which was also validated and corrected for over-optimism ($D_{xy} = 0.487$, *c-statistic* = 0.741). Next, we constructed a similar model, assigning scores using a similar methodology from the office-based model. We analyzed strata using Cox regression and using as a reference scores > − 1 but ≤4 we observed increased risk in patients with scores 5–8 (HR 2.28 95%CI 1.68–3.10), followed by scores 9–12 (HR 6.99 95%CI 5.04–3.69) and the highest risk for scores 13–16 (HR 18.69 95%CI 12.83–27.22). Evaluation between score categories

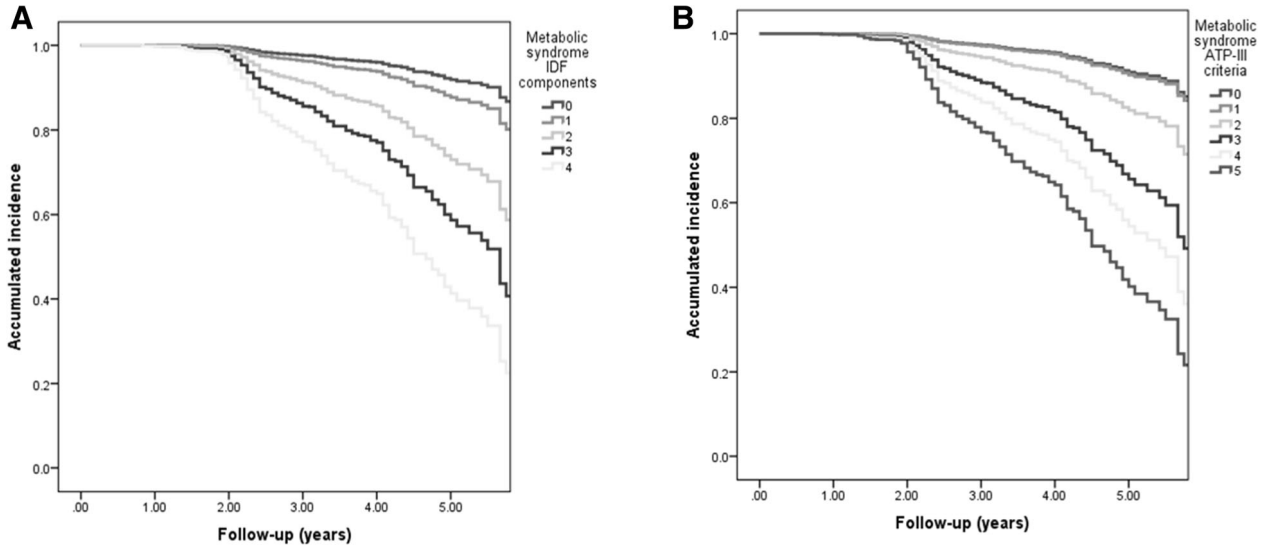

Fig. 2 Kaplan-Meier survival analyses for incident diabetes according to the number of components based on IDF (**a**) and ATP-III (**b**) criteria adjusted for age and sex. In both IDF and ATP-III criteria, we observed a significantly higher accumulated incidence for each additional MS component > 1 ($p < 0.001$) compared to none of the risk factors

showed different accumulated incidence (log-rank p < 0.001, Fig. 3). Overall, the biochemical model had a higher predictive accuracy (AUC = 0.752 95%CI 0.724–0.781), compared to FINDRISC (AUC = 0.634 95%CI 0.604–0.664) and the Cambridge risk score (AUC 0.654 95%CI 0.623–0.686) in our population.

Discussion

Our work is the first to estimate T2D incidence in central Mexico and the first in Latin America with sample large enough to develop predictive models in a high-risk, genetically-predisposed population. The only previous report about ID in adult Mexicans reported that 7% of 1244 adults who resided in a Mexico City neighborhood had hyperglycemia during a six-year observational period [9]. Even though direct comparisons between studies are not feasible, the incidence reported in our population is higher considering follow-up time, which reported 5.38% in a median of 2.4 years. FPG was the variable with the highest predictive value, followed by the WHtr, obesity diagnosed by BMI, hypertriglyceridemia > 150 mg/dL and HOMA2-IR values > 2.5. Despite the fact that FPG has been questioned as a detection method for type 2 diabetes, in our population it was a major prognostic factor for T2D.

The increase in diabetes-related mortality and the poor metabolic control in diagnosed individuals in Mexico represents a major concern [20]. Identifying risk factors for incident diabetes is of paramount importance for early

Table 4 Office-based and biochemical model for prediction of incident diabetes from Cox-proportional hazard regression models

Model parameters	Variable	β-coefficient	Wald	HR	95%CI	*p*-value	Points
Office-based model $X^2 = 76.64$ $p < 0.0001$ $D_{xy} = 0.2915$ c-statistic = 0.656	Age > 40 years	0.466	14.079	1.593	1.249–2.031	<0.001	2
	FDFH of T2D	0.242	4.560	1.273	1.020–1.590	0.033	1
	WHr > 0.5	0.725	5.809	2.065	1.145–3.725	0.016	3
	Arterial hypertension	0.503	18.471	1.654	1.315–2.080	<0.001	2
	BMI ≥30 kg/m2	0.388	11.500	1.474	1.178.1.845	0.001	2
Biochemical model $X^2 = 446.815$ p < 0.0001 $D_{xy} = 0.4723$ c-statistic = 0.752	Physical activity	−0.217	3.374	0.805	0.638–1.015	0.066	−1
	Age > 40 years	0.328	6.830	1.388	1.085–1.776	0.009	2
	TG > 150 mg/dL	0.517	14.932	1.677	1.287–2.185	<0.001	3
	Glucose 100–110 mg/dL	1.271	92.355	3.565	2.751–4.621	<0.001	4
	Glucose 111–125 mg/dL	2.097	176.167	8.138	5.971–11.091	<0.001	7
	Arterial hypertension	0.306	6.711	1.358	1.077–1.712	0.010	2
	Abdominal obesity (IDF)	0.422	5.576	1.525	1.074–2.165	0.018	2

Discrimination indexes from both regression models were obtained from k-fold cross-validation (k = 10) and were corrected for over-optimism

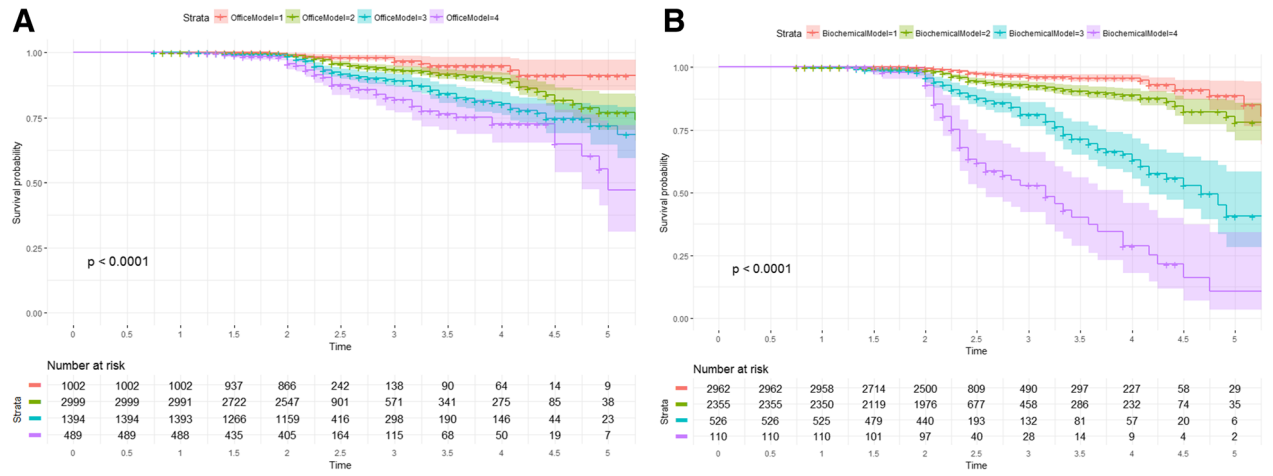

Fig. 3 Kaplan-Meier survival curves for incident diabetes according to stratified scores by quartiles obtained from our office-based and biochemical models for incident diabetes in the MS cohort (log-rank test $p < 0.001$)

detection of at-risk individuals, especially considering that T2D often has early-onset in our population, which leads to a higher incidence of adverse metabolic and cardiovascular outcomes [2, 8].

Several prognostic models and scores for type 2 diabetes risk have been developed based on identified risk factors including age, sex, obesity, diet, exercise, ethnicity, family history of diabetes amongst others. Our findings are similar to the FINDRISC study in Finland [21], which also included BMI, age and physical activity. However, the application of the FINDRISC score in our population does not have a high predictive accuracy. Our biochemical model was decidedly superior. The Australian AUSDRISK study [22] and UK-based Cambridge Risk Score, [23] also include age, sex, family history of diabetes, BMI and physical activity also underperformed in comparison to the biochemical model but were superior to the office-based model. The model reported here outscores other models (i.e. those derived from the ARIC [24] and the Framingham Offspring Study [25]), which include family history of diabetes and age and strongly differ from our proposed models.

Diabetes incidence in our study was among the highest reported in the literature for different ethnic groups, particularly considering the relatively short follow-up period. This high diabetes incidence could be attributable to the elevated prevalence of overweight and obesity across different age ranges in Mexican population as well as the high rate of inactivity combined with a high carbohydrate and fat intake. As reported by Stolerman et al., incorporation of genetic risk scores does not improve the prognostic performance of predictive models including clinical variables in a multiethnic cohort, which suggests that environmental risk factors could have a much greater impact in diabetes development in interaction with genetic risk factors [26]. Currently, there are several efforts to integrate -omics- technologies in risk prediction, which should be helpful to increase predictive performance of risk models with potential biomarkers of risk including genetic variants, RNA transcripts, peptides, lipids, small metabolites, cell markers and metabolic-driven products [27].

Our study had some strengths and limitations. First, we evaluated a large prospective effort to estimate diabetes incidence in a high-risk, not previously evaluated population, which allowed for identification of metabolic risk factors that predict ID. The loss to follow-up was relatively minor (19.6%), with no significant differences comparing individuals who did and did not complete follow-up, which allowed for an adequate estimate of diabetes incidence with enough statistical power to develop predictive models and validate metabolic measures [28]. Furthermore, we validated both our models using k-fold cross-validation and bootstrap to correct for over-optimism, which ensures validity of our observations. We also evaluated our proposed score against competing models constructed with similar variables and observed a superior predictive performance. The main limitations to be recognized is the lack of an external validation for calibration of the risk scores, which calls for further evaluations to assess the validity to implement our score in other Latin American populations. In addition, the inclusion criteria for this study could generate bias towards subjects with the highest risk, which calls for additional evaluations in low-risk populations with similar genetic profiles. Finally, given that T2D diagnosis was mainly based on previous diagnosis and a single abnormal FPG measurement, the true number of ID cases could have been underestimated if patients with undiagnosed T2D had FPG below the diagnostic threshold.

Conclusion

Type 2 diabetes incidence in apparently healthy middle-aged Mexican adults residing in urban centers in Mexico is currently at an alarming rate. FPG is the strongest predictor of incident diabetes, particularly in overweight and obese individuals. We constructed two models that can easily be implemented to predict diabetes risk in Mexican population, including age, BMI, WHtr, IFG, arterial hypertension, fasting hypertriglyceridemia and family history of diabetes, which represents an advantage given their availability in primary-care facilities and allows for large-scale implementations. Further studies are required for validation of these models in similar at-risk populations. Our study represents the largest prospective study regarding metabolic diseases in Latin-American population and the only current predictive model for diabetes in Mexican population.

Additional file

Additional file 1: Table S1. Assessment of anthropometric, demographic and biochemical risk factors for incident diabetes obtained through Cox-proportional hazard regression analyses in Mexican population, along with their predictive performance using area under the receiving operating characteristic curves. (DOCX 16 kb)

Abbreviations

95%CI: 95% confidence interval; ATP-III: Adult treatment panel III; AUC: Area under the curve; BMI: Body-mass index; FINDRISC: Finnish Diabetes Risk Score; FPG: Fasting plasma glucose; HDL-C: High-density lipoprotein cholesterol; HOMA2-IR: Homeostatic model assessment for insulin resistance 2; HR: Hazard ratio; ID: Incident type 2 diabetes mellitus; IDF: International Diabetes Federation criteria; IFG: Impaired fasting glucose; IR: Incidence rates; MS: Metabolic syndrome; T2D: Type 2 Diabetes Mellitus; WHr: Weight to hip ratio; WHtr: Weight to height ratio

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

OAC, DGV, OYBC, MTTL, RR, FJGP and CAAS developed the conceptualization of the study. OAC, DGV, MAMH, ICB, LMH, LEG, JJGG, UA, YOY, RCR, LSR, MEGS, JMMH, LMGR, RR, CAAS and MTTL conducted the studies in the different cohort centers, recruited patients and processed biological samples. OYBC, RR and CAAS performed and interpreted statistical analyses and developed predictive models. OAC, DGV, OYBC, RR and CAAS also participated in manuscript drafting and processing. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. All authors read and approved the final version of this manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición and all participants signed an informed consent form.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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The *SLC16A11* risk haplotype is associated with decreased insulin action, higher transaminases and large-size adipocytes

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Abstract

Objective: A haplotype at chromosome 17p13 that reduces expression and function of the solute carrier transporter SLC16A11 is associated with increased risk for type 2 diabetes in Mexicans. We aim to investigate the detailed metabolic profile of *SLC16A11* risk haplotype carriers to identify potential physiological mechanisms explaining the increased type 2 diabetes risk.
Design: Cross-sectional study.
Methods: We evaluated carriers (n = 72) and non-carriers (n = 75) of the *SLC16A11* risk haplotype, with or without type 2 diabetes. An independent sample of 1069 subjects was used to replicate biochemical findings. The evaluation included euglycemic–hyperinsulinemic clamp, frequently sampled intravenous glucose tolerance test (FSIVGTT), dual-energy X-ray absorptiometry (DXA), MRI and spectroscopy and subcutaneous abdominal adipose tissue biopsies.
Results: Fat-free mass (FFM)-adjusted M value was lower in carriers of the *SLC16A11* risk haplotype after adjusting for age and type 2 diabetes status (β = –0.164, P = 0.04). Subjects with type 2 diabetes and the risk haplotype demonstrated an increase of 8.76 U/L in alanine aminotransferase (ALT) (P = 0.02) and of 7.34 U/L in gamma-glutamyltransferase (GGT) (P = 0.05) compared with non-carriers and after adjusting for gender, age and ancestry. Among women with the risk haplotype and normal BMI, the adipocyte size was higher (P < 0.001).

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Conclusions: Individuals carrying the *SLC16A11* risk haplotype exhibited decreased insulin action. Higher serum ALT and GGT levels were found in carriers with type 2 diabetes, and larger adipocytes in subcutaneous fat in the size distribution in carrier women with normal weight.

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Introduction

The Slim Initiative in Genomic Medicine for the Americas (SIGMA) Type 2 Diabetes Genetics Consortium identified a genome-wide significant association of a haplotype on chromosome 17p13 with increased risk of type 2 diabetes (1). This risk haplotype is highly prevalent in populations with a native American background (e.g. Mexican mestizos), in whom the risk variants are present in more than 25% of the population. The associated haplotype credible set includes four missense and one silent variant in *SLC16A11* (V113I, L187L, D127G, G340S and P443T), as well as several non-coding variants in regulatory regions near the *SLC16A11* gene. SLC16A11 is most highly expressed in thyroid, liver and salivary gland. The type 2 diabetes-associated variants lead to lower SLC16A11 expression in liver and also disrupt the interaction between SLC16A11 and basigin, a chaperone protein important for plasma-membrane localization of SLC16A11 (2). Together, these variant effects result in less SLC16A11 at the cell membrane, thus reducing SLC16A11 function. Knockdown of SLC16A11 expression in primary human hepatocytes alters fatty acid and lipid metabolism, leading to increases in intracellular acylcarnitine, diacylglycerol and triacylglycerol levels (2). These metabolic changes are also observed in experimental models of lipotoxicity and insulin resistance and as part of the pathophysiology of type 2 diabetes (3, 4).

