



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
FACULTAD DE MEDICINA
BIOMEDICINA

**DETERMINACIÓN DE LA PROPORCIÓN CIRCULANTE DE MONOCITOS-
MACRÓFAGOS M1 Y M2 EN SUJETOS CON SOBREPESO Y OBESIDAD**

TESIS

(MODALIDAD: **POR ARTÍCULO CIENTÍFICO**)

QUE PARA OPTAR POR EL GRADO DE:

MAESTRA EN CIENCIAS BIOLÓGICAS

PRESENTA:

KARINA SUÁREZ ÁLVAREZ

TUTOR PRINCIPAL: DR. EUSTACIO GALILEO ESCOBEDO GONZÁLEZ, FACULTAD DE MEDICINA

**COMITÉ TUTOR: DRA. CLAUDIA VERÓNICA ZAGA CLAVELLINA, FACULTAD DE MEDICINA
DR. LUIS IGNACIO TERRAZAS VALDÉS, FACULTAD DE MEDICINA**

MÉXICO, D.F. JUNIO DEL 2013.



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Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
Presente

Me permito informar a usted, que el Subcomité de (Biología Experimental y Biomedicina), en su sesión ordinaria del día 11 de febrero de 2013, aprobó el jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS** de la alumna **SUÁREZ ÁLVAREZ KARINA** con número de cuenta 303693479, en la modalidad de **réplica oral del artículo científico**, con la tesis titulada **"DETERMINACIÓN DE LA PROPORCIÓN CIRCULANTE DE MONOCITOS-MACROFAGOS M1 Y M2 EN SUJETOS CON SOBREPESO Y OBESIDAD"**, bajo la dirección del **DR. EUSTACIO GALILEO ESCOBEDO GONZÁLEZ**:

Presidente: DR. LUIS IGNACIO TERRAZAS VALDÉZ
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Sin otro particular, me es grato enviarle un cordial saludo.

ATENTAMENTE
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F., a 09 de abril de 2013



DRA. MARÍA DEL CORO ARIZMENDI ARRIAGA
COORDINADORA

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Resumen

Recientemente se ha propuesto que la interleucina (IL-) 12 participa en el desarrollo de resistencia a la insulina en ratones obesos. Sin embargo, los niveles séricos de IL-12 no se han determinado en personas con sobrepeso y obesidad. Por consiguiente, el objetivo del presente estudio es determinar las concentraciones de IL-12 en sujetos mexicanos, examinando la relación que esto tiene con la presencia de un estado inflamatorio de bajo grado a nivel sistémico, así como con parámetros asociados a la disfunción metabólica. Un total de 147 individuos sanos participaron en el estudio, en el cual 43 sujetos presentaron un peso normal, 61 presentaron sobrepeso y 43 presentaron obesidad. En condiciones de ayuno, se determinaron los niveles circulantes de IL-12, el factor de necrosis tumoral alfa (TNF- α), la leptina, la insulina, la glucosa, el colesterol total y los triglicéridos, en los participantes del estudio. De manera complementaria, se determinó la circunferencia de la cintura y el porcentaje de grasa de cada participante. Los sujetos con sobrepeso y obesidad presentaron un incremento significativo en los niveles séricos de IL-12 con respecto a los individuos delgados. Los niveles séricos de IL-12 mostraron una relación positiva y significativa con el índice de masa corporal ($r=0.5154$), el porcentaje de grasa total ($r=0.5625$), los niveles séricos de glucosa ($r=0.3158$), los niveles séricos de triglicéridos ($r=0.3714$), los niveles séricos de TNF- α ($r=0.4717$) y la presencia de monocitos-macrófagos circulantes con fenotipo proinflamatorio ($r=0.2184$). Estos datos sugieren que los niveles séricos de IL-12 están aumentados en sujetos con sobrepeso u obesidad, y muestran una correlación positiva con marcadores de inflamación de bajo grado y parámetros asociados con disfunción metabólica. Este estudio podría ayudar a una mejor comprensión acerca del mecanismo por el cual la IL-12 participa en el desarrollo de alteraciones asociadas con la obesidad en humanos.

Abstract

Interleukin (IL-) 12 has been recently suggested to participate during development of insulin resistance in obese mice. Nevertheless, serum IL-12 levels have not been accurately determined in overweight and obese humans. We thus studied serum concentrations of IL-12 in Mexican adult individuals, examining their relationship with low-grade inflammation and obesity-related parameters. A total of 147 healthy individuals, 43 normal weight, 61 overweight, and 43 obese subjects participated in the study. Circulating levels of IL-12, tumor necrosis factor alpha (TNF- α), leptin, insulin, glucose, total cholesterol, and triglyceride were measured after overnight fasting in all of the study subjects. Waist circumference and body fat percentage were recorded for all the participants. Serum IL-12 was significantly higher in overweight and obese individuals than in normal weight controls. Besides being strongly related with body mass index ($r=0.5154$), serum IL-12 exhibited a significant relationship with abdominal obesity ($r=0.4481$), body fat percentage ($r=0.5625$), serum glucose ($r=0.3158$), triglyceride ($r=0.3714$), TNF- α ($r=0.4717$), and an increase in circulating monocyte-macrophages exhibiting a proinflammatory activation profile ($r=0.2184$). Thus, serum levels of IL-12 are increased in overweight and obese individuals, and show a strong relationship with markers of low-grade inflammation and obesity in the Mexican adult population. Further research is needed to understand the role of IL-12 in developing obesity-associated alterations in humans.

Introducción

La obesidad, definida como la acumulación excesiva de grasa corporal, se considera una enfermedad global y de etiología multifactorial, que representa un problema de salud pública a nivel mundial (1-3). Según la Organización Mundial de la Salud (OMS), aproximadamente 1,700 millones de adultos padecen sobrepeso y 312 millones padecen obesidad (1-4). En México, de acuerdo a la Encuesta Nacional y Nutrición (ENSANUT) 2006, la prevalencia de adultos con sobrepeso y obesidad fue de 39.7% y 29.9% respectivamente (1).

La obesidad es clasificada con base en el índice de masa corporal (IMC), el cual relaciona el peso y la estatura. El IMC se expresa como kg/m^2 y se calcula dividiendo el peso (kg) entre el cuadrado de la estatura (m^2) (3,5). De acuerdo a la OMS, un individuo con un IMC entre 18.5 y 24.9 kg/m^2 se considera delgado; un individuo con un IMC entre 25 y 29.9 kg/m^2 oscila en el sobrepeso y un individuo con un IMC superior a los 30 kg/m^2 clasifica con obesidad. Esta condición está relacionada con el aumento de hasta 5 veces la probabilidad de desarrollar diversas enfermedades como la Diabetes Mellitus tipo 2 (DM2) (1,3,6-9). Específicamente, esta predisposición se ha relacionado con la presencia de obesidad central, caracterizada por el acúmulo abdominal de tejido adiposo blanco y caracterizada por una circunferencia de cintura mayor de 88 cm para mujeres y 102 cm para hombres (1,8).

