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ORIGINAL ARTICLE

Gender differences in lipocalin 2 plasmatic levels are correlated with age and the triglyceride/high-density lipoprotein ratio in healthy individuals

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Abstract

Introduction: *The increment of lipocalin 2, also called neutrophil gelatinase-associated lipocalin, plasmatic levels is associated with cardiometabolic and nefrologic alterations. Nonetheless, there is much controversy about lipocalin 2 plasmatic concentrations among healthy individuals.* **Aim:** *The aim of this study was to quantify lipocalin 2 in plasma of healthy men and women and to assess a possible correlation with cardiometabolic risk factors.* **Methods:** *Fifty-three subjects (24 men and 29 women) were included. By means of an ELISA, a higher concentration of lipocalin 2 was observed in men than in women (91 ± 9 vs. 57 ± 7 ng/ml). Such difference was statistically significant ($p < 0.0001$).* **Results:** *Lipocalin 2 levels were significantly correlated with body mass index, homeostasis model assessment index-insulin resistance index, triglycerides, high-density lipoprotein, and age.* **Conclusion:** *Lipocalin 2 plasmatic concentrations present a gender-specific profile in healthy subjects and its circulating levels appear to be age-dependent and associated with several cardiometabolic risk factors, including the triglycerides/high-density lipoprotein cholesterol ratio, which has proven to be a reliable marker for cardiometabolic risk among the global population.* (Gac Med Mex. 2016;152:549-53)

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Introduction

Currently, it is known that body fat distribution presents at puberty, a sexual dimorphism caused by sex hormones action exerted on adipose tissue. This sexual dimorphism intervenes significantly in the distinct prevalence of cardiometabolic diseases between men and women[1]. In this regard, it is noteworthy that globally there is a greater risk of developing diseases associated with obesity in males than in females[2]. Studies that have compared the metabolic profile between men and women suggest that both insulin sensitivity and glucose uptake are higher in women than in men, even though women generally have a higher proportion of fat in their total mass and a higher percentage of lipid deposits[3]. This advantageous metabolic profile is due in part to the role estrogens play in fat metabolism.

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Estrogens protect against weight gain by increasing energy expenditure as well as fat oxidation. Moreover, estradiol suppresses food intake by stimulating the production of anorectic proteins, such as cholecystokinin, apolipoprotein A-IV, leptin, and brain-derived neurotrophic factor, and also by inhibiting orexigenic signals from melanin-concentrating hormone and ghrelin. In addition, estrogens promote lipolysis through the increment of sympathetic nerve activity in adipose tissue[1].

Even though the mechanisms that explain the link between obesity and cardiometabolic disorders have not been completely elucidated, the results reported by different research groups indicate that systemic inflammation may be an important mediator in the development of this physiopathological process[4,5], which is characterized by the infiltration of macrophages in adipose tissue and by altering the balance between pro-inflammatory and anti-inflammatory proteins secreted by hyperplastic adipocytes[6]. Similarly to what occurs with body fat distribution, a sexually dimorphic pattern in plasma concentrations of various adipokines has also been reported. It is known that from the beginning of puberty, there are sex differences in plasma levels of both leptin and adiponectin, which are higher in women than in men[7]. Also, gender differences in the circulating levels of ghrelin have been documented, being also higher in women than in men[8].

Among the cytokines of recent investigation is lipocalin 2, also called neutrophil gelatinase-associated lipocalin (NGAL)[9], this protein was initially identified in human neutrophils[10] by its covalent binding to the matrix metalloproteinase 9 (MMP9) present therein, whose discharge is activated when an infectious or inflammatory process occurs[11]. Likewise, an increment in lipocalin 2 protein levels in both urine and plasma of patients with acute kidney injury has been identified[12]. However, studies reporting potential sex-related differences in circulating levels of this adipokine in healthy subjects are scarce and contradictory. Moreover, the reference values for lipocalin 2 plasma levels in healthy individuals with respect to gender and age have not been established. We recently reported a statistically significant decrease in plasma levels of lipocalin 2 in Mexican patients with type 2 diabetes mellitus in comparison with the levels of lipocalin 2 observed in control subjects[13]. Here we describe that in the latter, the plasma levels of lipocalin 2 present a gender-specific profile, with the levels of lipocalin 2 being statistically higher in men than in women and they also present a significant correlation with sex, age, body mass index (BMI) and the triglycerides/high-density lipoprotein (HDL) ratio.