The findings from these molecular and cellular studies implicate a role for SLC16A11 in hepatic metabolism and suggest primary effects on type 2 diabetes through insulin-resistant mechanisms; however, the physiologic mechanisms explaining the association of the *SLC16A11* risk haplotype with hyperglycemia in humans have not been studied. Therefore, we conducted a deep phenotyping study in a sufficiently high number of risk haplotype carriers to comprehensively characterize the mechanisms through which variation in *SLC16A11* contributes to the pathophysiology of type 2 diabetes. We hypothesized that carriers of the *SLC16A11* risk haplotype will show lower insulin sensitivity in comparison with subjects without the risk haplotype.

Subjects and methods

We enrolled Mexican-mestizo men and women (with parents and grandparents born in Mexico), carriers (homozygous or heterozygous) or non-carriers of the risk haplotype at the *SLC16A11*, aged 20 to 79 years old, with a BMI between 18 and 34.9 kg/m^2. Individuals with type 2 diabetes with HbA_{1c} concentration <8% and without insulin treatment were eligible for the study. No subject smoked tobacco, had cardiovascular disease, diabetes complications or an acute infection. Subjects with more than a 3% weight loss in the last 3 months, taking medications or with conditions that could interfere with insulin secretion and action, high-performance athletes, with alcohol consumption more than 2 units per day in men or 1 unit in women were also excluded. Carriers and non-carriers were matched by gender, age (±5 years), BMI (±5 kg/m^2) and in the type 2 diabetes group also by HbA_{1c} (±1%). Subjects provided written informed consent before participating in this study, which was approved by the Comité de Ética en Investigación of the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ). All procedures were in accordance with the ethical standards of the Helsinki declaration.

Replication sample

To confirm some of the associations between phenotypes and the *SLC16A11* haplotype, we used a dataset that included an independent group of 1069 subjects in whom *SLC16A11* genotype was known. This dataset is composed of subjects seeking attention at the Diabetes, Obesity, Internal Medicine or Dyslipidemia outpatient's Clinics at the INCMNSZ. The inclusion criteria were the same as in the discovery sample.

Experimental procedures

Studies were conducted at the Unidad de Investigación de Enfermedades Metabólicas (UIEM) at the INCMNSZ,

whereas MRI studies were performed at the Centro Nacional de Investigación en Imagenología e Instrumentación Médica in Mexico City between 2015 and 2017. All evaluations were completed in the course of 1 month period.

To identify carriers of the risk haplotype of the *SCL16A11* variant samples were genotyped using a Quant Studio 12K Flex Real-Time PCR platform from Thermo Fisher Scientific.

Body composition: Body fat mass (FM) and fat-free mass (FFM) were determined using dual-energy X-ray absorptiometry (DXA) (GE Healthcare). Subcutaneous and intra-abdominal adipose tissue volumes were quantified using MRI, and the subcutaneous/intra-abdominal fat ratio was calculated. Intra-pancreatic and intrahepatic triglycerides content was determined using MRI spectroscopy (Philips Achieva 3 Teslas).

Insulin sensitivity

Participants were instructed to fast for 12 h before the study and admitted to the UIEM the day of the euglycemic–hyperinsulinemic clamp. Subjects with type 2 diabetes were instructed to suspend oral treatment 3 days before the procedure. The study was not performed if the fasting glucose concentration was >250 mg/dL. A catheter was inserted into a forearm vein to infuse dextrose and insulin, and a second catheter into a forearm vein in the contralateral hand was inserted in a retrograde fashion to obtain arterialized blood samples using a hot box. Insulin was infused at a rate of 50 mU/m^2 body surface area (BSA)/min (initiated with a priming dose of 200 mU/m^2/min for 5 min and then 100 mU/m^2/min for 5 min). Euglycemia (~100 mg/dL) was maintained by a variable infusion of 20% dextrose. During the clamp procedure, blood samples were drawn every 10 min during the final 30 min to determine glucose and insulin concentrations. Insulin sensitivity was determined as the glucose infusion rate (*M* value) during the final 30 min adjusted for weight and for the FFM (5).

Insulin secretory response: Participants were instructed to fast for 12 h before the frequently sampled intravenous glucose tolerance test (FSIVGTT). Two intravenous catheters were placed in antecubital veins (one in each arm). Blood samples were withdrawn at −10, −5, 0, 2, 3, 4, 5, 6, 8, 12, 14, 16, 19, 22, 24 and 25 min for measurement of serum glucose and insulin. Glucose was administered intravenously at a dose of 0.3 g/kg for 60 s beginning at time 0. The MINMOD Millenium computer package (6, 7) was used to estimate the acute insulin response to glucose (AIRg). The AIRg represents the acute insulin response and is defined as the area under the serum insulin curve between 0 and 10 min (7). The AIRg was adjusted by insulin sensitivity obtained in the clamp procedure (*M* value).

Adipose tissue morphometric analysis

Subcutaneous fat abdominal tissue biopsies were obtained nearby the umbilicus, in fasting conditions. For this report, adipocyte size was analyzed from subjects with normal weight. Thirteen controls (non-carriers: 7 women and 6 men) and 20 subjects with the *SLC16A11* risk haplotype (carriers: 16 women and 4 men) were included in the analyses. Paraffin-embedded subcutaneous fat sections of 5 µm thickness were mounted on poly- L-lysine pre-coated slides. After deparaffinization and rehydration, slides were stained with hematoxylin and eosin (Sigma-Aldrich). For each adipose tissue sample, 25 different fields were visualized with a Leica DM1000 LED (Leica Microsystems) microscopy and pictures taken in jpg format, using a LEICA ICC50 HD light microscope at 20× magnification. Subcutaneous fat cells were measured manually by delimiting the fat cell cross-sectional area in digital images using AxioVisio LE software real 4.8 versions (Zeiss copyright 2006–2010 Stuttgart-Germany). Data were obtained in 250 cells per subject. All histological measurements were performed by two independent observers without knowledge of the source of the tissues. Results are the averages of the two observers.

Laboratory methods: Plasma glucose concentration was measured by an automated glucose analyzer (Yellow Springs Instruments Co.). Serum insulin concentration was measured by using a chemiluminescent immunoassay (Beckman Coulter Access 2) and HbA_{1c} levels with HPLC (Variant II Turbo, Bio-Rad). Lipid concentrations (cholesterol, triglycerides and HDL cholesterol), apolipoprotein AI, apolipoprotein B, uric acid, creatinine, hepatic enzymes and C reactive protein were measured using colorimetric assays (Unicel DxC 600 Synchron Clinical System Beckman Coulter). LDL cholesterol was calculated with the Friedewald equation when the triglyceride concentration was <250 mg/dL (8). Thyroid-stimulating hormone (TSH) and free T4 were measured using electrochemiluminescence. For the TSH measurement a third-generation assay was used (Beckman Coulter). Plasma adiponectin, leptin and fibroblast growth factor (FGF)-21 concentrations were determined by performing ELISA assays (Merck Millipore).

Population stratification

A principal components analysis was performed on the 32 ancestry informative markers genotypes, previously validated against whole genomic data using EIGENSTRAT software (9). The top two principal components were used as covariates in the linear regression model to correct for ancestry.

Statistical analysis

Sample size was estimated to have adequate power (between 80 and 90%) to detect a difference between carriers and non-carriers of the *SLC16A11* risk haplotype on the main variables: log-transformed *M* value normalized for FFM and adjusted for the presence of type 2 diabetes; AIRg adjusted by age, sex and presence of type 2 diabetes and log-transformed ALT, AST and GGT. Continuous variables were tested for normality according the Kolmogorov–Smirnov test. Non-normally distributed variables are presented as medians and (interquartile ranges). Comparisons between carriers and non-carriers of the *SLC16A11* haplotype were performed with Student's *t* or Mann–Whitney *U* tests, as appropriate. Bivariate correlations were evaluated using Spearman coefficients, and adjusted correlations were also performed. In order to assess the effect of genotype on insulin sensitivity (FFM-adjusted *M* value) according diabetes status, stratified linear regression models adjusted for age were run. Before linear regression analysis non-normally distributed variables were log-transformed. In the replication sample, the association between genotype and transaminases was assessed separately for individuals with and without diabetes through two-step regression models. In the first step, the outcome was regressed on age and gender. Residuals from this model were normalized using inverse normal transformation. In the second step, the residuals were taken as the outcome and regressed on genotype. Sensitivity of the model to outliers was assessed by comparing the coefficients obtained from the model with and without outliers. The assumptions of the linear regression model were checked via residual diagnostics. Analyses were performed using SPSS software, version 21 (SPSS Inc.) and RStudio version 3.3.2.

Adipocyte size central tendency and data distribution

Adipocyte size differences among groups: normal weight female non-carriers ($n=1750$ cells/7 women) vs carriers ($n=4000$ cells/16 women) and normal weight male non-carriers ($n=1500$ cells/6 men) vs carriers ($n=1000$ cells/4 men) were compared by rank-sum test. Adipocyte size data from all groups were visualized in scatter plots.

Results

A total of 170 potential participants were screened for this study; 150 fulfilled the inclusion criteria and were invited to participate. Three participants withdrew consent and were discontinued. Seventy-five individuals were non-carriers and 72 were carries of whom 54 were heterozygous and 18 homozygous for the *SLC16A11* risk haplotype.

Metabolic characteristics

Table 1 shows the general characteristics of the participants. The biochemical and anthropometric characteristics were similar in groups with and without the risk haplotype. No differences were observed in the lipid profile, HbA_{1c} levels, adipokines and markers of low-grade inflammation. The independent sample of 1069 (449 non-carriers and 620 carriers of the *SLC16A11* risk haplotype) was composed of 25.2% individuals with type 2 diabetes with a median age of 44 years. Glucose and HbA_{1c} concentrations were higher in the carries of the *SLC16A11* risk haplotype (Table 2).

Insulin sensitivity

Insulin action (evaluated by the clamp procedure) was significantly lower in carriers of the *SLC16A11* risk haplotype: FFM-adjusted *M* value 9.7 (7.5–13.2) vs 12.2 (9.4–15.3) mg/kg FFM, $P=0.038$ (Fig. 1). Mean glucose and insulin concentrations at the end of the clamp were 99.5 ± 2.8 mg/dL and 88.4 (74.2–105.9) µUI/mL respectively. The risk *SLC16A11* haplotype ($\beta=-0.164$, $P=0.048$) and the presence of type 2 diabetes ($\beta=-0.333$, $P=0.003$) were significantly and independently associated with insulin sensitivity (FFM-adjusted M value) in an additive model adjusted for age ($R^2=0.188$, $F=10.3$, $P<0.0001$). In the replication sample, we observed a higher HOMA2-IR adjusted for age, sex and FFM evaluated with bioelectrical impedance analyses (BIA) in carriers without type 2 diabetes compared to non-carrier individuals with borderline statistically significance ($\beta=0.2713$, $P=0.058$).

Liver enzymes

Serum alanine transaminase (ALT) concentration was significantly higher in *SLC16A11* risk haplotype

Table 1 General characteristics of the carriers and non-carriers of the *SLC16A11* risk haplotype.

Variable	Non-carriers (*n* = 75)	Carriers (*n* = 72)	*P*
Female sex	38 (55.1)	40 (54.8)	0.957
Type 2 diabetes	35 (50.7)	37 (50.7)	0.996
Age, years	45.5 (28–57.8)	43 (29–56)	0.977
Weight (kg)	70.9 ± 11.8	72.2 ± 12.8	0.582
BMI (kg/m^2)	27.4 ± 3.5	28.1 ± 4.0	0.295
Glucose (mg/dL)	96 (87.5–114)	98 (88.5–110.5)	0.670
A1c (%)	5.7 (5.3–6.3)	5.6 (5.3–6.2)	0.698
Insulin (μU/mL)	9.8 ± 5.6	9.2 ± 6.0	0.276
Triglyceride (mg/dL)	122.5 (82.5–202.0)	122.0 (94.0–159.0)	0.488
Cholesterol (mg/dL)	181.0 ± 36.5	177.3 ± 35.4	0.522
LDL-C (mg/dL)	107.5 ± 27.3	104.9 ± 30.6	0.645
HDL-C (mg/dL)			
Women	45.5 (40.0–51.5)	46.5 (39.3–55.5)	0.779
Men	36.0 (30.0–44.0)	39.0 (34.5–46.0)	0.231
Apo A (mg/dL)	156.7 ± 34.0	151.4 ± 31.0	0.562
Apo B (mg/dL)	96.3 ± 25.3	95.0 ± 23.8	0.725
Uric acid (mg/dL)	5.6 ± 1.3	5.5 ± 1.2	0.983
Creatinine (mg/dL)	0.77 ± 0.25	0.70 ± 0.17	0.141
ALT (U/L)	20.5 (16.0–32.2)	28.0 (22.0–38.0)	0.039
AST (U/L)	23.0 (18.0–29.0)	26.0 (22.0–34.0)	0.067
GGT (U/L)	16.5 (12.0–26.3)	23.0 (15.0–31.0)	0.072
TSH (mIU/L)	1.76 (1.18–2.38)	1.97 (1.31–2.98)	0.271
Free T4 (pmol/L)	11.9 (10.8–12.7)	11.5 (10.9–13.0)	0.803
Adiponectin (ng/mL)	7.6 ± 3.7	8.9 ± 4.6	0.102
Leptin (ng/mL)	11.7 (7.2–23.8)	13.3 (8.2–20.3)	0.903
FGF-21 (ng/L)	154.3 (41.9–347.6)	96.8 (36.9–259.4)	0.151
PCR (nmol/L)	11.71 (6.76–22.09)	14.66 (7.80–46.76)	0.215

carriers (P=0.039). A non-significant similar trend was found for aspartate aminotransferase (AST) (P=0.067) and gamma-glutamyl transferase (GGT) (P=0.072). Subjects with diabetes and the *SLC16A11* risk haplotype demonstrated an increase of 8.76 U/L in ALT (P=0.02) compared with non-carriers after adjusting for gender, age and ancestry. Similarly, carriers with type 2 diabetes had an increase of 7.34 U/L in GGT after adjusting for gender, age and ancestry (P=0.05). The effect of the genotype was not significant in individuals without diabetes (P=0.448 and P=0.549, respectively). No effect of the genotype was found for AST. To increase the power and to replicate the association, the hypothesis was tested in an independent sample. Carriers of the *SLC16A11* risk haplotype with type 2 diabetes showed an increase in GGT and ALT in 11.2 (P=0.03) and 7.9 U/L (P=0.01) respectively.

Insulin secretory response

In the analysis of the FSIVGTT, no difference in the AIRg was observed between carriers and non-carriers of the risk *SLC16A11* haplotype in individuals with or without diabetes (263.2 (67.6–622.5) vs 260.9 (81.5–673.9), P=0.910 and 389.0 (205.5–883.2) vs 541.5 (315.0–931.9) μU/L^{-1}·min^{-1}, P=0.331). Analyzing the disposition index (AIR multiplied by the *M* value), no differences were found in individuals either with or without diabetes (P=0.598 and P=0.162, respectively).

Table 2 General characteristics of the carriers and non-carriers of the *SLC16A11* risk haplotype in the replication sample.