En respuesta a la ganancia de peso ($\text{IMC} \geq 25 \text{ Kg/m}^2$), el tejido adiposo se expande, aumentando su capacidad lipolítica, así como la pérdida gradual de la sensibilidad a la insulina (7,9-11). Además, el tejido adiposo hipertrófico aumenta significativamente su producción y liberación de factores proinflamatorios de afectación paracrina y endocrina, tales como las citocinas (9,10,12,13).

Debido al incremento en los niveles de secreción de agentes proinflamatorios y al reclutamiento de macrófagos en el tejido adiposo, la obesidad ha sido reconocida recientemente como un estado inflamatorio de bajo grado a nivel sistémico (4,9,14-19). Entre las citocinas proinflamatorias de mayor circulación en pacientes obesos se encuentran el TNF- α así como la interleucina (IL) 1 β , e IL-6 (20-21). Adicionalmente, en sujetos obesos se observan niveles altos de

leptina (hormona secretada en el tejido adiposo perinodal donde activa células inflamatorias como los macrófagos, induciendo síntesis de proteínas de fase aguda como INF- γ , TNF- α e IL-6), resistina (sintetizada en el humano por macrófagos residentes de tejido adiposo), MCP-1 (proteína quimioatrayente de macrófagos, también llamada CCL2) y proteína C reactiva (pCr) (9-11,16,17,20-26). Sin embargo, los niveles séricos de citocinas tipo Th1, mismas que podrían estimular la secreción de las citocinas proinflamatorias antes mencionadas, no se han estudiado en humanos en el contexto de obesidad y las alteraciones asociadas con ésta.

La IL-12 es una citocina producida principalmente por células dendríticas y macrófagos (27), la cual participa en la diferenciación de linfocitos T hacia el fenotipo Th1 (28). De manera reciente, se ha propuesto la posible participación de IL-12 en el desarrollo de resistencia a la insulina asociada con la obesidad en ratones sometidos a una dieta hipercalórica (29). Además, se ha demostrado que la producción de IL-12 puede ser estimulada en presencia de resistina, una adipocina elevada en ratones obesos y pacientes con DM2 (30). Sin embargo, los niveles séricos de IL-12 no se han estudiado en sujetos con sobrepeso u obesidad.

Por lo tanto, este estudio evaluó los niveles circulantes de IL-12 en sujetos mexicanos delgados y en aquellos que presentan sobrepeso y obesidad, evaluando si existe correlación entre esta citocina y diversos parámetros asociados con la obesidad (glucosa, insulina, HOMA-IR, colesterol total, triglicéridos, IMC, circunferencia de cintura y porcentaje de grasa total) y con la inflamación de bajo grado (leptina, TNF- α , y monocitos-macrófagos circulantes con fenotipo proinflamatorio).

Clinical Study

Serum IL-12 Is Increased in Mexican Obese Subjects and Associated with Low-Grade Inflammation and Obesity-Related Parameters

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Interleukin-(IL-) 12 has been recently suggested to participate during development of insulin resistance in obese mice. Nevertheless, serum IL-12 levels have not been accurately determined in overweight and obese humans. We thus studied serum concentrations of IL-12 in Mexican adult individuals, examining their relationship with low-grade inflammation and obesity-related parameters. A total of 147 healthy individuals, 43 normal weight, 61 overweight, and 43 obese subjects participated in the study. Circulating levels of IL-12, tumor necrosis factor- α (TNF- α), leptin, insulin, glucose, total cholesterol, and triglyceride were measured after overnight fasting in all of the study subjects. Waist circumference and body fat percentage were recorded for all the participants. Serum IL-12 was significantly higher in overweight and obese individuals than in normal weight controls. Besides being strongly related with body mass index ($r = 0.5154$), serum IL-12 exhibited a significant relationship with abdominal obesity ($r = 0.4481$), body fat percentage ($r = 0.5625$), serum glucose ($r = 0.3158$), triglyceride ($r = 0.3714$), and TNF- α ($r = 0.4717$). Thus, serum levels of IL-12 are increased in overweight and obese individuals and show a strong relationship with markers of low-grade inflammation and obesity in the Mexican adult population. Further research is needed to understand the role of IL-12 in developing obesity-associated alterations in humans.

1. Introduction

Obesity is a public health problem of global dimensions [1], with an alarming high prevalence among the Mexican population [2]. Particularly, abdominal obesity is known to directly augment the risk to develop type 2 diabetes (T2D),

cardiovascular disease, and other metabolic syndrome-associated alterations [3]. A growing body of evidence recently linked the pathogenesis of those disorders with a systemic low-grade inflammatory state [4], characterized by an increase in the circulating levels of several proinflammatory

mediators such as tumor necrosis factor- α (TNF- α), C-reactive protein (CRP), and leptin [5, 6]. TNF- α has been shown to increase with adiposity in mice and humans [7], promoting impaired insulin sensitivity in adipose tissue, liver, and muscle [8]. CRP exhibits high circulating levels in overweight and obese subjects, having a positive correlation with increased body mass index (BMI) and waist-to-hip ratio [9]. Leptin is known to regulate food intake and body weight [10], as well as exert immunostimulatory actions including macrophage and T cell activation, dendritic cell (DC) maturation, and releasing of interferon- γ (IFN- γ), TNF- α , and interleukin-(IL-) 6 [11]. Thus, circulating proinflammatory factors have received increasing attention since they seem to have a major role during development of obesity-related disorders such as nonalcoholic steatohepatitis, T2D, and arteriosclerotic vascular disease (AVD) [3, 12].

IL-12 is a heterodimeric class-I helical cytokine that is mainly produced by DC and macrophages and influences differentiation of T helper 1 (Th1) immune cells [13]. IL-12 has been recently suggested to participate during development of obesity-related insulin resistance in rodents, since it clearly increases in both the epididymal adipose tissue and adipose tissue-associated proinflammatory macrophages in high-fat-diet-fed mice [14]. Moreover, IL-12 production can be directly stimulated by resistin, a proinflammatory adipokine that shows an important elevation in obese mice and T2D patients [15]. Nevertheless, serum IL-12 levels have not been precisely determined in subjects with a high metabolic risk such as both overweight and obese individuals.

We therefore evaluated circulating levels of IL-12 in Mexican normal weight, overweight, and obese individuals, examining their correlation with obesity-related parameters (fasting glucose, fasting insulin, insulin resistance, total cholesterol, total triglyceride, BMI, waist circumference, and body fat percentage) and low-grade inflammation markers (serum TNF- α and leptin).