Methods

Study design and patients

A non-randomized cross-sectional assay was conducted on 53 healthy individuals (24 men and 29 women). Written informed consent was obtained from all subjects before screening was undertaken. Exclusion criteria included: acute or chronic infectious diseases as well as any other disease. The present study was authorized by the Bioethics Committee of the Instituto Mexicano del Seguro Social in accordance with the ethical guidelines comprised in the 1975 Declaration of Helsinki (1964).

All biochemical and anthropometric parameters were registered according to the methodology described previously[14]; measurements of weight and height from all subjects were obtained using a fixed scale with stadimeter (Tanita® TBF-215, Tokyo, Japan). Increments of measurements for weight and height were 0.1 kg and 0.01 m, respectively. Body mass index was calculated as weight (kg) divided by height (m) squared. Obesity was defined according to a BMI of 30 kg/m^2 or more, and overweight according to a BMI of 25 kg/m^2 or more, but less than 30 kg/m^2. Blood pressure measurements were taken in a supine position after five minutes of rest, following guidelines recommended in the Sixth Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure[15].

Assays

In order to obtain plasma concentrations of total cholesterol, low- and high-density lipoproteins (LDL and HDL, respectively), triglycerides, as well as the corresponding fasting plasma glucose, a venous blood sample was drawn from each subject after an overnight fasting of 8-10 hours; plasma was obtained after centrifugation at 857 xg for 10 minutes at 10 °C (Beckman Gs-15R, GMI, Inc., Ramsey, Min., USA) and immediately stored at –70 °C for subsequent assays. Plasma glucose was quantified employing the glucose-oxidase method. Triglycerides, total cholesterol, LDL, HDL, and glycated hemoglobin (HbA1c) were assayed using a Technicon® RA1000 analyzer (Bayer Diagnostics, Puteaux, France). Insulin was centrally assayed on serum by specific radioimmunoassay employing I^{125} (Linco Research Inc., St Charles, MO, USA). Intra- and inter-assay coefficients of variation for all measurements were < 7%. Insulin resistance (IR) was estimated using the homeostasis model assessment index-insulin resistance

Table 1. Anthropometric and biochemical characteristics of male and female subjects

Variables	Total (n = 53)	Male	Female
Sex (Male/Female)	(24/29)	24	29
Age (years)	43 ± 10	37.5 ± 11	44.5 ± 10
BMI (kg/m^2)	23.4 ± 1.0	24 ± 2	23 ± 2
Total cholesterol (mg/dl)	165.0 ± 2.6	167 ± 4	163 ± 4
LDL (mg/dl)	95.4 ± 2.7	98 ± 4	93 ± 3
HDL (mg/dl)	50.0 ± 1.2	48 ± 2	52 ± 2
Triglycerides (mg/dl)	107.7 ± 5.4	120 ± 7	96 ± 8
TG/HDL ratio	2.24 ± 0.8	2.6 ± 0.7	1.9 ± 0.8
Fasting glucose (mg/dl)	88.9 ± 1.5	90 ± 3	88 ± 2
Fasting insulin (µU/ml)	10.6 ± 0.5	11 ± 1	10 ± 1
HOMA-IR	2.3 ± 0.1	2.5 ± 0.5	2.2 ± 0.5
HbA1c (%)	5.4 ± 0.6	5.2 ± 0.5	5.2 ± 0.5
SBP (mmHg)	111 ± 9.3	119 ± 8	112 ± 5
DBP (mmHg)	72 ± 7	75 ± 8	70 ± 5

BMI: body mass index; DBP: diastolic blood pressure; HbA1c: glycated hemoglobin; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment index of insulin resistance; LDL: low-density lipoprotein; SBP: systolic blood pressure; TG: triglycerides.

(HOMA-IR). HOMA-IR is defined as fasting glucose (mg/dl) multiplied by fasting insulin (µU/ml), divided by 405. Dyslipidemia is defined as any of the following: total cholesterol > 200 mg/dl, HDL < 40 mg/dl, LDL > 130 mg/dl, or triglycerides > 150 mg/dl.

Lipocalin-2 circulating levels were detected with a neutrophil gelatinase-associated lipocalin (NGAL) ELISA kit (BioPorto Diagnostics, Denmark); absorbance was quantified in a multi-detector (Victor 3 1420, Perkin Elmer, Turku, Finland). ELISA experiments were determined in duplicate as recommended by the manufacturer. Intra– and inter-assay coefficients of variation for all measurements were < 10%. Also, a standard curve was included within each assay.