Variable	Non-carriers (*n* = 449)	Carriers (*n* = 620)	*P*
Female sex	298 (60.0)	392 (63.6)	0.252
Type 2 diabetes	105 (21.1)	181 (29.4)	0.002
Age (years)	44 (29–55)	44 (32–54)	0.606
Weight (kg)	71.1 (61.3–82)	71.95 (61.8–81.9)	0.913
BMI (kg/m^2)	27.4 ± 4.8	27.8 ± 4.89	0.169
Glucose (mg/dL)	93 (86–105)	95 (87–109)	0.030
A1c (%)	5.5 (5.2–6.0)	5.6 (5.3–6.2)	0.004
Insulin (μU/mL)	7.8 (4.9–11.3)	7.90 (5.30–12.5)	0.263
Triglyceride (mg/dL)	128 (88.5–190.5)	135 (93.0–200.0)	0.110
Cholesterol (mg/dL)	192.5 ± 43.4	188.4 ± 41.4	0.106
LDL-C (mg/dL)	114 (92.8–134)	112 (89.4–134)	0.224
HDL-C (mg/dL)			
Women	44.0 (37.7–53.0)	44.0 (37.0–53.0)	0.136
Men	45.0 (38.0–53.0)	43.0 (37.0–53.0)	0.532
Apo B (mg/dL)	103.8 ± 27.7	104.6 ± 26.4	0.665
Uric acid (mg/dL)	5.2 (4.3–6.1)	5.2 (4.4–6.3)	0.658
Creatinine (mg/dL)	0.72 (0.61–0.86)	0.71 (0.59–0.84)	0.197
ALT (U/L)	24.0 (18–33)	24.0 (18.0–35.0)	0.219
AST (U/L)	23.0 (20.0–28.0)	23.0 (20.0–28)	0.807
GGT (U/L)	20.0 (14.0–29)	20.0 (14.0–32.0)	0.247

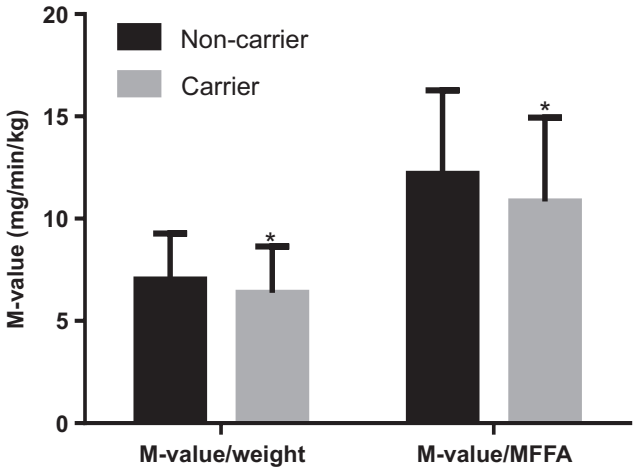

Figure 1
Comparison of median weight and fat-free mass adjusted *M* values obtained by the euglycemic–hyperinsulinemic clamp between carriers (*n* = 75) and non-carriers (*n* = 72) of the risk *SCL16A11* haplotype. Mann–Whitney *U* test was run and a significantly lower *M* value was observed in carriers of the risk haplotype for both weight and fat-free mass adjusted *M* values ($P < 0.05$).

Adipose tissue histology

To avoid the confounding effect of excess adiposity, analyses of adipose tissue histology were performed in participants with a BMI ≤25 kg/m^2. The distribution of the adipocyte size was shifted to higher values among risk haplotype carriers, an effect that was significant ($P<0.001$) only in women. The proportion of total adipocytes represented by smaller-sized fat cells (<5th percentile) was 10 times lower among the women with the risk haplotype, whereas the representation of big-sized fat cells (>95th percentile) was five times greater. Among men, only a two-fold increase in the proportion of the large-sized fat cells was found (Fig. 2).

MRI spectroscopy assessment of intrahepatic, intra-pancreatic, intra-abdominal and subcutaneous fat

No differences were found between carriers and non-carriers of the *SCL16A11* risk haplotype in the intrahepatic, intra-pancreatic, intra-abdominal or subcutaneous fat content (Table 3). However, in the subgroup of women with BMI ≥30 kg/m^2 ($n=17$), carriers of the risk haplotype had a greater amount of intra-abdominal fat (147.3 vs 64.0 cm^2, $P=0.01$) and a lower subcutaneous/intra-abdominal fat ratio (4.9 vs 2.3, $P=0.03$) than non-carriers. In the replication cohort, we observed significantly higher visceral fat content obtained from BIA in carriers compared to non-carriers adjusted for age, sex, type 2 diabetes status and FFM ($P=0.019$).

DXA assessment of body composition

No difference in body composition was found between the risk haplotype carriers and non-carriers (Table 4). The mean or median FFM, FM and bone mineral content were not different between groups.

Discussion

The SIGMA Type 2 Diabetes Genetics Consortium has previously shown that a *SLC16A11* variant haplotype confers ~30% higher risk of type 2 diabetes per copy of the allele and is highly prevalent in populations

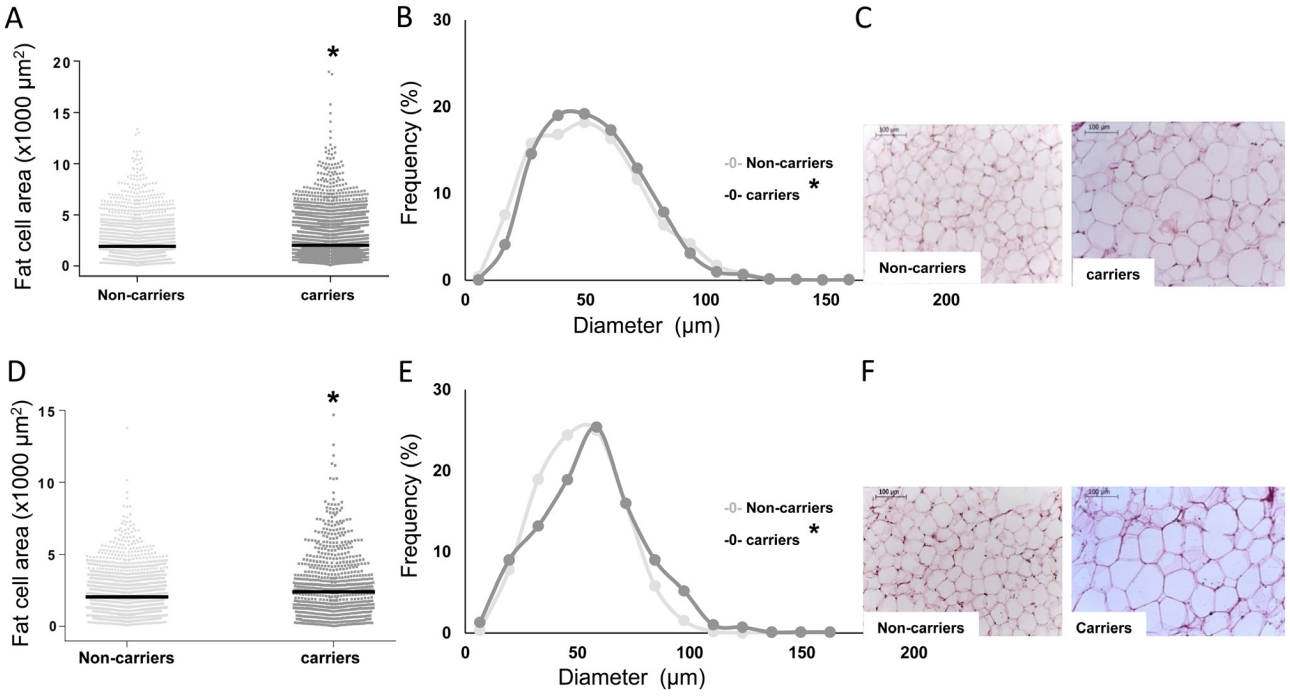

Figure 2

Adipose tissue histology between carriers and non-carriers of the SLC16A11 risk haplotype. A and D. Median adipocyte size and dispersion are represented in the scatter plots ((A) Female, (D) male)). (B and E) Relative frequency histograms for non-carriers (grey) and carriers (black) ((B) Female, (E) male)). (C and F) Microphotography of subcutaneous adipose tissue H&E stained at 20x magnification ((C) Female, (F) male)) *$P<0.01$ carriers vs non-carriers.

Table 3 Intrahepatic, intra-pancreatic, intra-abdominal and subcutaneous fat in carriers and non-carriers of the *SLC16A11* risk haplotype.

Variable	Non-carriers (*n* = 56)	Carriers (*n* = 60)	*P*
Intrahepatic fat (%)	2.0 (0.68–5.8)	2.4 (1.1–5.9)	0.346
Intra-pancreatic fat (%)	2.4 (1.1–8.2)	2.9 (1.3–6.7)	0.934
Intra-abdominal fat (cm^2)	78.4 (51.0–109.7)	76.5 (56.5–123.1)	0.493
Subcutaneous fat (cm^2)	205.0 (175.0–305.0)	237.8 (184.2–315.2)	0.657
Subcutaneous/intra-abdominal fat	2.9 (1.9–4.9)	3.0 (1.9–4.9)	0.866

with a native American background. Carriers of the risk haplotype develop type 2 diabetes at a younger age and with a lower BMI compared to non-carriers (1). In this report, we provide a detailed clinical profiling of *SLC16A11* risk haplotype carriers. Individuals with the *SCL16A11* risk haplotype have impaired insulin sensitivity and higher predominance of large diameter adipocytes in subcutaneous fat. The risk haplotype was associated with higher ALT and GTT in individuals with type 2 diabetes. These observations suggest that decreased SLC16A11 function changes hepatic and adipose tissue functionality, organs whose dysfunction condition type 2 diabetes pathophysiology.

SLC16A11 is a bidirectional solute carrier capable of transporting monocarboxylates such as pyruvate (10). While SLC16A11 transport of pyruvate has been experimentally shown, it may transport additional, as-yet-unidentified substrates.

The haplotype under study is associated with decreased SLC16A11 function conferred by the combined effects of lower SLC16A11 expression in liver and reduced localization of SLC16A11 transporters at the cell membrane (2).

Increased hepatic *de novo* lipogenesis is a major source for the intra-hepatocellular lipids that cause lipotoxicity in type 2 diabetes (i.e. diacylglycerol and acylcarnitines), inducing insulin resistance (11, 12). The decreased insulin action found in risk haplotype carriers is consistent with findings from previous experimental studies, in which knockdown of *SLC16A11* expression in primary human hepatocytes alters fatty acid and lipid metabolism resulting in an increase in intracellular acylcarnitines, diacylglycerols and triacylglycerol levels (2). Thus, it is expected that insulin resistance would be magnified in *SLC16A11* risk haplotype carriers when they are exposed to a chronic caloric overload.

Cell transporters (mainly the related family member SLC16A11) export lactate from muscle or liver to other tissues (13). It can be hypothesized that lactate as a gluconeogenesis precursor may contribute to increased hepatic glucose production. Hence, this compensatory mechanism may contribute, in combination with the lipid-induced hepatic insulin resistance, to the appearance of hyperglycemia and type 2 diabetes. Furthermore, lactate is a lipolysis inhibitor by its interaction with GPR81. The receptor is activated in the presence of physiological lactate concentrations (14, 15). Chronic inhibition of lipolysis by lactate may contribute to the appearance of large-sized adipocytes, as found in the *SLC16A11* risk allele carriers.

De novo lipogenesis in adipose tissue is reciprocally regulated with hepatic *de novo* lipogenesis. It is positively associated with insulin action and its activation protects against hepatic steatosis (16). *De novo* lipogenesis in adipocytes plays a role as an alternative source to store carbon molecules and calories besides the liver. Adipose tissue *de novo* lipogenic capacity is reduced in obese individuals, contributing to the metabolic abnormalities linked to excessive adiposity (17). The SLC16A11 deficiency may alter the balance between lipid synthesis in the fat and the liver. Additional studies are needed to confirm this hypothesis. Interestingly, we observed that women with the *SLC16A11* risk haplotype have a greater amount of intra-abdominal fat and a lower subcutaneous/intra-abdominal fat ratio.

Table 4 Body composition and mineral content evaluated using DXA in carriers and non-carriers of the *SLC16A11* risk haplotype.

Variable	Non-carriers (*n* = 59)	Carriers (*n* = 64)	*P*
Total mass (kg)	70.5 ± 11.5	72.3 ± 12.6	0.413
Fat-free mass (kg)	38.5 (36.0–49.5)	40.7 (37.7–52.6)	0.346
Fat mass (kg)	25.40 ± 6.73	26.15 ± 7.31	0.553
Fat mass (%)	37.25 ± 7.74	37.40 ± 7.63	0.916
Bone mineral content (g)	2271.0 (2038.0–2653.0)	2379.5 (1923.0–2787.0)	0.671
Visceral adipose tissue (cm^3)	1118.0 (686.0–1667.0)	1156.5 (636.2–1821.7)	0.759
Visceral adipose tissue (g)	1055.0 (647.0–1572.0)	1090.5 (600.2–1719.0)	0.759

Furthermore, among normal weight participants, the risk haplotype was associated with a remarkable increment in the number of large-sized adipocytes, an abnormality associated with adipose tissue dysfunction (18).

Another major finding of our study is the higher serum ALT concentrations found in the *SLC16A11* risk haplotype carriers. Insulin resistance is a likely explanation for the abnormal ALT concentration, condition in which increased expression of *ALT2* has been reported (19, 20). An alternative explanation is hepatocellular damage due to hepatic steatosis. Though no difference in fat content was found between haplotype groups, the techniques used in humans *in vivo* may not be sensitive enough to capture intracellular lipid content. In addition, ALT catalyzes the transfer of an amino group from alanine to ketoglutarate in the cytoplasm, producing L-glutamate and pyruvate (21), potentially exacerbating haplotype-related cellular changes in pyruvate metabolism.

Limitations of the present study should be acknowledged including a relatively small sample size, although power was calculated for the main outcomes and this was sufficiently large and while we report nominal *P* values, an independent sample was used to replicate the findings. Although statistically significant, the magnitude of the differences in the ALT concentrations and adjusted M value between carriers and non-carriers of the risk haplotype was modest. However, greater differences are unlikely to occur in polygenic disorders (as type 2 diabetes). Therefore, the search for other contributors to the increased risk for type 2 diabetes in carriers of the SLC16A11 risk haplotype along with the confirmation of the findings in this work should continue. We did not perform clamps in the replication sample; therefore, we use the HOMA-IR index as a surrogate for the evaluation of insulin sensitivity. Nevertheless, we found a tendency for a higher insulin resistance in population with the risk haplotype without type 2 diabetes. We recognize that no single test is sufficient for a complete characterization of beta-cell function; however, the AIR represents the most widely used index. In addition, the AIR is dependent on insulin sensitivity thus comparison of AIR in populations with different insulin sensitivity may lead to inappropriate conclusions; therefore, we adjusted the AIR using the M value obtained in the clamp procedure. Finally, evaluation of the AIR allows an assessment of the first phase insulin secretion, and this is only one of the ways of response of the beta-cell and insufficient to characterize beta-cell function comprehensively. The relatively small number of homozygotes for the *SLC16A11* risk haplotype precluded us to search for a dose–response relationship. Even though the histological analysis was performed in a small subset of the population, 250 cells from each subject were analyzed. In histological analysis, only normal weight subjects were included to avoid confusing changes due to overweight/obesity; therefore, these results might not be extrapolated. In addition, we did not evaluate selective hepatic insulin sensitivity, but we consider the high dose euglycemic–hyperinsulinemic clamp as the best approach for the overall evaluation of insulin resistance.

The detailed clinical characterization of the study sample and the successful matching process between carriers and non-carriers allowed us to detect subtle differences associated with the *SLC16A11* risk haplotype. However, additional mechanistic studies and parallel exploration in relevant animal models are needed to explore the hypotheses here proposed for the phenotypes associated with SLC16A11 deficiency.