2. Materials and Methods

2.1. Subjects. A total of 147 healthy Mexican adult volunteers from the south-central region of Mexico were included in the study. All of the participants provided written informed consent, previously approved by an institutional review board of the General Hospital of Mexico, which guaranteed that the study was conducted in accordance with the principles described at the Helsinki Declaration. Subjects were excluded from the study if they had previous or recent diagnosis of diabetes mellitus, cardiovascular diseases, chronic hepatic or renal disease, blood pressure higher than 140/90 mm Hg, inflammatory or autoimmune disorders, acute or chronic infectious diseases, cancer, and endocrine disorders. We additionally excluded pregnant or lactating women, subjects under any kind of cardiometabolic medication including anti-inflammatory, antiaggregant, and antihypertensive drugs, and subjects without having an overnight fasting of 8–12 hours. All of the individuals enrolled into the study received full medical evaluation, including achievement of clinic history and physical examination by a physician.

2.2. Anthropometric Measurements. According to the World Health Organization criteria for BMI, all of the participants were divided into three groups: normal weight subjects as the control group (BMI 18.5–24.9 kg/m²), overweight individuals (BMI 25–29.9 kg/m²), and obese subjects (BMI \geq 30 kg/m²), where BMI is the result of dividing weight by height squared (kg/m²). Waist circumference was obtained from each study subject, considering the midpoint between the lower rib margin and iliac crest, using a conventional tape in centimeters (cm). For women, abdominal obesity was considered when their waist measurements were 80 cm or higher, whereas for men, abdominal obesity was considered when their waist measurements were 90 cm or higher. Body fat percentage was individually recorded by means of using a body composition analyzer (TANITA, Body Composition Analyzer, Model TBF-300A, Tokyo, Japan).

2.3. Biochemical Measurements. Blood samples were individually taken after overnight fasting and collected into pyrogen-free tubes (Vacutainer, BD Diagnostics, NJ, USA) at room temperature. Collection tubes were then centrifuged at 1000 g/4°C for 30 min, and serum samples were obtained and stored at –80°C in numerous aliquots until use. Total cholesterol and triglyceride were individually measured in triplicate by an enzymatic assay according to manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Serum insulin levels were individually determined in triplicate by means of the enzyme-linked immunosorbent assay (ELISA), following the manufacturer's instructions (Abnova Corporation, Taiwan). Serum glucose levels were individually determined in triplicate by the glucose oxidase assay, following the manufacturer's instructions (Megazyme International, Ireland). All of the biochemical measurements were performed at the same time in order to avoid procedural variations. The estimate of insulin resistance was individually determined by means of the HOMA-IR as follows: fasting insulin concentration (mU/L) \times fasting glucose concentration (mmol/L) divided by 22.5.

2.4. IL-12, TNF- α , and Leptin Measurements. Blood samples were individually taken after overnight fasting and collected into pyrogen-free tubes (Vacutainer, BD Diagnostics, NJ, USA) at room temperature. Collection tubes were then centrifuged at 1000 g/4°C for 30 min, and serum samples were obtained and stored at –80°C in numerous aliquots until use. Serum levels of IL-12, TNF- α , and leptin were determined in triplicate by ELISA, following the manufacturer's instructions (Peprotech, Mexico). All of the cytokine measurements were performed at the same time in order to avoid procedural variations.

2.5. Statistical Analysis. Data from BMI, waist circumference, body fat percentage, fasting glucose, fasting insulin, HOMA-IR, total cholesterol, triglycerides, serum TNF- α , and leptin are expressed as mean \pm standard deviation, using one-way ANOVA followed by a post hoc Tukey test for determining significant differences. Data from IL-12 are expressed as median and interquartile range in a box plot analysis, using the Kruskal-Wallis test or the Mann-Whitney *U* test for

TABLE 1: Anthropometric and biochemical characteristics of the study subjects.

	BMI			P value
	18.5–24.9 ^a	25–29.9 ^b	≥30 ^c	
Gender (W/M)	26/17	30/31	21/22	N. S.
Age (years)	30.3 ± 10.3	32.4 ± 10.2	34.7 ± 10.8	N. S.
BMI (kg/m ²)	22.7 ± 1.8	27.4 ± 1.2	33.6 ± 3.4	^{b,c} P < 0.0001
Waist circumference (cm)	80.1 ± 6.9	91.2 ± 6.8	107.2 ± 9.7	^{b,c} P < 0.0001
Body fat percentage	24.2 ± 8.1	30.3 ± 6.1	37.5 ± 6.9	^b P < 0.05, ^c P < 0.0001
Fasting blood glucose (mg/dL)	78.9 ± 5.2	88.7 ± 3.6	106.1 ± 7.4	^b P < 0.05, ^c P < 0.0001
Fasting blood insulin (mU/L)	12.23 ± 2.65	15.30 ± 7.94	16.84 ± 8.20	N. S.
HOMA-IR	2.58 ± 0.51	3.24 ± 0.36	3.81 ± 0.59	^c P < 0.05
Total cholesterol (mg/dL)	187.2 ± 7.6	195.8 ± 6.9	203.5 ± 8.1	^c P < 0.05
Total triglyceride (mg/dL)	136.5 ± 9.4	172.3 ± 11.8	251.3 ± 19.5	^{b,c} P < 0.0001
TNF-α (ng/mL)	256 ± 16.6	289.7 ± 37.5	341.2 ± 45.5	^b P < 0.05, ^c P < 0.0001
Leptin (ng/mL)	558.1 ± 43.5	545.5 ± 58.8	1246.3 ± 149.6	^c P < 0.0001

W: women; M: men; BMI: body mass index; HOMA-IR: homeostatic model assessment-insulin resistance; TNF-α: tumor necrosis factor-alpha.

Data are presented as mean ± standard deviation. Differences were considered significant when $P < 0.05$, as comparing overweight (b) and obesity (c) groups with normal weight subjects (a).

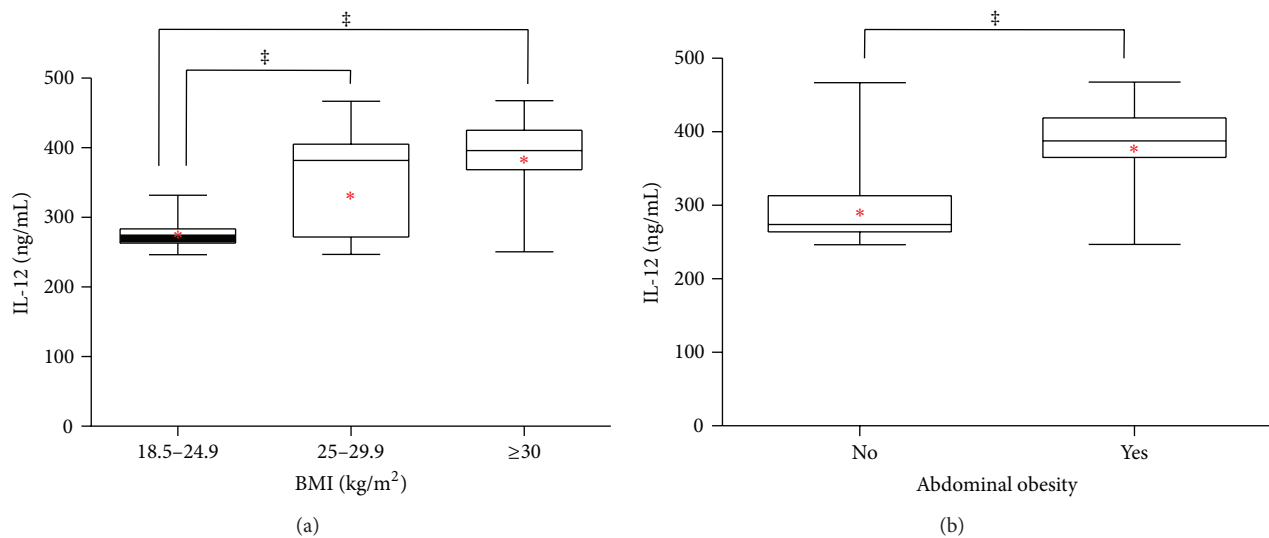


FIGURE 1: Serum levels of IL-12 in obese and nonobese individuals. Circulating levels of IL-12 (a) were assessed in normal weight, overweight, and obese subjects, according to the World Health Organization criteria for body mass index (BMI). In the study population, serum concentrations of IL-12 (b) were also evaluated in terms of abdominal obesity. Serum IL-12 was elevated in overweight and obese individuals, as well as in subjects exhibiting abdominal obesity. For women, abdominal obesity was considered when the waist measurement was 88 cm or higher, whereas for men, it was considered when the waist measurement was 102 cm or higher. Data are expressed as median and interquartile range in a box plot analysis. The mean value of IL-12 is graphically showed in a red asterisk. Differences were considered significant when $P < 0.05$. [‡] $P < 0.0001$.

determining significant differences. The Spearman's correlation coefficient was performed for evaluating the relationship of IL-12 with anthropometric, biochemical, and inflammatory parameters. All of the studied groups were matched by gender and age. Statistical analysis was performed using the GraphPad Prism 5 software. Differences were considered significant when $P < 0.05$.

3. Results

A total of 147 participants were included into the study (43 normal weight controls, 61 overweight individuals, and 43

obese subjects). No significant differences were observed in age (for normal weight controls, mean age 30.3 ± 10.3 years; for the overweight group, mean age 32.4 ± 10.2 years; for the obesity group, mean age 34.7 ± 10.8 years) and woman and man proportion (26 women and 17 men in the normal weight control group; 30 women and 31 men in the overweight group; 21 women and 22 men in the obesity group) among all of the studied groups (Table 1). On the contrary, waist circumference, body fat percentage, fasting blood glucose, triglyceride concentration, and serum TNF-α showed a clear increase in overweight and obese individuals as compared with normal weight controls (Table 1). Total cholesterol,

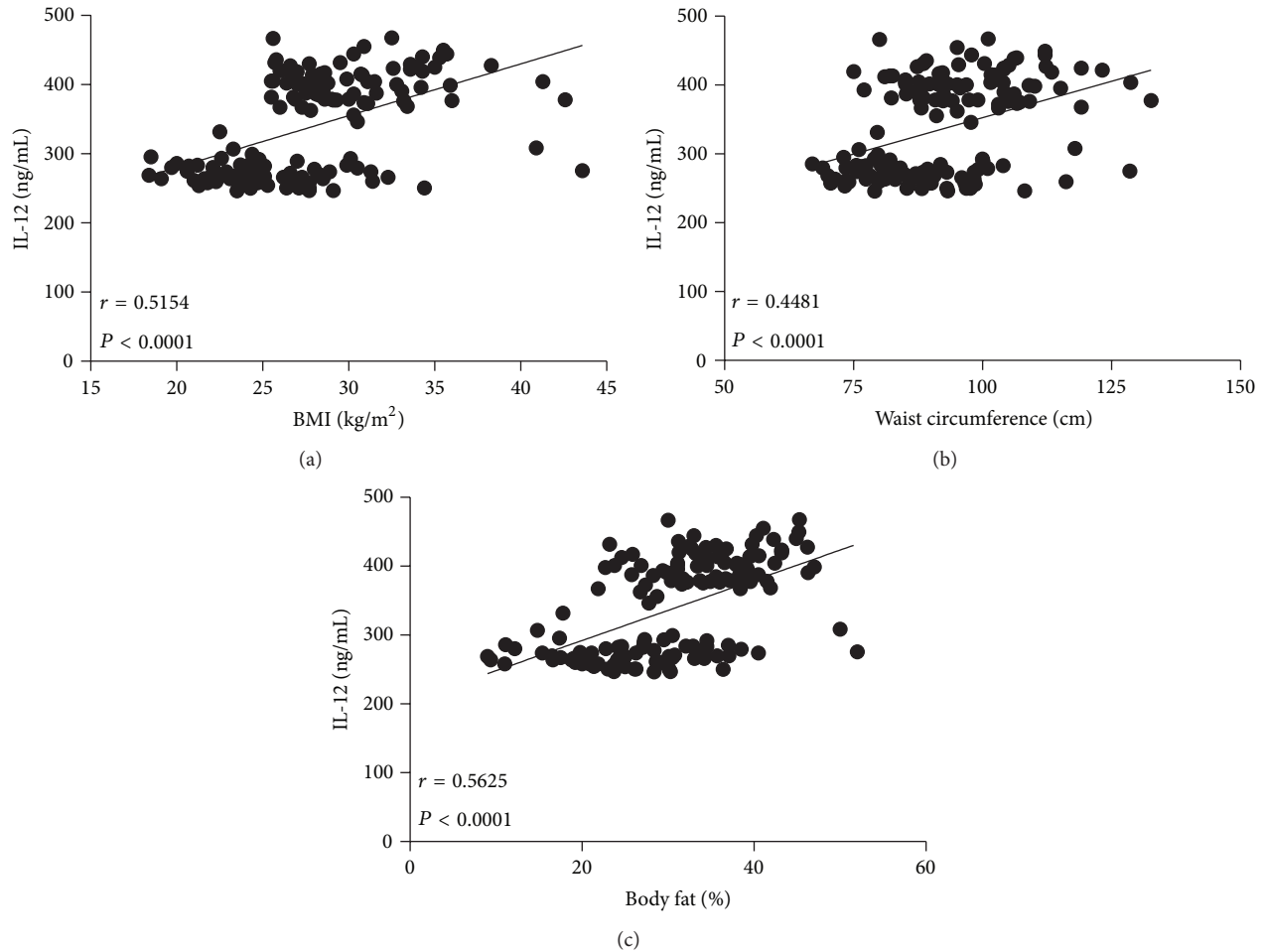


FIGURE 2: Relationship between serum levels of IL-12 and anthropometric parameters of obesity. Serum IL-12 values were positively associated with body mass index (BMI) (a), waist circumference (b), and body fat percentage (c). Coefficients (r) and P values were calculated by the Spearman's correlation model. The correlation level was considered significant when $P < 0.05$.