Individuals with the *SLC16A11* risk haplotype have reduced insulin action. Subjects with type 2 diabetes and the risk haplotype demonstrated an increase serum ALT and GGT. In women carriers with normal weight the proportion of large-sized adipocytes in subcutaneous fat was higher. Additional studies are needed to describe the consequences of the SLC16A11 deficiency on the pathogenesis of the diabetes-related chronic complications and the response to therapy.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

All authors contributed to experimental design, data acquisition and analysis and writing the manuscript. All authors approved the final version. P A wrote the manuscript and researched data. D V G researched data. O A C researched data. O Y B contributed to the manuscript. M D S contributed to the manuscript. T V R researched data. A J M R researched data. C J B performed histological analyses. L M H researched data. I C B researched data. H M performed statistical analyses, reviewed/edited the manuscript. A H researched data. K G R researched data. G A W reviewed/edited the manuscript. S B R J contributed to discussion and reviewed/edited the manuscript. L E G P researched data. M L O researched data. E R researched MRI data. J A researched MRI data. J F researched data. P C researched DXA data. M H H analyzed histological data. J C F contributed to discussion and reviewed/edited the manuscript. M T T L E Z researched V S contributed to discussion and reviewed/edited manuscript. C A A wrote the manuscript. CAS is the guarantor of this work.

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Long-term effectiveness of a type 2 diabetes comprehensive care program. The CAIPaDi model

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ABSTRACT

Aims: To evaluate the effectiveness of a comprehensive care program to achieve and maintain goals in patients with type 2 diabetes.

Methods: The CAIPaDi program includes 9 interventions delivered in 7 h. It seeks to achieve metabolic goals, identify and resolve barriers that would make implementation difficult, and provide self-efficacy and empowerment to patients by identifying personal profiles to establish individualized strategies. The program consists of a 4 intervention visits (1, 2, 3, and 4 months) and two follow up visits (12 and 24 months). Outcomes are compared between every visit. Main outcome was the attainment of the USA National Committee for Quality Assurance treatment goals.

Results: 1104 patients completed the first 4 visits, 545 the 12 month evaluation, and 218 the 24 month evaluation. After the conclusion of the four monthly sessions, 80.6% had HbA1c <7%, 72.1% had BP <130/80 mmHg and 71.6% had LDL- cholesterol <100 mg/dl. After twelve months, the percentage of goals achieved were 65.9%, 67.7% and 43.3% respectively ($p < 0.001$). For the 2-year evaluation the percentages were 61.0%, 70.6%, and 40.8% respectively ($p < 0.001$). All patients had renal, eye, foot and dental evaluations. Empowerment and quality of life showed significant changes; anxiety and depression scores remained low at annual follow-ups.

Conclusions: The CAIPaDI program results in sustained improvement and maintenance of treatment goals.

1. Introduction

Healthcare systems face big challenges to provide effective and high quality diabetes care. Achievement of the treatment goals is low, especially in the developing world. Type 2 diabetes is a major challenge for the Mexican Healthcare System due to its high prevalence, high rate of disabling complications and concerning direct and indirect costs [1–4].

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Patient-centered, comprehensive care programs are among the top actions to decrease the burden of the disease. These efforts are a work in progress. They have moved from purely informative sessions to multidisciplinary interventions designed to bring benefits in obtaining metabolic control goals, and reducing hospitalizations, emergency services visits and mortality rates [5–7].

These interventions include patient-centered medical care, mental health evaluations, the adoption of a healthy lifestyle and diabetes education programs. The core and processes of such programs are diverse, depending on available resources and characteristics of the target population (as reviewed by Lim and coworkers) [8]. However, the interventions' effects are transient if empowerment (getting essential competencies for self-care) is not promoted by the program [8,9]. Patient empowerment ensures capability to take the best decisions with available resources to accomplish control in patient's conditions [10].

In 2013, the Center of Comprehensive Care for the Patient with Diabetes (CAIPaDi – an acronym for its name in Spanish) was created with the purpose of developing a patient-centered, multidisciplinary model focused on the resolution of the most common barriers that preclude adherence to therapy and the attainment of treatment goals [11]. It is composed by nine structured interventions implemented in a single visit executed by a multidisciplinary team. The program's target population are patients within their first 5 years after diagnosis and free of chronic, disabling complications. Empowerment is considered as a primary goal in this program and the standardized protocols focus on self-efficacy and co-responsibility. The program also includes elements of the World Health Organization chronic disease care model such as the use of procedure manuals, treatment algorithms based on available resource, usage of an electronic registry system and the evaluation of quality indicators of medical care [12].

The aim of this report is to provide results about the effectiveness of a comprehensive care program (CAIPaDI) based upon empowerment techniques to achieve metabolic goals, in recently diagnosed type 2 diabetes, after 2 years of program participation.

2. Materials and methods

This is a program evaluation study. The CAIPaDi program consists of two phases (Fig. 1). The first comprises an initial and 3 visits one month apart (visits 1, 2, 3 and 4 respectively), each one taking place in a single 7 h shift. The interventions are: medical care, diabetes education, nutrition, physical activity, psychological evaluation, psychiatric assessment, eye exam, foot and dental care. These are delivered by one nurse, two endocrinologists, a diabetes educator (DE), a nutritionist, an ophthalmologist, a psychologist, a psychiatrist, a physical activity instructor and a dentist. Each intervention follows a procedure manual and has: (1) a specific goal, (2) a self-management strategy and (3) prespecified indicators. Each session is 30 to 60 min long; some of them are group meetings in which a predesigned dynamic is executed. Blood test, EKG, weight and height are obtained at arrival; blood test results are available in 2 h and attached to every medical record so specialists can adapt and adjust the treatment according to their results.

The second phase consists of 2 annual evaluations (visits 5 and 6) where all interventions from the initial phase are reinforced. During each annual visit, prespecified outcomes are measured [11]. A continuous at-distance support system was implemented to maintain communication with patients via e-mail, phone calls, text messages, and through the hospital's webpage. (http://innsz.mx/opencms/contenido/departamentos/CAIPaDi).

The main outcome is the achievement of treatment goals defined by the National Committee for Quality Assurance criteria (NCQA): HbA1c, blood pressure (BP) and LDL-cholesterol. NCQA parameters provide an integrative score of the program performance [13]. Secondary outcomes include the percentage of patients: (1) achieving the 3 metabolic goals, (2) with diabetes-related complications and (3) treated with aspirin, antihypertensive and lipid-lowering drugs.

2.1. Study population

The patients for this study were enrolled from November 1st, 2013 to June 30th, 2018. Inclusion criteria were: type 2 diabetes patients, ≤5 years of diagnosis, without disabling complications (blindness, renal failure, stroke, limb amputations, ischemic heart disease) and non-smokers; when smokers, patients attended a Smoking Cessation Clinic as part of the treatment for 6 months before entering the program given the negative impact of smoking in diabetes [14,15]. If selected, patients received a phone call and an e-mail with the information of their first visit appointment and questionnaires (mentioned later in this section) to be answered in each visit.

2.2. Procedures

The Institutional Ethics and Research Committees from the National Institute of Medical Sciences and Nutrition Salvador Zubirán (INCMNSZ for its name in Spanish) approved this study (Ref 1198) and it was registered in ClinicalTrials.gov (NCT02836808). All patients signed an informed consent form.

Each visit was held at the CAIPaDi Center. Patients could participate in groups of 10 people in individual sessions depending on the intervention, with a close relative being encouraged to participate with them. Every one of these interventions followed a procedure manual and included a checklist of the main actions to be implemented and variables to be measured. The aim of visit 1 was to obtain a complete assessment of the patient and provide basic information to start the required changes. On visit 2, patients underwent a problem-oriented evaluation, where the recommendations were selected based on patient´s profile. Visit 3 focused on the identification of potential barriers that may impede metabolic control achievement and visit 4 aimed to reinforce the knowledge already acquired and evaluate the initial results of interventions. During visits 5 and 6, the barriers and their proposed solutions were reviewed. In summary, a collaborative, iterative process was applied in each intervention. To

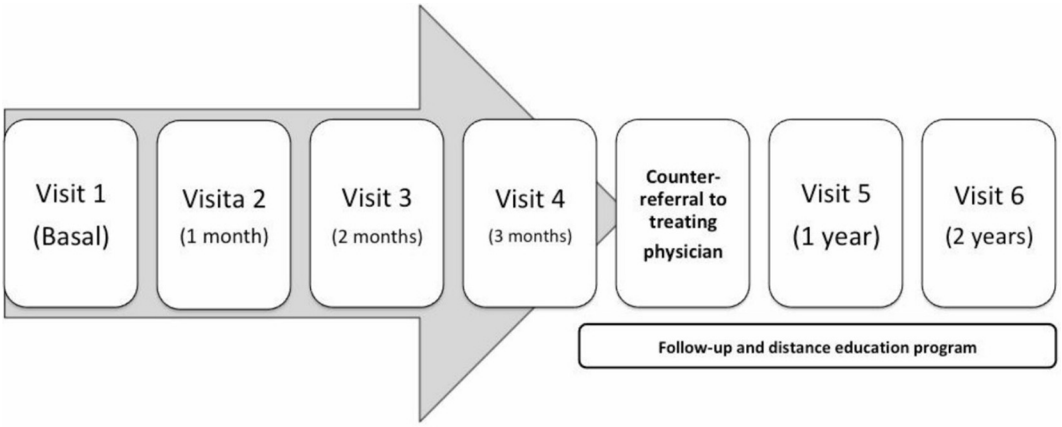

Fig. 1 – The CAIPaDi program is conformed of a baseline and 3 monthly visits (phase 1) and then a reevaluation at 1 and 2 years (phase 2).

evaluate the competencies acquired in every visit, a structured exam was applied to each patient asking them to undertake activities related to self-care (check their feet, glucose monitoring, toothbrushing. . .). All interventions were applied in every visit.

Although many aspects were reviewed in each intervention, the strategies applied to empower patients were directed to their needs, beliefs (regarding diet and exercise according to geographic area and preferences) and resources. Table 1 summarizes the 9 interventions and the health professional delivering each part of the program. When not evaluated by the program (time between visits), patients were regularly checked by their personal general physician.

2.3. *Outcome measurements*

Fasting concentrations of glucose, creatinine, lipids and HbA1c (Bio-Rad Variant II Turbo HbA1c Kit 2, with HPLC method) were assessed in each visit. Albuminuria/creatinuria ratio (ACR) (SYNCHRON CX system with colorimetric method) was used for screening diabetic nephropathy at baseline and annual visits. The laboratory is certified by ISO 90001:2015 and the College of American Pathologist. Body composition was assessed by bioimpedance (body composition analyzer JAWON medical ioi353).

Validated questionnaires were applied for: empowerment (The Diabetes Empowerment Scale-Short Form [DES-SF]) [16], anxiety and depression symptoms (Hospital Anxiety and Depression Scale [HADS]) [17,18], quality of life (Diabetes Quality of Life Measure [DQoL]) [19,20], diabetes-specific emotional distress (Problem Areas in Diabetes Questionnaire [PAID]) [21] and Diabetes Knowledge Scale [22]. To retrieve information about fitness, the International Physical Activity Questionnaire [IPAQ] was answered [23], and the 6-minutes walking test was done in every visit [24]. Patients completed a 3-days food record to register calories consumed per day [25].

Evaluation of social support was classified into 3 categories: (1) Functional: included family or friends providing emotional and/or economic support and being involved in treatment strategies; daily activities did not affect adherence. (2) Partial: family or friends giving partial emotional and/or economic support, were not completely involved in treatment strategies and patients' daily activities affected adherence. (3) Dysfunctional: family or friends not aware of diagnosis or patients had activities with negative impact in health.

The clinical diagnosis of periodontal disease was established according to the criteria of the American Academy of Periodontology [26]. The classification included periodontal health, gingivitis and periodontitis. Likewise, chronic periodontitis was classified as slight (1–2 mm of clinical attachment loss [CAL]), moderate (3–4 mm CAL) and severe (>5 mm CAL) and according to extension as localized (<30% of sites are involved) and generalized (≥30% of sites are involved).

The ankle/brachial index was evaluated according to the Guidelines of the American College of Cardiology/American Heart Association [27].

2.4. *Statistical analysis*

Results were reported as means (±SD) if they followed a normal distribution or medians and interquartile ranges (25–75) if they did not have a normal distribution, according to Kolmogorov-Smirnov test. Percentages were used for discrete values. Changes in the NCQA scores were compared using McNemar test, and for comparing categorical variables or Chi-square test. Analysis by protocol was performed and included T-test for related samples of changes in scores of questionnaires and laboratory tests. Non-parametric values were log-transformed for regression models. Analysis included T-test or U-Mann Whitney for related samples when appropriate. We performed a principal component analysis (PCA) to evaluate variables explaining target goals reached using varimax rotation on the coefficients to assess consistency. The number of components was evaluated using sedimentation graphs.

We evaluated through an explanatory model the association of the components with metabolic goals. This analysis included variables from visits 4, 5 and 6. The variables were included using two-step logistic regression models. In the first model, components obtained from PCA analyses considered if

Table 1 – Interventions and members of the CAIPaDi team.

Member of CAIPaDi team	Intervention
Endocrinologist	(1) Checked metabolic outcomes. Adjusted drug treatment (following treatment algorithms for glucose, lipids and blood pressure control, depending on patient's resources). Evaluated any potential dermatological, neurological and vascular complication
Diabetes educator	(2) Provided individual or group sessions depending on the topic to be reviewed: glucose monitoring, timely detection and adequate treatment of hypoglycemia, foot care (patients were taught about proper use of footwear, cream, powder and nail clipping to prevent injuries), eradicate diabetes-related myths and proper actions during a sickness day
Nutritionist	(3) Prepared diet plan depending on patients' preferences and resources, based initially on a "simplified plan" (start avoiding the most deleterious customs) and then escalates to improve their feeding choices. Elaborated specific dietary cards to help patients adhere to their plan if barriers as: "having to eat outside home" or "at work" or "no place to have healthy snacks" were identified
Psychologist	(4) Searched for anxiety, depression or any other emotional factor that could limit adherence to treatment. Addressed social support, cognitive resources and emotional status for helping patients overcome barriers in different areas and solve daily problems
Dentist	(5) Performed general dental exam and treated specific diseases if identified. Empowered patients by teaching them dental health topics, the correct technique for toothbrushing and usage of dental floss. Initial non-surgical treatment of periodontitis and referral to more specialized treatment
.Psychiatrist	(6) Detected personality traits that may alter response to therapy. Treated depression, anxiety or eating disorders. Prescription of drugs for the treatment of psychiatric disorders
Physical therapist	(7) Explained differences between physical activity and exercise. Start avoiding sedentarism (increase steps per day) and start exercise programs including aerobic and strength activities. Identified barriers to do exercise and proposed activities to help patients increase daily steps
Foot Care	(8) Evaluated dermatological, neurological, vascular factors for foot health. A session for abnormal pressure points included step analysis, where individualized soles were indicated when necessary
Ophthalmologist	(9) Evaluated vision acuity, ruled-out diabetic retinopathy and macular edema using a no-mydriatic camera for retinal review. Pupillary pharmacological dilation was performed when photographs had poor quality

the patient achieved metabolic goals as a dependent variable. For the second step, models were adjusted for age, sex, baseline HbA1c, body mass index (BMI), medications for diabetes, hypertension and lipid control. Harrell's C-statistic evaluated the predictive capacity of the models and 95% confidence intervals. These latter were computed using the DeLong method. SPSS Statistics version 21 was used for data analysis and a p-value < 0.05% was considered as significant.

3. Results

A total of 1837 patients were enrolled within study's time period. From these, 444 patients (24.1%) abandoned the program in the initial four visits and 150 patients are still taking part in the first phase, so 1243 patients finished the first phase and were included for analysis. Of this total, 262 (20.9%) did not attend to their 1-year follow-up. For the visit 5 analysis we included 628 patients (353 patients are still ongoing for this visit). At visit 6, 99 (15.7%) of 628 did not attend to their appointment, and 241 are still ongoing this visit. In this report we included 1243 patients who finished visits 1 to 4, 628 who completed visit 5 and 288 for visit 6 (Fig. 2).