HOMA-IR, and leptin levels were significantly elevated in obese subjects as compared with normal weight individuals, but not with overweight subjects (Table 1). Furthermore, in our study population, BMI was strongly associated with waist perimeter ($r = 0.896$, $P < 0.0001$) and percentage of body fat ($r = 0.700$, $P < 0.0001$), while the latter ones also exhibited a positive significant relationship ($r = 0.613$, $P < 0.0001$).

In terms of BMI, serum levels of IL-12 exhibited a significant increase in overweight and obese subjects as compared to normal weight controls (Figure 1(a)). The mean value of IL-12 in overweight and obese individuals was 354.93 ± 68.39 and 382.04 ± 58.52 , respectively, whereas in the normal weight group, it was 273.94 ± 16.05 (data expressed as mean \pm standard deviation) (Figure 1(a)). Interestingly, serum IL-12 had a clear tendency to be significantly elevated in subjects exhibiting abdominal obesity, when compared with individuals showing a healthy waist circumference (Figure 1(b)). In this form, the mean value of IL-12 in subjects with abdominal obesity was 378.60 ± 66.86 , while it decreases to 301.62 ± 59.81 in individuals with normal waist perimeter (data expressed as mean \pm standard deviation) (Figure 1(b)).

It was clear that while BMI and waist circumference augmented, circulating levels of IL-12 also increased in the study population. This result was denoted by a strong positive correlation between serum IL-12 and BMI ($r = 0.5154$, $P < 0.0001$) (Figure 2(a)), as well as IL-12 and waist circumference ($r = 0.4481$, $P < 0.0001$) (Figure 2(b)). Serum values of IL-12 also exhibited a positive relationship concerning percentage of body fat ($r = 0.5625$, $P < 0.0001$) (Figure 2(c)). Furthermore, circulating concentrations of IL-12 showed a clear association with other obesity-related parameters, including high serum glucose ($r = 0.3158$, $P = 0.046$) (Figure 3(a)) and increased triglyceride levels ($r = 0.3714$, $P = 0.013$) (Figure 4(a)). In contrast, no significant associations were observed among serum levels of IL-12 and fasting blood insulin ($r = 0.1286$, $P = 0.208$), insulin resistance denoted as HOMA-IR ($r = 0.2472$, $P = 0.147$), and total circulating cholesterol ($r = 0.1843$, $P = 0.054$) (Figures 3(b), 3(c), and 4(b), resp.).

In a similar way that in the case of anthropometric and biochemical parameters of obesity, serum IL-12 showed a significant coefficient of association with circulating levels

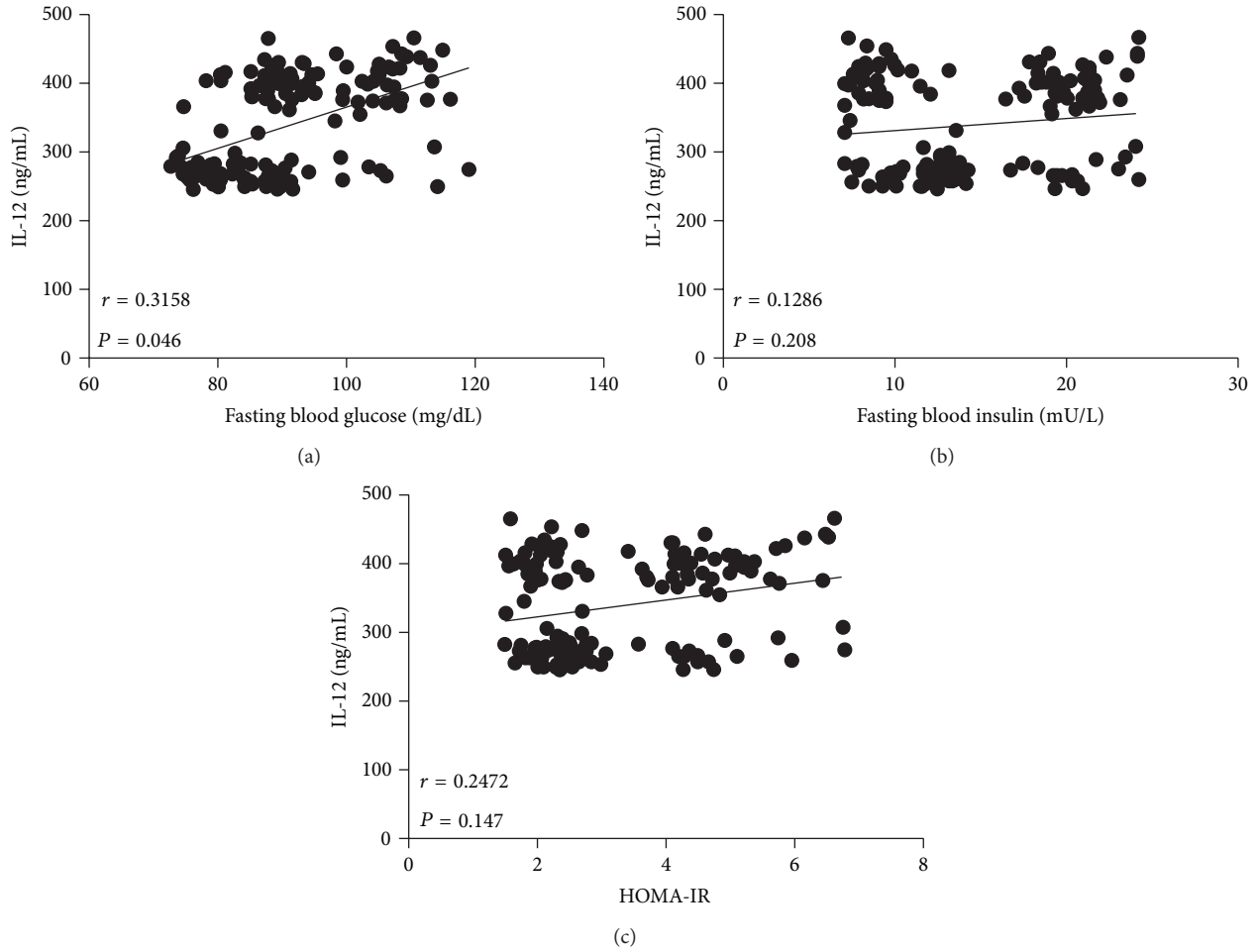


FIGURE 3: Relationship of the serum levels of IL-12 with fasting blood glucose, fasting blood insulin, and HOMA-IR. Serum IL-12 showed a positive significant association with high glucose levels (a), but not with insulin (b) or insulin resistance (c). The level of insulin resistance was estimated using the HOMA-IR index, which results from the next equation: fasting insulin concentration (mU/L) \times fasting glucose concentration (mmol/L) divided by 22.5. Coefficients (r) and P values were calculated by the Spearman's correlation model. The correlation level was considered significant when $P < 0.05$.

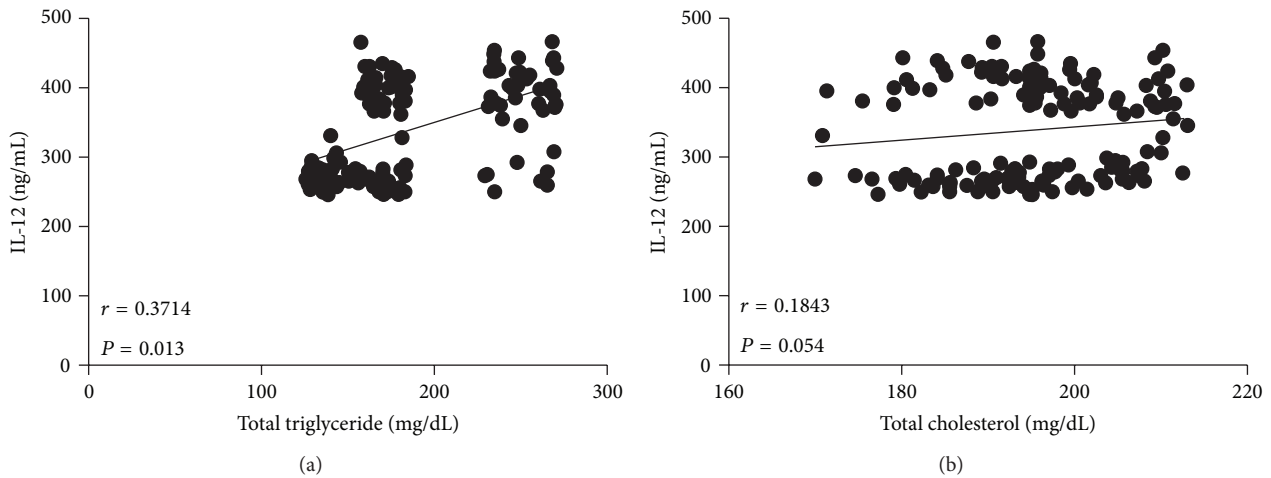


FIGURE 4: Relationship of the serum levels of IL-12 with total triglyceride and cholesterol levels. Serum IL-12 showed a positive significant association with high triglyceride levels (a), but not with total cholesterol (b). Coefficients (r) and P values were calculated by the Spearman's correlation model. The correlation level was considered significant when $P < 0.05$.

of TNF- α , a typical inflammatory marker ($r = 0.4717$, $P < 0.0001$) (Figure 5(a)). On the contrary, despite the fact that leptin significantly increased in obese individuals as compared with control and overweight subjects (Table 1), no significant relationship was observed between this proinflammatory adipokine and IL-12 ($r = -0.171$, $P = 0.264$) (Figure 5(b)).

4. Discussion

IL-12 is one of the most representative members of the Th1 cytokine family, with well-known functions in inducing production of IFN- γ and differentiation of type 1 T cells, promoting in this way a linkage between innate response and adaptive immunity [13]. Nevertheless, recent experimental evidence from high-fat-diet-fed mice suggests that IL-12 could have an additional role in the systemic low-grade inflammation and the concomitant advent of obesity-related disorders, such as insulin resistance [14, 15]. For this reason, it is of much relevance to study the systemic levels of IL-12 in humans that show high metabolic risk, such as obese individuals. In this sense, it has been previously reported that circulating concentrations of IL-12 are significantly increased in subjects with metabolic syndrome [16] and type 2 diabetic patients undergoing cardiovascular complications [17]. Furthermore, peripheral blood mononuclear cells (PBMCs) from T2D patients are able to produce higher levels of IL-12 in response to lipopolysaccharide stimulation than those cells from healthy subjects [18]. In a similar sense, increasing IL-12 secretion has been also observed in human macrophages treated with resistin, a proinflammatory adipokine clearly elevated in obese individuals and T2D patients [15]. Interestingly, our data demonstrate that circulating levels of IL-12 start to increase in the overweight and have a strong relationship with central obesity, one of the key factors to develop obesity-related disorders such as metabolic syndrome and type 2 diabetes [1, 3]. Concomitantly, a recent study in a rural population of Mexican women suggests that the risk of having elevated serum IL-12 diminishes in women with high plasma concentrations of zinc, a micronutrient that has been related with a reduced risk for being obese [19]. In this sense, our results reveal that circulating concentrations of IL-12 increase at the same time that with parameters of obesity, including body mass index, body fat accumulation, and high glucose and triglyceride levels.

An interesting finding in this work is the relationship between serum levels of IL-12 and TNF- α in overweight and obese individuals. As a classical Th1 cytokine, IL-12 has the ability to induce IFN- γ production in differentiated T cells [13]. Interestingly, IFN- γ has been shown to increase in obese mice and humans [20, 21]. Thus, in view of the fact that it has been previously demonstrated that IFN- γ is able to promote releasing of TNF- α [4], it is plausible to expect that increasing levels of IL-12 correlate with high TNF- α levels in obese subjects. Our results seem to be consistent with this hypothesis, since serum IL-12 shows a strong positive relationship with circulating levels of TNF- α in both overweight and obese individuals. In this low-grade inflammation milieu, TNF- α has been extensively

studied as a key inflammatory factor, capable to impair insulin signaling in murine adipocytes, through activation of negative regulators of the insulin receptor substrate 1- (IRS-1), including p38 mitogen-activated protein kinase and protein-tyrosine phosphatase 1B [22]. However, the serum levels of cytokines with the ability to induce production of TNF- α had not been precisely determined in obese humans to date. Therefore, as the low-grade inflammation seems to be determinant in the advent of obesity-related diseases such as type 2 diabetes and AVD [3, 4], further studies are needed in order to determine the role of IL-12 in the production of IFN- γ and TNF- α in obese subjects.