The mean age was 51.1 ± 10.3 years, 56.2% were women, with time since diagnosis 1 (0–5) year. The mean BMI was 29.5 ± 5 kg/m^2. All patients had renal, dental, foot and eye exam. Renal evaluation was performed with albuminuria/creatinuria ratio at the visits 1, 5 and 6. The basal median of ACR was 7.4 (4.2–18.8) mg/g and 16.3% had >30 mg/g. We observed 80 patients with gingivitis and 431 with periodontitis at visit 1. Periodontal disease (gingivitis and periodontitis) was present initially in 92.3% of the patients. In foot evaluation, 30.2% of the patients had an altered tuning fork test. Also, in vascular evaluation, we found 2% with an altered ankle/brachial index. The eye exam included an evaluation for retinopathy and macular edema. At visit 1, 14.1% of the patients had any level of retinopathy, and 3.3% had macular edema.

3.1. *Outcomes*

We evaluated the performance of the program using the approach proposed by the NCQA [13]. Table 2 shows the total score and each one of the NCQA parameters. The program reached all the NCQA goals after the first 4 visits. As a result, the maximal score (100 points) was achieved and remained the same at visit 5.

At visit 1, only 8.1% of the patients had met the 3 main goals (HbA1c, blood pressure, and lipids). In contrast, at the end of visit 4, 47.4% of the patients achieved the 3 goals (p < 0.001). 24.3% and 23.2% of patients met the 3 goals in visits 5 and 6 respectively (p = 0.003). The effect of the intervention in the metabolic parameters is shown in Table 3.

The absolute change in HbA1c was −1.2 (−3.4 to 0.3)% after the initial four visits and it remained −0.4 (−2.0 to 0.2)% at visit 5 and −0.1 (−1.5 to 0.5)% at visit 6. The same trend was

observed for fasting glucose, blood pressure, and lipid concentrations. All changes are statistically significant ($p < 0.001$).

3.2. *Lifestyle modifications obtained in the CAIPaDi program*

As measured by IPAQ, patients achieved a remarkable increase of the minutes devoted to moderate physical activity. They reported moving from 0 (0–151) to 180 (120–300) minutes/week ($p < 0.001$) after the fourth visit. The minutes decreased to 150 (0–240) minutes/week in visit 5, but still in agreement with the minimal goals ($p < 0.001$ vs baseline). At visit 6, the minutes reported were 150 (0–245) minutes/week ($p < 0.001$ vs baseline). The results of the 6-minute test in treadmill increased from 418 (337–470) meters to 464 (400–500) meters in 4 months ($p < 0.001$). At visit 5, it changed to 450 (386–492) meters ($p < 0.001$), and 450 (386–498) meters ($p = 0.04$) in visit 6. The average of calories consumed reported in visit 1 was 1581 ± 437 kcal. At the end of the first phase, patients consumed 1373 ± 266 kcal ($p < 0.001$). In visit 5, the consumption of calories was 1411 ± 304 calories/day ($p < 0.001$, compared with the first visit) and 1392 ± 304 kcal at visit 6 ($p < 0.001$, compared with the first visit). Despite the changes in physical activity and caloric intake, the weight change was marginal. Important parameters that help in weight follow-up are lean and fat mass, which relate to metabolic control. The patients in the program had important changes in both parameters, losing fat and maintaining lean mass (Table 3).

Empowerment scores changed from 72.6 ± 17 to 82.4 ± 12.7 ($p < 0.001$) and 82.3 ± 13.6 at visits 5 and 6 ($p < 0.001$). Up to 38.9% of the patients had depression in the first visit. This percentage changed to 12.4% at visit 4 and 21.3% and 15.1% at visits 5 and 6, respectively ($p < 0.001$ for all visits). At the beginning of the program, 46.4% of the patients had anxiety, which diminished to 16.3% at visit 4 and 20.8% and 15.1% for visits 5 and 6 ($p < 0.001$ for all visits). The DQoL score reduced 24% and continued as such in the annual visits (Table 3). DQoL scores changed from 90.9 ± 24.5 to 71.0 ± 17.8 ($p < 0.001$) and 72.5. ± 18.6 at visit 5 and 71.4 ± 18.2 at visit 6. ($p < 0.001$).

In the Diabetes Knowledge Scale, 72.7% of the patients had adequate knowledge (adequate being >18 points). At visits 4, 5 and 6 zero patients had inadequate knowledge. Ninety seven percent of the patients had adequate knowledge ($p < 0.001$ compared with basal) in visit 4. For visits 5 and 6, 98% and 95% had adequate knowledge ($p < 0.001$, both visits compared with baseline).

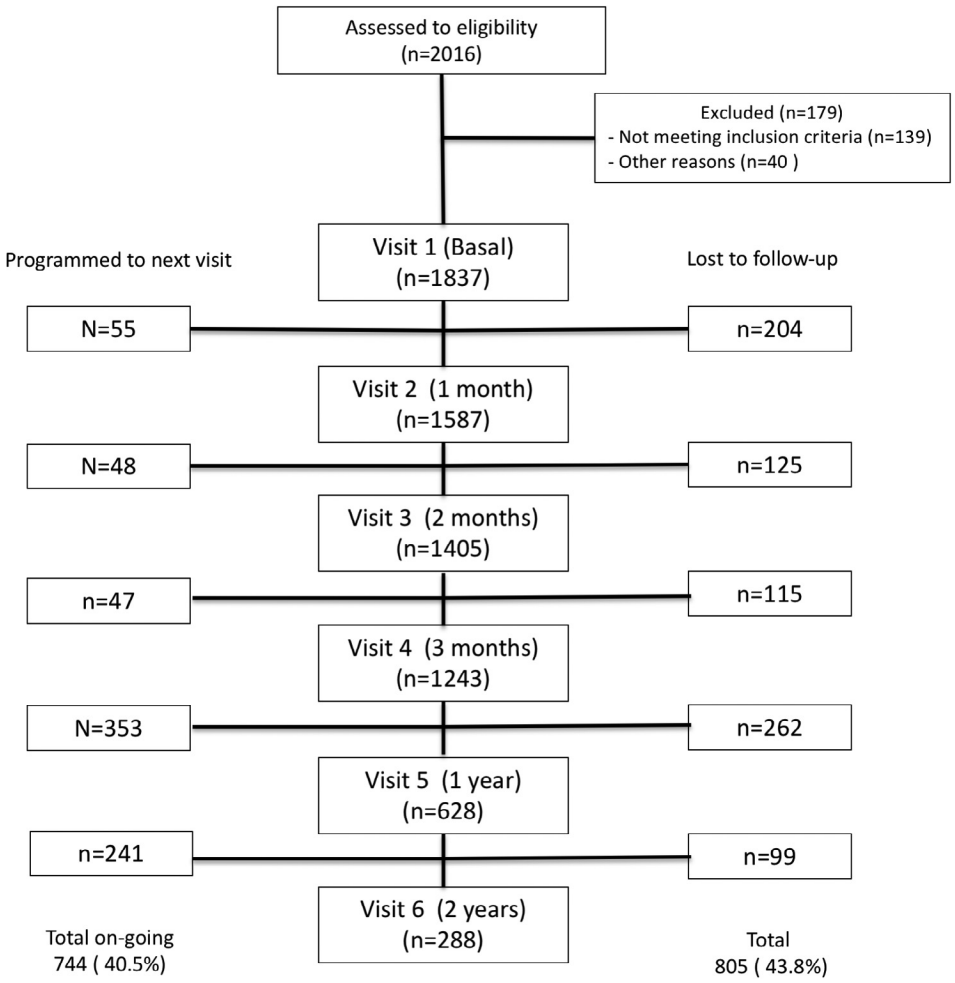

Fig. 2 – Flow chart of patients enrolled in the CAIPaDi program, dropouts and ongoing patients.

Table 2 – National Committee for Quality Assurance parameters in the CAIPaDi program.

Parameter	Goal (% of patients)	Visit 1 (n = 1837)	Visit 4* (n = 1243)	Visit 5* (n = 628)	Visit 6*,+ (n = 288)
HbA1c > 9%	≤15%	35.3	2.2	9.0	11.1
HbA1c < 8%	>60%	52.4	93.0	82.1	79.1
HbA1c < 7%	>40%	37.0	79.8	65.7	59.3
BP ≥ 140/90	≤35%	17.9	5.0	7.3	5.5
BP < 130/80	>25%	50.6	83.7	65.6	68.7
LDL-c ≥ 130	≤37%	32.9	3.8	18.6	15.9
LDL-c < 100	>36%	34.3	82.6	57.3	59.3
Eye exam	60%	ND	100	100	100
Foot exam	80%	ND	100	100	100
Renal evaluation	80%	ND	100	100	100
Smoking Status and Cessation Advice or Treatment	80%	ND	100	100	100

BP: Blood pressure, HbA1c: glycated haemoglobin, LDL-c: Low-density lipoprotein cholesterol, ND: Non-determined.
* $p < 0.001$ for differences in HbA1c, BP and LDLc between 3 months vs basal, 1-year vs basal and 2 years vs basal.
+ $p = 0.004$ for differences in HbA1c between 2 years vs basal.

3.3. *Changes in the use of pharmacological treatment*

According to the ADA Guidelines, all patients with diabetes and >50 years old are candidates for antiplatelet treatment [28]. Following this recommendation, the use of antiplatelet agents moved from 9.74% before starting the program to 54.4% after the first visit. In visit 4, 64.9% of patients received antiplatelet treatment, 64.9% and 68.4% at visit 5 and 6 respectively ($p < 0.001$ for all visits). Before starting the program, only 14.0% of patients were receiving statin therapy. This is a low percentage considering that 64.6% of patients had LDL values that qualified for pharmacological treatment [28]. At visit 4, 75% of patients received statin therapy ($p < 0.001$). Only 26.8% remained above the LDL target despite moderate-intensity statin therapy.

For blood pressure drugs, 24.5% of patients were taking antihypertensive drugs before starting the program. This percentage increased to 37.0% patients receiving treatment in the first visit. At visit 4, 42.6% patients received antihypertensive drugs. At visits 5 and 6, the percentages of patients with antihypertensive drugs increased to 44.8% and 46.8% respectively.

Before starting the program, 13.9% of patients were not taking any type of hypoglycemic drug. Up to 54% were taking only 1 drug, 30.6% were taking a combination of 2 drugs and 1.7% were using 3 glucose-lowering drugs. In the first visit, 93.7% had treatment indicated to achieve glycemic control. The number of hypoglycemic agents per patient was 1 (0–3), being metformin the most common. In the fourth visit, 5.26% of patients were controlled without taking any hypoglycemic drugs. At visit 5, 32 patients (5.0%) were controlled without hypoglycemic drugs. At visit 6, only 17 patients (5.9%) were controlled without hypoglycemic treatment.

3.4. *Logistic regression models*

A PCA for visit 4 identified 11 components that explain 13.78% the variance for subjects who reached all three goals. Step-wise logistic regression identified three components associated with target goals in this visit. The first associated component had a significant correlation with empowerment (rho = −0.519). The second component had a significant correlation with dietary fat intake (rho = 0.715). The third component was psychological evaluation (rho = 0.779). The adjusted model was statistically significant ($r2 = 0.081$, $p < 0.001$) and had a good performance identifying patients who reached goals at visit 4 (AUC 0.639, 95% CI 0.610–0.668).

For the fifth visit, we identified 12 components that explained 14.04% of the variance to identify subjects who reached goals. Logistic regression analyses identified three components associated. These were cognitive/emotional resources (rho = 0.632), PAID questionnaire (rho = 0.769), and social support (rho = −0.417). The adjusted model explained 7.7% of the variance ($r2 = 0.077$, $p < 0.001$), with a good performance (AUC 0.693 95% CI 0.646–0.740).

For the sixth visit the components associated were social support (rho = −0.558), nutritional status (rho = −0.411), motivation stage (rho = 0.536), and empowerment (rho = 0.550). The adjusted model explained 16.8% of the variability in identifying subjects who reach goals ($r2 = 0.168$, $p < 0.001$) with good performance (AUC 0.820, 95% CI 0.768–0.872). The models are shown in Supplementary data.

4. Discussion

In several reports it has been shown that with the comprehensive approach that includes the use of strategies centered on the patient with diabetes produce better metabolic results and reduce complications [5]. The efforts that have been made in several sites with models of comprehensive care require greater complexity of operation but have provided a great opportunity to innovate [29]. Knowledge, motivation and competencies are three main components of the treatment in diabetes. On this basis, our program aimed to improve the quality of life of patients with diabetes and reduce disabling and costly complications such as amputations, blindness and renal failure. For this, the main activities are the identification and solution of barriers to reach the control goals, to promote self-efficacy and co-responsibility in the treatment, the identification of patient profiles to

establish specific approaches, and the application of cost-effective strategies based on evidence and feasibility according to the resources of each patient.

According to the evaluation of the diabetes care quality standards established by the NCQA, the CAIPaDi program achieved a high score and shows an improvement in the majority of the target goals. Most important is that the beneficial effect of the intervention remained significant after one and two years. The program maintained 20% of the 3 control goals compared to 1% of a previous report in Mexican male patients [30].

Some features of the CAIPaDi program should be highlighted. The multidisciplinary interventions concentrated in the same place and in the same day ensures compliance by avoiding appointments of separate consultations as carried out in the traditional care model. A strength of the model is that renal, ophthalmological, dental and foot evaluations are ensured for all patients with diabetes. This allows the establishment of appropriate treatment and referral strategies. The program has a strong behavioral intervention, planned to stimulate empowerment and self-care. Workshops, group dynamics, and participation of a close relative were strategies applied. The progression of complications and related factors will be data for analysis in a different publication.

Empowerment is a determinant of long-term effects of the treatment of chronic diseases [31–37]. The CAIPaDi program has a remarkable positive effect on empowerment, knowledge of diabetes, anxiety, depression, and quality of life as shown in Table 3. High scores were an independent predictor for reaching metabolic goals in the first phase (Table 3, Supplementary data). All these areas impact reducing the interference of the disease with the daily life of patients, as seen with the PAID evaluation.

The short-term effect of CAIPaDi is similar to what is described in other programs [36]. The major changes observed in CAIPaDi were in the HbA1c levels. Drug therapy is an important area in diabetes treatment. In the first-visit, the prescription of statins increased to 75% of the patients, more than 40% of the patients had antihypertensive treatment, and 95% had hypoglycemic treatment. The percentage of patients receiving these pharmacological treatments is high compared with national surveys [2], but the model uses the most common and least expensive treatments to achieve goals. The results in weight reflect the complex nature of the treatment of obesity. In CAIPaDi, physical activity and reduced caloric intake are maintained for 2 years.

In the traditional diabetes care model, in most cases the patients are treated only by the general practitioner or a family physician, and sometimes by nutritionists. Unfortunately, most of the complementary consultations are directed to specialists who treat complications (cardiologists, neurologists, angiologists, nephrologists, etc.) [38]. For patients, CAIPaDi program is affordable in costs and time. Also, it only takes them 1 day to have all the laboratory tests and evaluations, which makes it easier for work permissions. A cost-effectiveness and cost-benefice analysis needs to be examined in the future. It will show the importance of multidisciplinary interventions, including the cost of drugs and medical consultations.

A great concern is that even with less than 5 years of evolution, 14% already have retinopathy, 16% albuminuria and 30% altered sensitivity to vibration, for which preventive strategies become more compelling.

A limitation of this report is the lack of replication of intervention in different settings. We did not include patients with complications or more than 5 years of diagnosis. Our group focused the strategies in newly diagnosed patients to avoid disabling complications in the mid-term. Other limitations are the lack of a control group and the high dropout rate seen after the first visit. From visits 2 to 4, the number of patients attending the program is consistent. As expected, the second dropout seen is for the annual visit since some patients feel they didn't do well in a year and don't wanted to get checked again. This has been a great area of opportunity and to make efforts for improving quality of care.

In conclusion, according to the proposed model to consider Diabetes Centers of Excellence [39], the CAIPaDi program has the infrastructure and abilities across the medical team necessary to guide a comprehensive care. It is a health-care system focused on quality improvement, outcome assessment, education and dissemination.

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Group of Study CAIPaDi

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Table 3 – Analysis by protocol of changes in metabolic parameters, body measurements and questionnaire scores from the first visit to the fourth, fifth and sixth evaluations.

	N = 1, 243		N = 628		N = 288	
	Basal	Visit 4	Basal	Visit 5 (1st year)	Basal	Visit 6 (Second year)
Glucose (mgl/dl)	135 (107–188)	107 ± 29	147.8 ± 66.4	112 (98–134)	143.1 ± 63.1	124.9 ± 41.7
HbA1c (%)	8.5 ± 2.5	6.4 ± 0.9	8.07 ± 2.36	7.0 ± 1.5	7.77 ± 2.22	7.16 ± 1.62
Triglycerides (mg/dl)	177 (128–253)	116 (93–150)	177 (128–253)	142 (107–195)	177 (128–253)	138 (106–197)
LDL cholesterol (mg/dl)	115 ± 37	87 ± 24	115.7 ± 37.4	109 ± 33**	114.9 ± 37.9	114.94 ± 37.98***
Systolic BP (mmHg)	126 ± 16	119 ± 13	127.9 ± 15.75	122 ± 12.5	128.9 ± 16.4	120.86 ± 11.83
Diastolic BP (mmHg)	78 ± 8	73 ± 7.1	77.9 ± 7.76	75 ± 7	78.4 ± 7.87	74.06 ± 6.86
BMI (kg/m^2)	29.5 ± 5	28.7 ± 4.6	29.0 ± 4.50	28.8 ± 4.3	29.19 ± 4.27	28.8 ± 4.2
Waist circumference women (cm)	97.3 ± 12.5	93.6 ± 12	96.1 ± 11.3	94.2 ± 11.5	96.5 ± 10.6	94.52 ± 11.45
Waist circumference men (cm)	100.7 ± 12.2	98 ± 11.4	100.6 ± 11.5	99.3 ± 10.9**	99.6 ± 10.6	98.69 ± 10.26^
Lean mass women (kg)	39.6 ± 5.7	38.9 ± 5.4	38.8 ± 5.09	38.3 ± 5.3**	39.04 ± 5.05	38.61 ± 5.17**
Lean mass men (kg)	52.5 ± 7.3	52.1 ± 7.6*	52.08 ± 7.08	52.4 ± 6.7	51.81 ± 7.14	51.77 ± 5.97
Fat mass women (kg)	28.2 ± 8.4	26.7 ± 7	27.36 ± 7.67	26.9 ± 7.5^	27.72 ± 7.73	27.89 ± 7.59
Fat mass men (kg)	26.7 ± 9.1	25.1 ± 8	26.33 ± 8.06	25.7 ± 7.5*	26.03 ± 7.46	25.06 ± 7.22ς
HAD anxiety (%)	46.4	15.7	46.4	20.8	46.4	15.1
HAD depression (%)	39.5	19.3	39.5	21.3	39.5	16.5
PAID	37.5 (20–55)	11.2 (5–22.5)	36.7 ± 23.1	12.5 (3.7–26.2)	35.38 ± 22.10	15.47 ± 15.58
DQoL	93 ± 25	71 ± 18	89.1 ± 23	72 ± 18	86.82 ± 22	71.41 ± 18
Empowerment	72.6 ± 17	82 ± 13	73.1 ± 16	82 ± 14	73.36 ± 18	82.34 ± 13

BMI: Body Mass Index. BP: Blood pressure. DQoL: Diabetes Quality of Life Measure. HAD: Hospital Anxiety and Depression Scale. HbA1c: glycated haemoglobin, LDL-c: Low-density lipoprotein colesterol. PAID: Problem Areas in Diabetes Questionnaire.

All $p < 0.001$.

* $p = 0.01$.

** $p = 0.002$.

*** $p = 0.005$.

ς $p = 0.03$.

^ NS.

Conflict of interest statement

No potential conflicts of interest relevant to this article were reported.

Author contribution

S.H.J. and A.C.G.U. wrote the manuscript. A.C.G.U. and O.Y.B.C made the statistical analysis. The remaining authors revised the manuscript critically for important intellectual content. The group of study CAIPaDi are all the health-care professionals attending the patients.

S.H.J. is the guarantor of this work and takes responsibility for the integrity of the data.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diabres.2019.04.009.

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ORIGINAL ARTICLE

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Quality of life is significantly impaired in both secretory and non-functioning pituitary adenomas

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Summary

Objective: To evaluate the quality of life (QoL) in patients with pituitary adenomas in comparison with healthy Mexican population QoL scores.

Design & Measurements: Cross-sectional study using the short form 36 questionnaire (SF-36) in 175 patients with pituitary adenomas grouped by adenoma subtype and disease activity, and compared them with the healthy Mexican population normative QoL scores.

Patients: A total of 44 patients with non-functioning pituitary adenomas (NFPA), 48 with acromegaly, 53 with prolactinomas and 30 with Cushing disease (CD) were enrolled in this study.

Results: Mental and physical components scores (MCS & PCS) of SF-36 questionnaire were lower in patients with active disease in all adenoma subtypes ($P < 0.03$). A significant negative relationship between prolactin levels and MCS ($r = -0.30$, $P < 0.01$) and PCS ($r = -0.41$, $P < 0.01$) were found in prolactinomas. Patients with CD showed 24 hours urine-free cortisol levels negatively correlated with MCS ($r = -0.43$, $P < 0.01$) but not with PCS. No significant correlation was found between IGF-1 ULN and QoL scores in acromegaly. NFPA patients had lower QoL scores than patients with controlled CD, acromegaly or prolactinoma ($P < 0.02$). Active CD and prolactinoma have lower QoL scores in comparison of NFPA ($P < 0.05$). Having an adenoma, secretory or non-functioning, decrease QoL scores in comparison of results in the healthy Mexican population register. Using an adjusted-multivariate model, we confirmed that disease activity in all secretory adenomas is an independent risk factor, reducing SF-36 scores significantly.

Conclusion: Activity in all secretory pituitary adenomas' patients decrease mental and physical QoL. However, independently of disease activity, secretory and NFPA significantly decrease QoL in comparison with healthy Mexican population QoL register.

KEYWORDS
acromegaly, Cushing disease, non-functioning pituitary adenomas, pituitary adenomas, prolactinoma, quality of life, short form 36 questionnaire

1 | INTRODUCTION

Quality of life (QoL) is defined as patient's health well-being related to physical, emotional and social aspects, implying an objective and subjective judgment about how an individual feels, functions and responds in daily life.[1,2] In the past years, QoL has been explored in patients with pituitary tumours. The purpose is to evaluate issues and interventions that are not routinely explored, providing information about the clinical impact of the disease, and leading to multidisciplinary treatments to improve the perception of the tumour pathology. There are two types of validated questionnaires to evaluate QoL in patients with pituitary adenomas. First, the generic questionnaires that can be applied to general population and results can be compared between patients with different diseases. Examples are the Nottingham Health Profile, the Psychological General Well Being Scale (PGWBS), and the Short Form Questionnaire 36 (SF-36).[1] The other group of questionnaires are more disease-specific. In patients with pituitary tumours, we have the Acro-QoL,[3] the Cushing-QoL,[4] and the Leiden Bother and Needs (LBNQ-Pituitary)[5] questionnaires. These evaluations are more sensitive to detect QoL problems related to the disease itself, but the disadvantage is that results are not comparable with other diseases or with results in general population.

Several studies have concluded that QoL is decreased when patients have a pituitary adenoma.[4,6-12] However, results are inconsistent about improvement of QoL after disease control. Some studies reported better QoL scores[1,4,13] whereas others reported QoL scores without change.[11,12,14-16] While some studies showed a negative correlation between serum hormone levels related to disease activity and lower QoL scores,[11,13] others found no significant correlation.[3,17] Such inconsistent results increased the debate, and currently, it is unknown the magnitude of QoL impairment between secretory and non-functioning pituitary adenomas (NFPAs). Also, it is unclear the independent factors significantly associated with lower QoL,[10,18,19] and if having a NFPA impairs QoL in comparison with otherwise healthy population without pituitary disease. Therefore, this study aimed to evaluate QoL scores of patients with secretory pituitary adenomas and compared such scores with NFPA patients. In addition, we compare all QoL scores of patients with secretory and NFPA with those in the healthy Mexican population normative.

2 | MATERIALS AND METHODS

2.1 | Patients

We conducted a comparative, cross-sectional study, with prolective evaluation in all cases. Included patients were those with confirmed pituitary adenoma diagnosed by magnetic resonance imaging, attending to the Neuroendocrinology outpatient clinic, at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, from August 2016 to December 2017. Flow diagram of subject progress through the study is shown in Figure S1. We exclude patients without adenoma or who had nontumoral pathology (ie, pituitary abscess, hypophysitis, pituitary apoplexy, craniopharyngioma or Sheehan syndrome). Elimination criteria included psychiatric disorder and inability to answer the survey (ie, physical or intellectual disability). All subjects gave their written-informed consent before inclusion to the study. The Institutional Human Biomedical Research Committee approved the study, and written-informed consent was obtained from all participants. This clinical research was carried out in accordance with the principles expressed in the Declaration of Helsinki.

2.2 | Biochemical criteria for disease activity

Patients with hormone hypersecretion due to pituitary adenoma were grouped according to disease activity. Patients with acromegaly were considered controlled when insulin-like growth factor-1 (IGF-1) serum levels were less than 1.2× the upper limit value (ULN) adjusted for gender and age, together with a growth hormone (GH) nadir below 0.4 mg/L during 2 hours glucose tolerance test (TTOG) after 75 g load. Patients could be under cabergoline treatment, after surgery or radiotherapy (Table 1). Serum GH and IGF-1 were measured with an ultrasensitive chemiluminescence immunoassay (ACCESS, Beckman Coulter®, Germany). Patients with Cushing disease were considered controlled when 24 hours urinary free cortisol was below the upper assay reference value of 140 μg/day, together with morning cortisol level of <1.8 mcg/mg at 8:00 hours after 1 mg dexamethasone at 23:00 hours in the previous night. Free cortisol was measured using a competitive union immunoenzymatic assay (ACCESS cortisol reagent pack, Beckman Coulter®, Germany). Criteria for biochemical control in patients with prolactinoma were serum prolactin (PRL) below 26 ng/mL for women and 20 ng/mL for men. The PRL determination was performed with a chemiluminescence immunoassay (ACCESS prolactin Beckman Coulter®) with a detection limit of 0.25-20 000 ng/mL. No cases with thyrotropinoma were included. Those patients with a secreting pituitary adenoma who did not meet these criteria were considered with active disease. Lastly, patients with pituitary adenoma on MRI, without a clinical syndrome or biochemical hypersecretion, were diagnosed with NFPA. NFPA patients were used as first group for statistical comparison because of lacking hormone but having symptomatology related to harbouring a pituitary tumour, which may reduce QoL. After comparing NFPA patients with and without surgery and/or medical therapy (radiotherapy, and/or cabergoline), we did not

TABLE 1 Baseline characteristics of patients studied grouped by subtype of adenoma and disease activity

n = 175	NFPA	Cushing disease		Acromegaly		Prolactinoma	
	44	23	7	25	23	25	28
	-	Controlled	Active	Controlled	Active	Controlled	Active
Age at diagnosis (y)	44 (36-54)	29 (25-37)	27 (19-38)	36 (27-51)	37 (34-48)	30 (25-39)	27 (21-34)
Female	35 (79.5)	22 (95.7)	7 (100)	11 (44)	10 (43.5)	21 (84)	26 (92.9)
BMI (kg/m^2)	28.3 ± 4.1	29.3 ± 5.0	32 ± 3.8	31 ± 5.3	29.7 ± 5.3	27 ± 4.1	28.1 + 8.4
Panhypopituitarism[c]	8 (18.2)	3 (13)	1 (14.3)	5 (20)	2 (8.7)	1 (4)	5 (20.3)
GH deficiency	7 (15.9)	1 (4.3)	1 (14.3)	1 (4)	0 (0)	1(4)	4 (14.3)
Central hypocortisolism	11 (25.0)	10 (43.5)[a]	0 (0)[a]	8 (32)	5 (21.7)	3 (12)[a]	13 (46.4)[a]
Central hypothyroidism	6 (13.6)	1 (4.3)	2 (28.6)	3 (12)	1 (4.3)	1 (4)	5 (20.3)
Central hypogonadism	7 (15.9)	4 (17.4)	2 (28.6)	5 (20)	3 (13)	2 (8)	8 (28.6)
Macroadenoma	13 (29.5)	0 (0)	1 (14.3)	2 (8)[a]	13 (56.5)[a]	3 (12)	5 (17.9)
Invasion (MRI)	5 (11.4)	0 (0)	0 (0)	2 (8)	6 (26.1)	4 (16)	4 (14.3)
Visual defects	15 (34.1)	0 (0)[a]	2 (28.6)[a]	2 (8)	5 (21.7)	1 (4)	6 (21.4)
Neurosurgery (1-3)[d]	12 (27.2)	21 (91.2)	7 (100)	19 (76)[a]	11 (47.8)[a]	2 (8)	4 (14.3)
Cabergoline	19 (43.2)	6 (26.1)	3 (42.9)	10 (40)	15 (65.2)	22 (88)	27 (96.4)
LINAC radiotherapy[e]	5 (11.4)	7 (30.4)	3 (42.9)	15 (60)[a]	5 (21.7)[a]	1 (4)	1 (3.6)
Tumour volume (mm3)	86 (23-320)	30 (9-74)	36 (4-125)	24 (3-61)[b]	125 (26-400)[b]	22 (4-98)	27 (12-142)

NPFA, non-functioning pituitary adenoma. Invasion: Knosp >1. Macroadenoma: >1 cm. MRI, magnetic resonance imaging. Tumour volume = 0.5 (lengt h × width × height) = mm^3. GH, Growth hormone.
[a]Pearson's chi-squared test ($P < 0.05$).
[b]Mann-Whitney U test ($P < 0.05$).
[c]Panhypopituitarism was defined with all hormone deficiencies.
[d]A total of 76 patients underwent pituitary neurosurgery, 1 surgery = 63; 2 surgeries = 9; and 3 surgeries = 4.
[e]Patients with NFPA that received radiotherapy had residual tumour after surgery.

identify any clinical or biochemical statistical differences between them. Therefore, all NFPA patients were used as unique group.

2.3 | Evaluation of clinical variables

Baseline patient characteristics were evaluated in addition to complete physical examination (ie, blood pressure, weight, height, visual campimetry, acanthosis nigricans, acne, hirsutism and arthralgias). In order to adequately complete SF-36 questionnaires, some comorbidities like diabetes mellitus, arterial hypertension, obesity, lipids alterations, osteoporosis, stroke, autoimmune diseases, cardiopathy and malignant cancer were also registered in all patients as binary variables (present/absent). Time from beginning of symptomatology, from diagnosis of pituitary adenoma, and from first medical or surgical treatment were also considered. Medical, surgical and radiation treatments for the pituitary adenoma were also documented. Imaging findings were taken from the closest magnetic resonance imaging (MRI) to the questionnaire evaluation, within the last 6 months. Tumour volume was evaluated using ellipsoid formula = 0.5 (length × width × height) = (mm^3).[21] For cavernous sinus invasion, we used Knosp classification, consisting in five grades depending on invasiveness of the adenoma. Grade 0 is when the adenoma is not invasive; grade 1 when adenoma extends less than 25%, and it does not reach the median line; grade 2 when tumour extends 50%, usually beyond the median line, but does not extend beyond lateral line; grade 3 when tumour extends about 75%, usually beyond the lateral line; and finally, the grade 4 when the adenoma encase carotid artery.[22] Laboratory tests included GH, IGF-1, thyrotropin, total thyroxine, prolactin, adrenocorticotropin (ACTH), serum morning cortisol, luteinizing hormone (LH), follicle stimulating hormone (FSH), oestradiol in women, and testosterone in men. Hormone levels were measured, without treatment. Hypogonadism was diagnosed based on patients' clinical symptoms, low testosterone in men, or low oestrogen in women, together with inappropriate normal or low gonadotropins (Table 1). Hormone replacement therapy was given in order to correct symptoms and biochemical abnormalities. Adrenal insufficiency was diagnosed clinically and biochemically as well, with low cortisol level, and low or inappropriate normal ACTH level. Steroid replacement was given after diagnosis. Hypopituitarism was defined with one or more hormone deficiencies, and panhypopituitarism in those cases with deficit of all anterior pituitary hormones. We completed biochemical evaluation with 25-OH-vitamin D, creatinine, glycated haemoglobin (A1C), and fasting insulin, since these parameters when abnormal may also decrease QoL scores.