On the other side, unexpectedly, we did not observe a significant statistical correlation between serum IL-12 and leptin, despite the fact that leptin showed a clear increase in obese subjects. Nevertheless, it has been shown that leptin does not always correlate with other low-grade inflammation markers. For instance, a previous study conducted in Serb obese women reported a significant augmentation in the plasma levels of the proinflammatory cytokines IL-17 and IL-23, independently of the increase in leptin [23]. In a similar way, a Th1-immune profile characterized by an increase in the circulating proportion of IFN- γ -secreting PBMC has been described in Italian obese children, without a significant relationship with leptin levels [24]. Consistent with previous studies [19], present results suggest that leptin may not be significantly related with the systemic mild-grade inflammation milieu in this study population. However, additional research taking into consideration the influence of age-, sex-, and ethnicity-associated factors upon leptin levels is required in order to draw major conclusions. A similar result was observed in the absence of significant association of IL-12 with insulin, total cholesterol, and insulin resistance. Regarding this issue, it has been previously reported that the relationship of IL-12 with elevated insulin resistance and dyslipidemia increases in subjects with type 2 diabetes and AVD [17]. Since we have shown that IL-12 does not seem to have a significant relationship with insulin resistance and total cholesterol in healthy obese subjects, it is probable that IL-12 could be more related with the advent of obesity-related complications than with obesity itself. Nevertheless, further studies using a larger study population of healthy, prediabetic, and diabetic obese subjects are necessary for a more accurate determination of the relationship of IL-12 with insulin resistance and hypercholesterolemia in humans.

Finally, it is worth to mention that serum levels of IL-12 seem to be grouped in two clusters in our study population, characterized by high and low production of IL-12. Interestingly, there are no high producers of IL-12 among normal weight subjects. However, increased production of IL-12 seems to start since the overweight, reaching a plateau in the obesity group. It is important to underline that more than 70% of the high producers of IL-12 also showed increased levels of triglycerides (data not shown). Since there was no difference in the serum levels of IL-12 between women and men (data not shown), we hypothesize that high production of IL-12 may be a marker related to body weight gain and triglyceridemia. Nevertheless, taking into consideration that several factors are able to influence high and low production

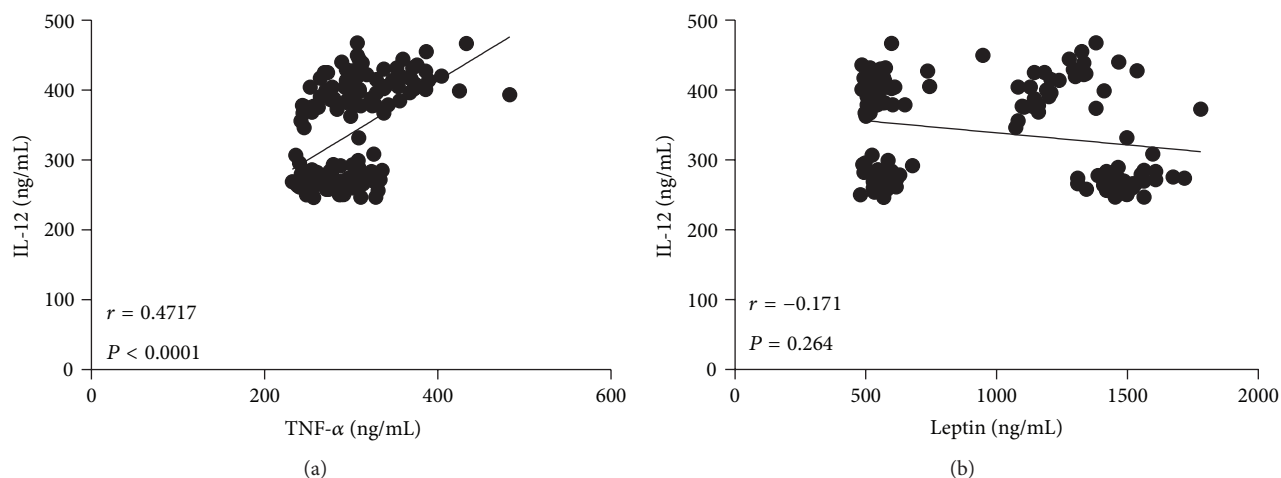


FIGURE 5: Relationship of the serum levels of IL-12 with systemic low-grade inflammation markers. Serum IL-12 showed a positive significant association with circulating levels of TNF- α (a), but not with leptin (b). Coefficients (r) and P values were calculated by the Spearman's correlation model. The correlation level was considered significant when $P < 0.05$.

of cytokines [25–27], further research is required to conclude whether body weight gain and triglyceridemia are determinant factors in the production of IL-12 in humans.

5. Conclusions

In conclusion, the present study demonstrates that serum levels of IL-12 are significantly higher in overweight and obese individuals than in normal weight subjects. In the Mexican adult population, serum levels of IL-12 show a strong relationship with systemic low-grade inflammation and obesity-related markers, including TNF- α , abdominal obesity, percentage of body fat, and high glucose and triglyceride levels. Additional research should be addressed in order to understand the role of Th1 cytokines in developing of insulin resistance, dyslipidemias, cardiovascular disease, and other obesity-associated alterations in humans.

Conflict of Interests

The authors declare that they do not have conflict of interests.

Acknowledgments

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Discusión general y conclusión

La IL-12 es una proteína proinflamatoria, considerada el miembro más representativo de la familia de las citocinas Th1. Una vez producida, la IL-12 induce la producción de IFN- γ al diferenciar los linfocitos T hacia Th1, promoviendo una asociación entre la respuesta inmune innata y la respuesta adaptativa (28). Sin embargo, existe evidencia reciente que sugiere un papel adicional de IL-12 en la inflamación de bajo grado a nivel sistémico y el concomitante advenimiento de desórdenes asociados a la obesidad, como el desarrollo de resistencia a la insulina en ratones sometidos a una dieta hipercalórica (29,30). Con base en lo anterior, es de gran relevancia estudiar los niveles de IL-12 a nivel sistémico en los humanos que muestran mayor predisposición a desarrollar desórdenes metabólicos, como en individuos que presentan obesidad. Existen reportes que demuestran un incremento significativo en los niveles de IL-12 en sujetos que presentan síndrome metabólico (31) y en pacientes con DMT2 que padecen complicaciones cardiovasculares (32). Además, los monocitos de pacientes con DMT2 son capaces de producir niveles elevados de IL-12 en respuesta a estímulos con lipopolisacáridos que aquellos monocitos provenientes de individuos sanos (27). De manera similar, se ha observado mayor secreción de IL-12 en macrófagos humanos tratados con resisitina, una adipocina proinflamatoria claramente elevada en individuos con obesidad y en pacientes con DMT2 (30).