2.4 | Healthy Mexican population registry (HMPR)

We compared the QoL scores in patients with secretory and NFPA with those QoL scores reported in the Healthy Mexican Population Registry (HMPR) as reference information. These data come from the Mexican survey of access. This survey evaluated quality of health services in two states of Mexico, which was conducted from 1999 to 2000.[20] The survey was based on a multistage randomized sampling that considered four stages and encompassed both rural and urban areas. A total of 1200 dwellings were randomly selected in rural areas and 3000 in urban areas. In both urban and rural areas, an additional 10% was evaluated in order to ensure sample size. The SF-36 questionnaire was answered by 5961 normal-weight individuals, over 25 years of age, with at least one individual per household. Exclusion criteria for this group included any patient with diagnosis or treatment for chronic or acute illness disease at the moment of questionnaire evaluation. Women have 46.6 ± 13.4 years, and men 47.9 ± 13.8 years. Since the majority of our patients with pituitary adenoma were female (75%), we considered necessary to compare the baseline characteristics and QoL scores in all HMPR vs only the group of females in the HMPR. No significant differences were found (Table S1). Therefore, our HMPR reference full group is suitable for statistical comparison with our cohort of patients with pituitary adenomas even though the majority were females.

2.5 | Assessment of QoL

Quality of life was assessed using the self-administered generic instrument SF-36 health questionnaire in the translated and validated version for Mexican population.[20] The questionnaire items are formulated as statements to evaluate eight specific health scales which are physical functioning, physical pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, emotional well-being, social functioning, energy/fatigue and general health perceptions. Each item can be answered by choosing from five possible responses. A higher score value indicates a better health. Scales result calculating the average of each item's scores, so the lowest and highest scores are 0 and 100, respectively. Scales are classified in physical (PCS) and mental component scores (MCS). Using Cronbach's alpha coefficient, the SF-36 survey showed high internal consistency reliability of 0.93-0.78, which is greater than the minimum recommended of 0.70.[23] QoL was evaluated once the patients were with the necessary hormone replacement therapy. The questionnaire was self-answered when patients attended their medical appointment to our clinic. Average time to answer the questionnaire is 7 minutes.

2.6 | Statistical analyses

Continuous data with normal and non-normal distribution is expressed with arithmetic means and standard deviations (SD), or medians and interquartile ranges, respectively. Categorical variables are expressed with frequencies and proportions. Linearity, normality, homoscedasticity and absence of multicollinearity were checked. Differences in sociodemographic, treatment, comorbidities, biochemical, imaging and SF-36 scales among adenomas subtypes grouped by disease activity were assessed with Student's *t* test, Mann-Whitney *U* test, and chi-squared test, as appropriate. Kruskal-Wallis test was used to analyse differences in QoL across secreting adenomas subtypes, grouped by MCS and PCS. Then, we performed Spearman's correlation to assess the linear association between hormones related to disease activity in each adenoma subtype with MCS or PCS scores. Those variables with a significant correlation were then included on stepwise multiple linear regression models to identify independent parameters related to impaired QoL. SF-36 scores were also compared with those scores obtained from Mexican normative values of the SF-36 questionnaire.[20] We performed a single radar chart to evaluate median scores of each SF-36 scale in Mexican population survey with those QoL scores in each secretory and non-functioning pituitary adenoma (Figure 4). A two-tailed level of $P < 0.05$ was considered significant. We used the Statistical Package for Social Sciences software (SPSS, version 24.0, Chicago, IL).

3 | RESULTS

3.1 | Baseline characteristics

We evaluated 175 patients with secretory and non-functioning pituitary adenomas (Table 1). They were grouped according to disease activity. Mean age was 44 ± 14 years. Pituitary adenoma was diagnosed at 36 ± 14 years, with a median disease duration of 7 (1-10) years. No significant difference was found in age between subtypes of pituitary adenomas (Table 1). The majority of our patients were females (n = 132, 75%). More than half of patients (n = 130, 74%) had overweight or obesity. Disease was under control in 117 patients (67%) at study evaluation, and 125 patients (71%) were already with replacement therapy related to their pituitary dysfunction. In addition to neurosurgery (n = 76, 1 surgery, n = 63; 2 surgeries, n = 9; 3 surgeries n = 4), and stereotactic radiotherapy (n = 37), available medical therapy for patients with pituitary adenoma or its related hypersecretion were cabergoline and ketoconazole. Patients with NFPA that received radiotherapy had residual tumour after surgery. This and other baseline characteristics are summarized in Table 1.

3.2 | Quality of life in secreting pituitary adenomas

Patients with biochemical uncontrolled laboratories because of pituitary hormone hypersecretion and disease activity showed significant lower mental (MCS) and physical components scores (PCS) in the SF-36 survey, than those cases with controlled disease (all $P < 0.03$). These were consistent for all subtypes of pituitary adenomas. Active Cushing disease (CD) showed lower MCS in comparison of active prolactinomas (Figure 2, $P < 0.01$). Active CD and acromegaly showed significant lower PCS in comparison of active prolactinomas

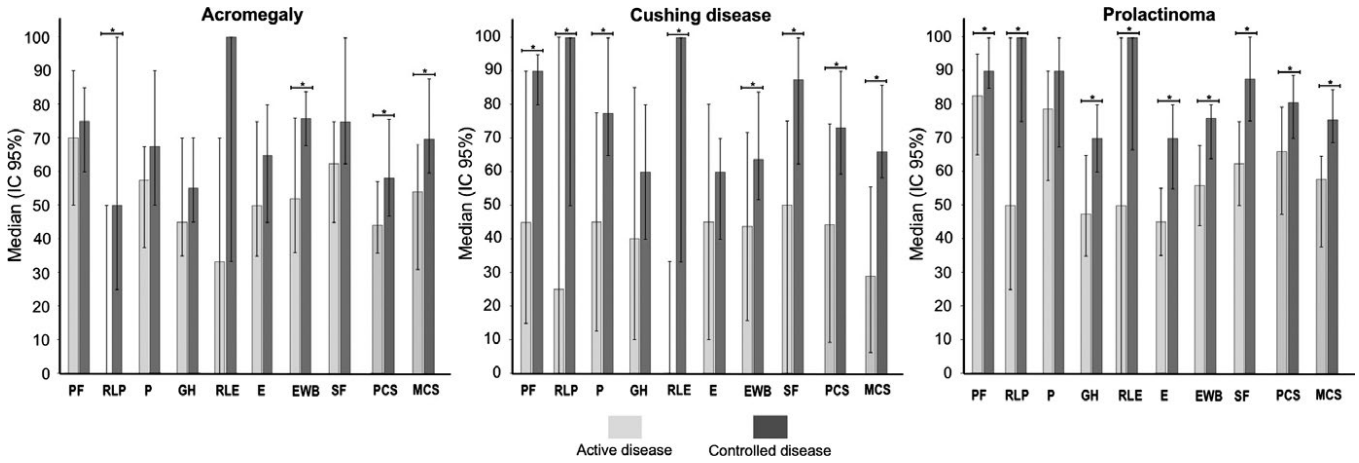

FIGURE 1 SF-36 scales and global scores of patients with secretory adenomas grouped by disease activity. *Mann-Whitney *U* test $P < 0.05$. E, energy; EWB, emotional well-being; GH, general health; MCS, mental component score; P, pain; PCS, physical component score; PF, physical functioning; RLE, role limitations due to emotional problems; RLP, role limitations due to physical health; SF, social functioning

or NFPAs (Figure 2, $P < 0.03$). NFPAs therefore have higher PCS vs active CD or acromegaly ($P < 0.01$, Figure 2). Interestingly, patients with controlled prolactinomas and controlled CD showed higher scores in 6 out of 8, and 7 out of 8 scales, respectively ($P < 0.03$, Figure 1). This was not the case in acromegaly since it persisted with low scores in 5 out of 8 scales, despite active or controlled disease. The three scales that showed higher scores in controlled acromegaly patients were in the limitations due to physical (RLP) and emotional (RLE) problems, in addition to the emotional well-being scale (EWB, Figure 1). All secretory adenoma but acromegaly showed higher QoL scores after disease control, however, NFPAs persisted with significant lower MCS (Figure 2, $P < 0.03$), and less PCS in comparison of CD and prolactinomas. Table S1 summarized the mean of PCS and MCS for each subtype of pituitary adenoma grouped by disease activity.

3.3 | Correlation between biochemical serum parameters and QoL scores

Prolactin levels showed a significant negative correlation with QoL in both MSC ($r = -0.31$, $P = 0.01$) and PCS ($r = -0.41$, $P = 0.002$, Figure 3) in patients with prolactinomas. Similarly, in patients with CD, urinary 24 hours free cortisol correlated inversely with MCS ($r = -0.43$, $P = 0.01$), and we saw a statistical trend with PCS ($r = -0.32$, $P = 0.08$). No significant correlation was found between IGF-1 levels (adjusted for age and gender) and MCS ($r = -0.23$, $P = 0.11$) or PCS ($r = -0.21$, $P = 0.14$) in acromegaly (Figure 3).

3.4 | Independent parameters determining impaired QoL

We performed a stepwise linear regression analyses grouped by subtype of pituitary adenoma to identified significant independent parameters that may explain the reduced QoL (Table 2, Figure S2). PCS was lower in acromegaly with previous pituitary surgery ($\beta = -13.5$, $P = 0.01$), and diagnosis of hypogonadism ($\beta = -17.6$, $P < 0.01$). MCS was lower with active acromegaly ($\beta = -13.7$, $P = 0.03$). Male gender ($\beta = 21$, $P < 0.01$), and control of acromegaly activity ($\beta = 19.8$, $P = 0.04$), significantly determined higher MCS and PCS, respectively. In active CD, the 60% of the impaired MCS was explained because of lower HDL ($\beta = -0.98$, $P < 0.01$), pituitary surgery ($\beta = -30.3$, $P = 0.04$), and visual defects in campimetry (2 out of 7 patients, 28%, $\beta = -45.6$, $P < 0.01$, $R^2 = 0.60$, $P < 0.001$). The linear multivariate regression analyses showed that active CD ($\beta = -22$, $P = 0.04$), high HbA1c ($\beta = -18.5$, $P = 0.01$), and lower HDL level ($\beta = -0.77$, $P = 0.03$) determined lower PCS. In prolactinomas, PCS and MCS were lower because disease activity ($\beta = -16.7$, $P = 0.002$) and visual defects ($\beta = -21$, $P = 0.009$). Central adrenal insufficiency also caused decreased PCS ($\beta = -12.5$, $P = 0.01$). Having a micro vs. macroadenoma showed better MCS ($\beta = 21$, $P < 0.01$). Independent parameters related to lower PCS in patients with NFPAs were younger age ($\beta = -0.38$, $P = 0.03$), BMI ($\beta = -1.37$, $P = 0.04$), and previous diagnosis of malignant cancer ($\beta = -27$, $P = 0.03$). No significant associations were identified to predict lower MCS. Radiotherapy was not associated with lower QoL in any pituitary adenoma.

3.5 | QoL between pituitary adenomas vs Healthy Mexican Population Registry (HMPR)

SF-36 results of our cases with pituitary adenomas were compared with those SF-36 results in the HMPR (Figure 4). These otherwise healthy subjects responded the SF-36 quite similar throughout the eight scales and their components. In summary, harbouring a secretory or non-functioning pituitary adenoma decrease both PCS and MCS (Table S1, and Figure 2) even after biochemical control. Interestingly, patients with NFPA had more QoL impairment than cases with controlled prolactinoma. This information is summarized in Table S1.

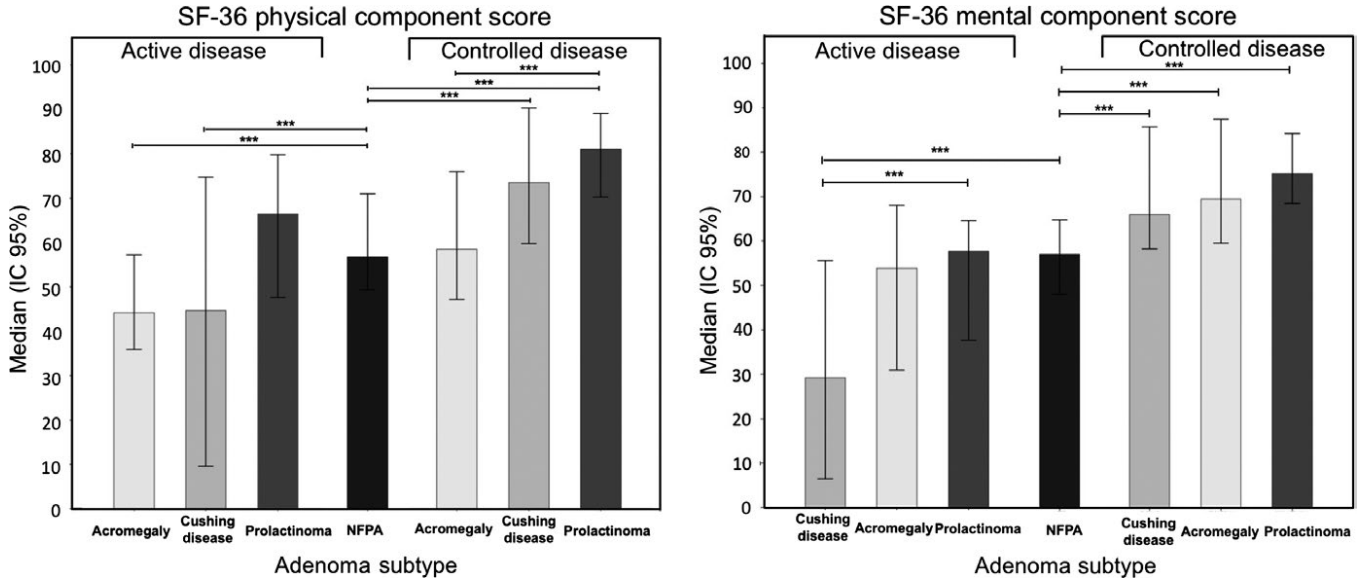

FIGURE 2 SF-36 global MCS and PCS comparison among all adenoma's subtypes. Secretory adenomas comparisons are made between active and controlled disease, respectively. NFPA scores are compared with both groups. *Mann-Whitney *U* test $P < 0.05$. MCS, mental component score; NFPA, non-functioning pituitary adenoma; PCS, physical component score;

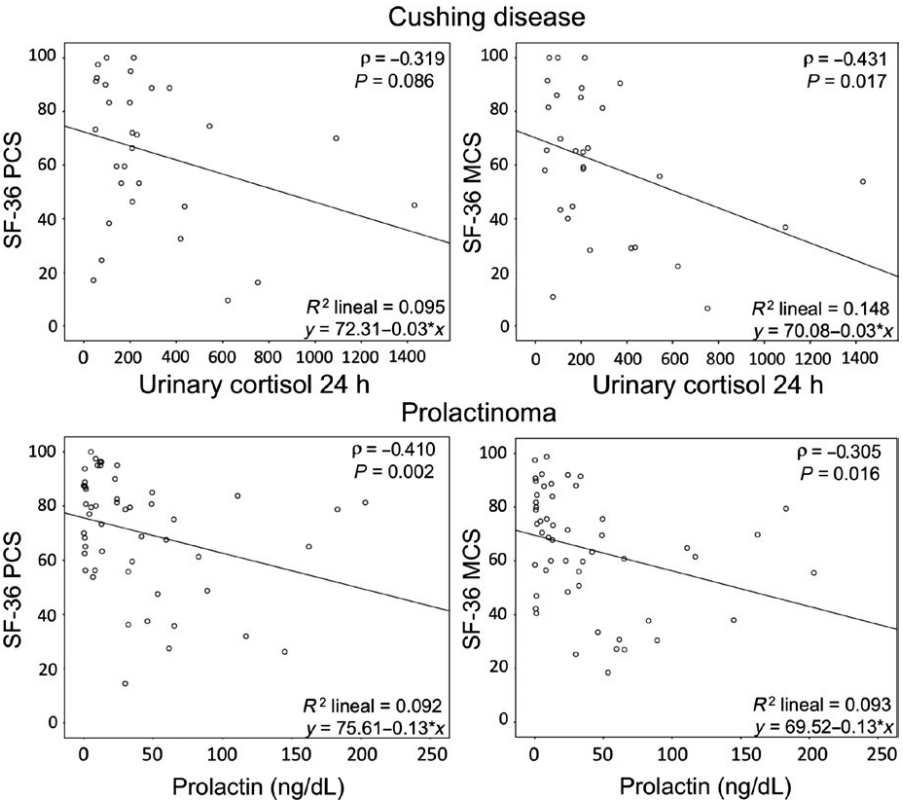

FIGURE 3 Spearman correlation between biochemical parameters and SF-36 scores grouped by mental (MCS), and physical component score (PCS)

4 | DISCUSSION

We evaluated QoL in a cohort of 175 patients with secretory and non-functioning pituitary adenomas grouped by disease activity. Then, we compare our results with those registered in the HMPR.[20] We confirmed that all subtypes with active pituitary adenomas have reduced QoL. However, despite normalization of hormone hypersecretion, QoL remain lower than Mexican general population. In fact, NFPAs also showed lower QoL than healthy people. Therefore, harbouring a pituitary adenoma, secretory or not-secretory, significantly impaired the QoL of a given individual. The independent and significant parameters related to reduction of QoL were also explored, and we found specific and novel parameters for each type of pituitary adenoma.

TABLE 2 Stepwise multiple linear regression analyses to evaluate independent parameters related to lower physical (PCS) and mental component scores (MCS) in each adenoma subtype

PCS				MCS			
Related Factor	***β***	**95% CI**	***P***	**Related Factor**	***β***	**95% CI**	***P***
Acromegaly							
Active disease	−17.646	−30.7 to −4.5	0.009	Active disease	−13.708	−26.59 to −0.82	0.038
Pituitary surgery	−13.575	−24.7 to −2.4	0.018	Male	21.152	7.86-34.44	0.003
Hypogonadism	−8.647	−2.2 to −19.1	0.046	Hypogonadism	−5.985	−22.69 to 10.72	0.474
Hypertriglyceridemia	10.196	−1.1 to 21.5	0.116	-	-	-	-
$F = 5.4$, $P = 0.001$, $R^2 = 0.39$, $y = 39 + (-13.6)X_1 + (-17.6)X_2 + (-8.6)X_3$				$F = 6.7$, $P = 0.001$, $R^2 = 0.32$, $y = 68 + (-13.7)X_1 + (21.2)X_2$			
Cushing disease							
Active disease	−21.815	−42.9 to −0.72	0.043	Active disease	−20.791	−39.75 to −1.82	0.033
A1c	−18.490	−33.6 to −3.2	0.019	HDL	−0.987	−1.61 to −0.359	0.003
HDL	−0.773	−1.4 to −0.56	0.036	Pituitary surgery	−30.307	−60.09 to −0.51	0.046
Visual defects	−24.67	−70.6 to 21.3	0.280	Visual defects	−45.671	−75.38 to −15.95	0.004
$F = 7.3$, $P = 0.001$, $R^2 = 0.46$, $y = 212.2 + (-21.8)X_1 + (-18.5)X_2 + (-0.8)X_3$				$F = 9.5$, $P < 0.001$, $R^2 = 0.60$, $y = 141.6 + (-20.8)X_1 + (-0.98)X_2 + (-30.3)X_3 + (-45.7)X_4$			
Prolactinoma							
Active disease	−16.757	−27.2 to −6.2	0.002	Active disease	−20.722	−31.0 to −10.4	0.000
ACTH deficiency	−12.523	−21.3 to −3.3	0.010	Microadenoma	20.939	7.3-34.5	0.003
Visual defects	−21.128	−36.7 to −5.5	0.009	Visual defects	−13.627	−28.52 to 1.2	0.072
$F = 9.3$, $P < 0.001$, $R^2 = 0.34$, $y = 79.5 + (-16.8)X_1 + (-12.5)X_2 + (-21.1)X_3$				$F = 10.5$, $P < 0.001$, $R^2 = 0.41$, $y = 71.7 + (-20.7)X_1 + (20.9)X_2$			
Non-functioning pituitary adenoma							
Age	−0.387	−0.75 to −0.02	0.039	None	-	-	-
BMI	−1.374	−2.6 to −0.6	0.041	-	-	-	-
History of cancer	−27.7	−52.9 to −2.5	0.039	-	-	-	-
$F = 6.191$, $P = 0.001$, $R^2 = 0.17$, $y = 172.4 + (-0.387)X_1 + (-1.374)X_2 + (-27.7)X_3$							

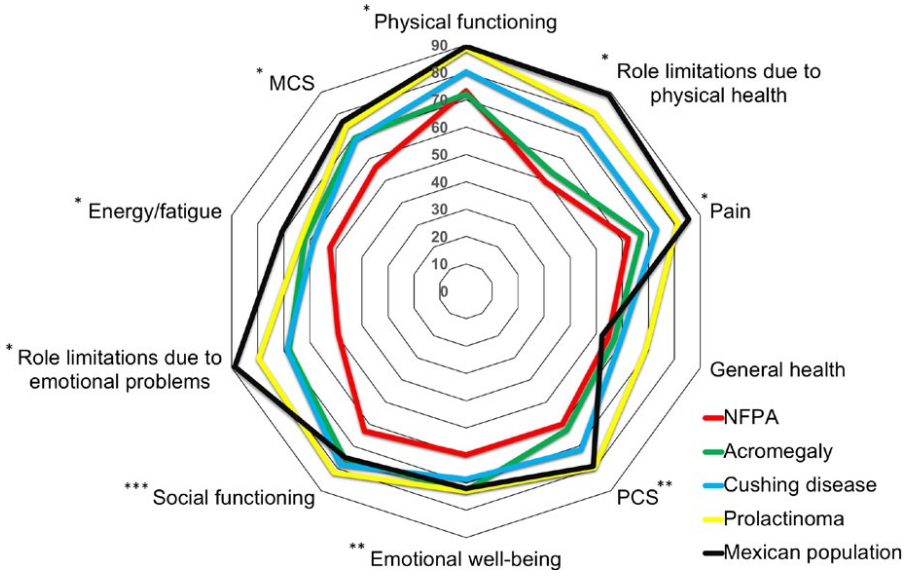

FIGURE 4 Single radar chart to evaluate mean scores of each SF-36 scale in HMPR with those QoL scores in each secretory and non-functioning pituitary adenoma (age- and sex-paired). *Student's *t* test $P = 0.001$ for Mexican scores vs scores in others secretory adenomas. **Student's *t* test $P = 0.001$ for Mexican scores vs scores in others secretory adenomas except prolactinomas. ***Student's *t* test $P = 0.001$ for Mexican score vs NFPA QoL scores

4.1 | Decrease in physical component score (PCS)

Physical component score was particularly low in patients with acromegaly. Because of the acral overgrowth, musculoskeletal pain, arthritis,[10] fatigue and soft tissue swelling,[18] it is feasible to explain these consistent results in our and other publications. However, no significant negative correlation was found between higher serum IGF1 levels and lower PCS. Such clinical complications are

consequence of active acromegaly but usually do not improve after biochemical control having irreversible effects; therefore, patients remained with poor PCS. CD, however, usually improves after successful treatment, and symptomatology improves together with normalization of serum or urinary cortisol levels. As a result, significant negative correlation was identified between 24 hours urinary free cortisol with PCS. Similar benefit was seen in patients with prolactinoma, since reduction in symptomatology as consequence of medical treatment and prolactin normalization was significantly associated with improvement of PCS.

4.2 | Decrease in mental component score (MCS)

Mental component score was especially low in patients with CD and prolactinoma. Although sometimes cortisol or prolactin levels do not correlate with disease severity, previous studies have implicated both prolactin and cortisol on human behaviour with multiple psychosomatic implications.[13,26] For example, reduction in hippocampus volume, with progressive cognitive decline, increased risk of developing psychiatric illnesses such as depressive symptoms, anxiety-related disorders and borderline personality disorder.[19,27,28] Also, higher cortisol levels have been significant related to poor self-esteem, low internal locus of control,[29,30] loneliness and sleep deprivation[31]; while reduction in serum cortisol has been related to positive affect demonstrated after aggregating momentary experiences throughout a working or leisure day.[32] Additionally, PRL hypersecretion increased anxiety in one study[24] by producing an imbalance of related neurotransmitters like serotonin, GABA and dopamine,[24] which also influences mood, and attitude. Clinical studies in humans have also indicated a significant correlation between higher PRL levels and psychological distress.[25] Female patients with hyperprolactinemia usually report more symptoms of anxiety and hostility than control female subjects, and additionally, patients with prolactinomas have more perception of pain, affecting their social functioning and emotional status. These variables significantly correlated with prolactin circulating levels.[13]

Taking these findings together it could explain why higher serum cortisol and prolactin significantly correlated with poor QoL results in our patients, particularly in components like social relations, mental health and emotional role limitations. Despite significant negative correlation (Figure 3), we believe PRL was not too high because our cases were uncontrolled under insufficient treatment with cabergoline. Regression model showed that having a microprolactinoma was significant and independent parameter related to higher QoL in MCS (Table 2). Therefore, it is possible that in addition to the negative significant correlation of PRL with QoL (Figure 3), tumour size also impacted QoL in prolactinomas (Table 2).

Non-functioning pituitary adenomas also caused significant reduction in MCS. Although NFPA may cause hyperprolactinemia because mass effect and compression of infundibulum, prolactin usually have a more slightly to moderate elevation making necessary to find additional explanation to this outcome. Also, NFPA-related hypopituitarism may cause less QoL but usually this is when it remains symptomatic because of lack or incomplete treatment. In our clinic, a complete evaluation, treatment and follow-up of hypopituitarism are given to all of our patients, and therefore, it is less likely that NFPA-related hypopituitarism caused poor MCS. In contrast, patients with NFPA has been associated with reduced QoL too in other studies. Van der Klaauw et al reported worse physical ability and body pain in patients with pituitary adenomas after treatment, including NFPA.[9] However, we evaluated NFPA patients after treatment but also under clinical observation because of a stable tumour without growing. In both clinical scenarios, harbouring a NFPA significantly reduced MCS, therefore, further risks factors may be identified. In addition to chronic headaches, for example, scales related to MCS may be impaired because of patients are now aware that have a "head tumour," which will need long-term follow-up that perhaps starts growing and require surgical treatment or radiotherapy, it may compress optic chiasm, invade carotid artery, or sometimes is complicated with an emergency called apoplexy. All this information might cause anxiety to patients with NFPA, decreasing well-being and energy, low self-stem, or depression. This scenario could also explain why all secretory pituitary adenomas remained with low QoL even with good disease control. In addition, having hormone deficits associated with NFPA may also contribute to low QoL. Usually, all anterior pituitary hormones should be evaluated to treat any deficiency, however, sometimes it is expensive, like GH-replacement therapy, or affects energy, fertility, sexuality, or increase risk for metabolic diseases, which also have been reported to decrease QoL. Recently, Andela et al proposed that impaired quality of life in patients with NFPA results from a multi-scale situation that can be explained by the Wilson-Cleary biopsychosocial model, which states that health and QoL can be considered as a continuum of increasing biological, psychological and social complexity, with pure biological measures and general health perceptions.[34] Therefore, NFPA is a tumour that should not be considered with similar QoL as general healthy population,[10,18,35] and clinical research using such patients as "control group" may yield misleading results.

In summary, patients with pituitary adenoma and disease activity or disease control, significantly have worse QoL outcomes.[4,13,33]

4.3 | Independent parameters significantly associated with lower QoL

Identifying independent factors related to lower QoL in patients with pituitary adenomas have been motive of constant research.[1,3,8,11,36,37] All this information help to understand why the pituitary adenoma patients have low QoL. In our patients, we found novel risk factors that showed positive or negative impact on QoL depending on adenoma subtype and disease activity. Firstly, in acromegaly, males were less aggravated in MCS, independently of disease activity. Although the positive impact of male gender has been previously described in acromegaly and CD,[1,9,38] this was not evaluated with or without disease activity. We found here that male gender is an independent factor related to better

MCS even in patients with active acromegaly. Secondly, hypopituitarism has been significantly and independently associated with lower QoL in patients with pituitary adenoma.[9,37,38] Consistently, gonadotropin and ACTH deficiencies impacted negatively in global QoL scores mainly in acromegaly and prolactinomas. This result was despite good replacement therapy with oestrogen in women, testosterone in male, or steroids for central adrenal insufficiency. In addition to the symptomatology of acromegaly, and hyperprolactinemia, it should be considered less QoL in such patients when hypogonadism or central adrenal insufficiency is diagnosed. The two main risk factors that decrease QoL scores in NFPA were age at diagnosis, and BMI, decreasing PCS. Also, these should be remembered as additional significant clinical features to impair QoL. Previous pituitary surgery and visual defects were also independent and significant risk factors to reduced QoL. In our study, we do not find stereotactic radiotherapy with LINAC as a significant variable to decrease QoL. This is emphasized because previous reports have found the conventional radiotherapy as an important negative factor related to lower QoL outcomes in acromegaly.[8] However, it is important to consider that it was only used in few patients (n = 37).

4.4 | SF-36 Mexican normative comparison

Quality of life is chronically affected in patients with pituitary adenoma even after disease control and remained lower when compared with general healthy population.[20] However, other studies reported similar QoL in healthy people and patients with NFPA.[6,15] All of the secretory or non-functioning pituitary adenomas showed lower QoL than healthy Mexican population, and this was seen with active or disease control. Moreover, patients with NFPA also shown lower QoL than general healthy population. These results highlight that patients with pituitary adenomas, secretory or with NFPA, do not have same QoL to healthy general population and this QoL could even be more reduced when patient has active disease.

Our study has some limitations that should be stated. First, the cross-sectional design only allowed us to reached significant associations but not specific cause-effect conclusions. However, these results contribute importantly to the specific attention that patients with pituitary adenomas should receive in mental and physical components. Another limitation is that we only used a general QoL questionnaire like the SF-36 which lacks specific questions that may have a greater sensitivity in the symptomatology of patients. Nevertheless, we do not use specific QoL questionnaires for acromegaly or Cushing disease (ie, AcroQoL or CushingQoL) in order to compare QoL between different pituitary adenomas. Finally, we evaluate a relatively small sample of patients, however, considering that some of these pituitary adenomas are quite rare we showed here sufficient number of cases to reach statistical power to do comparisons among them.

We can conclude that QoL is reduced in active pituitary disease, that persisted low in patients after controlled pituitary hormone hypersecretion, and that NFPA showed significant impairment in QoL too, despite been a non-functioning tumour. It is also important to note that although patients got higher and therefore better QoL score when they reached disease control, they do not reach a QoL similar to the one scored in healthy Mexican normative system. Identifying and treating the associated independent factors is also important in order to reduce or correct the mental and physical impairments as good as possible.

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CONFLICT OF INTEREST

The authors have nothing to declare.

AUTHOR CONTRIBUTIONS

AVB, VMEE, DCR: Research idea and study design; AVB, VMEE, TRTV, FDMS, MCPG, JMHA, HDES, ALS: Data acquisition; AVB, DCR, OYBC, VMEE: Statistical analysis; AVB, DCR, MAGS, FGP: Data analysis/interpretation; AVB, DCR, VMEE, OYBC, DCR, MGAS, FGP: Manuscript drafting; DCR: Supervision and mentorship. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

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SUPPORTING INFORMATION

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