En el presente estudio se muestra por primera vez que los niveles séricos de IL-12 empiezan a incrementar desde el sobrepeso y que esto tiene una asociación fuerte con la obesidad central, la cual es considerada un factor de riesgo para desarrollar desórdenes relacionados con la obesidad tales como el síndrome metabólico y la DMT2 (33,34); además muestra una correlación positiva con la presencia de monocitos-macrófagos M1. De igual manera los resultados obtenidos de un estudio realizado en mujeres mexicanas de poblaciones rurales sugieren que el riesgo de presentar niveles séricos incrementados de IL-12 disminuye en aquellas mujeres con concentraciones plasmáticas elevadas de zinc, un micronutriente que se ha relacionado con reducir el riesgo de desarrollar obesidad (35).

En este sentido, nuestro estudio revela que niveles séricos de IL-12 incrementan con otros parámetros asociados con la obesidad, como el IMC, la acumulación de grasa corporal, altos niveles de glucosa y triglicéridos. Por otro lado, este estudio muestra también una relación directa

entre los niveles séricos de IL-12 y TNF- α en individuos con sobrepeso y obesidad. La IL-12, al ser una citocina Th1, tiene la capacidad de inducir la producción de IFN- γ al activar las células T, diferenciándolas hacia Th1. De manera interesante, el IFN- γ se encuentra elevado en ratones obesos (36,37). Considerando que el IFN- γ promueve la liberación de TNF- α (38), es plausible esperar una correlación entre los niveles incrementados de IL-12 y un aumento en los niveles de TNF- α en individuos con obesidad. Nuestros resultados mostraron consistencia con la hipótesis estipulada en este artículo, ya que la IL-12 sérica muestra una relación positiva con los niveles circulantes de TNF- α en individuos con sobrepeso y obesidad.

En cuando al escenario de un estado inflamatorio de bajo grado, TNF- α ha sido extensivamente estudiada como un factor clave en la inflamación, capaz de inhibir la señalización de la insulina en los adipocitos murinos, a través de la activación de reguladores negativos del sustrato receptor a insulina-1 (IRS-1), incluyendo la proteína cinasa activada por el mitógeno p-38 y la proteína tirosina fosfatasa 1B (39). Sin embargo, los niveles séricos de las citocinas que inducen la producción de TNF- α no han sido determinados en sujetos con sobrepeso. Por lo tanto, a pesar de que el estado inflamatorio de bajo grado parece ser determinante en el desarrollo de patologías asociadas con la obesidad como DMT2 y la enfermedad vascular arteriosclerótica (34,38), se requieren estudios posteriores para determinar el papel de IL-12 en la producción de IFN- γ y TNF- α en individuos con obesidad.

Por otra parte, no se observó una correlación significativa ente IL-12 y la leptina. Si bien la leptina mostró un incremento notable en sujetos con obesidad, en individuos delgados o con sobrepeso no se observó esta elevación. En este sentido, diversos estudios sugieren que los niveles séricos de leptina no siempre correlacionan con marcadores asociados al estado inflamatorio de bajo grado. Un estudio realizado en mujeres con obesidad en Serbia reportó un incremento significativo en los niveles plasmáticos de las citocinas proinflamatorias IL-17 e IL-23, que ocurría independientemente de un incremento en la leptina (40). De manera similar, un estudio en niños italianos con obesidad describió que bajo un perfil inmune Th1, caracterizado por el aumento en los niveles circulantes de IFN- γ producido por monocitos, no existe una relación significativa con los niveles de leptina (41).

Considerando la evidencia mostrada, los resultados presentados en este artículo sugieren que la leptina no está relacionada con el microambiente presente en el estado inflamatorio de bajo grado. Sin embargo, será necesario realizar más estudios, considerando la influencia de la edad, el género y factores asociados a la etnicidad, para obtener conclusiones sólidas concernientes a los niveles circulantes de esta adipocina.

De igual manera, se observó que no existe asociación entre la IL-12 y la insulina, el colesterol total y el HOMA-IR. Previamente se reportó que la relación entre la IL-12 y el desarrollo de resistencia a la insulina y dislipidemia aumentaba en sujetos con DM2 y la enfermedad vascular arteriosclerótica (31). Al mostrar que IL-12 no tiene asociación con el desarrollo de resistencia a la insulina y el colesterol total en sujetos sanos con obesidad, se sugiere que IL-12 puede estar relacionada con el desarrollo de las complicaciones asociadas con la obesidad más que con la obesidad per se. Sin embargo, se requieren estudios posteriores usando una población más grande de sujetos con obesidad sin disfunción metabólica, prediabéticos y diabéticos para determinar la relación que existe entre IL-12 con la resistencia a la insulina y la hipercolesterolemia en humanos.

Cabe mencionar que los niveles séricos de IL-12 parecen estar agrupados en dos grupos en nuestra población de estudio, caracterizada por una alta y una baja producción de IL-12. Es muy interesante observar que los sujetos delgados no producen niveles elevados de IL-12, mientras los sujetos con sobrepeso empiezan a mostrar indicios de un incremento en los niveles de esta citocina, los cuales aumentan dramáticamente en la obesidad. Es importante mencionar que más del 70% de los sujetos con niveles de IL-12 elevados, mostraron niveles incrementados de triglicéridos. Al analizar que no existía diferencia en los niveles séricos de IL-12 entre hombres y mujeres, se hipotetizó que una producción elevada de IL-12 puede ser un buen marcador relacionado con la ganancia de peso y la trigliceridemia. Sin embargo, es importante considerar que existen diversos factores que pueden influir en la alta o baja producción de citocinas (42-44), por lo que se sugiere llevar a cabo más estudios para concluir si la ganancia de peso y la trigliceridemia son factores determinantes en la producción de IL-12 en humanos.

En conclusión, el estudio presentado demuestra que los niveles séricos de IL-12 son significativamente mayores en individuos con sobrepeso y obesidad, con respecto a sujetos de peso normal. En la población mexicana, los niveles de IL-12 muestran una relación significativa con la presencia del estado inflamatorio de bajo grado y con marcadores asociados a la obesidad, incluyendo TNF- α , la población circulante de monocitos-macrófagos proinflamatorios, la obesidad abdominal, el porcentaje de grasa total y los niveles elevados de glucosa y triglicéridos. Es necesario estudiar con mayor profundidad el papel de las citocinas Th1, como la IL-12, en el desarrollo de resistencia a la insulina, dislipidemias, enfermedades cardiovasculares y otras alteraciones asociadas con la obesidad en humanos.

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