Statistical analysis

Data are presented as mean value of each group and the respective standard error (SE); for categorical variables, number and percentage were determined. In order to analyze both anthropometric and biochemical differences between male and female groups, we employed a Mann-Whitney test for independent samples. Also, to assess a possible correlation among the quantitative variables, a Pearson correlation test was performed. A p value ≤ 0.05 was considered as statistically significant. All statistical analyzes were performed with the Graph Pad Prism version 5.1.

Results

A non-randomized cross-sectional assay was performed on 53 middle aged healthy individuals (24 men and 29 women). The average age in the female group was higher than the one reported for males (44.5 ± 10.0 vs. 37.5 ± 11.0 years). Similarly, plasma HDL levels were higher in women than in men (52 ± 2 vs. 48 ± 2 mg/dl). In contrast, the average value of the remaining parameters was higher in men than in women and only the average value of glycated hemoglobin was the same for both sexes: 5.2 ± 0.5% (Table 1). Lipocalin 2 plasma concentration was assessed in both groups. To our surprise, the average value of the corresponding plasma levels of lipocalin 2 was significantly higher in men than the one obtained for women (91 ± 9 vs. 57 ± 7 ng/ml, respectively). This gender difference was statistically significant (p < 0.0001) (Fig. 1).

In order to determine a possible association between lipocalin 2 circulating levels and any of the anthropometric or biochemical parameters analyzed, a Pearson

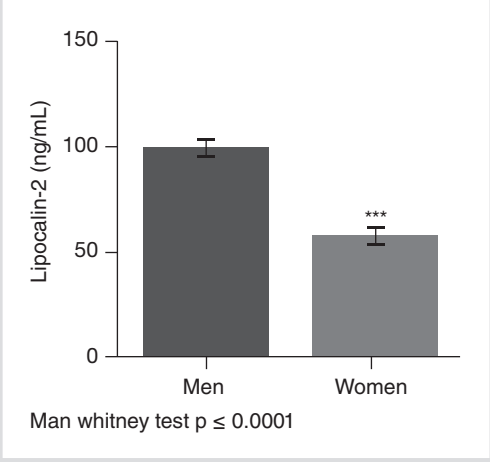

Figura 1. *Lipocalin 2 concentration in plasma of healthy men and women.*

correlation test was performed, which indicated that the levels of lipocalin 2 have a tendency to directly correlate with age, BMI, HOMA-IR, and triglyceride levels, and to correlate inversely with HDL levels ($r = –0.40$; $p \leq 0.03$) (Table 2).

Discussion

Currently, it is well established that the regulation of various metabolic components is gender-specific. In this context, several studies have shown sex differences in the levels of different adipocytokines, but studies suggesting sex differences in plasma lipocalin 2 levels are scarce and contradictory[13]. Thrailkill, et al. (2010)[16] reported a higher concentration of lipocalin 2 levels in the urine of both healthy and type 1 diabetic women, compared to the values observed in healthy and diabetic male patients (9,364 vs. 1,254 ng/g, respectively). Likewise, the study conducted by Luque-Ramirez, et al. (2013)[7] reported a higher average value of lipocalin 2 in plasma of healthy women with respect to the one observed in male subjects (3.3 ± 2.0 vs. 2.7 ± 0.7 nmol/l), but on the other hand, higher levels of this adipokine were observed in obese men than the ones reported for obese women (4.4 ± 2.3 vs. 3.8 ± 2.3 nmol/l). On the contrary, and in agreement with our results, the study conducted by Wang, et al. (2007)[17] reported a higher average value of lipocalin 2 plasma levels in thin men than the one presented in thin women (72.1 vs. 57.6 mg/l, respectively). This was also observed when comparing lipocalin 2 levels between men and women with obesity (117.7 vs. 92.9 mg/l). In this respect it is noteworthy that unlike the aforementioned studies where no correlation between lipocalin 2 and age is described, our results show a statistically significant correlation between the levels of lipocalin 2 and this parameter. Moreover, studies in which higher plasma concentrations of lipocalin 2 were reported in females compared with the concentration observed in males, included subjects with an age range of 20-35 years. Conversely, both the results described by Wang, et al. (2007)[17] and ours indicated a higher plasma concentration of lipocalin 2 in men than in women, whose age range was 37.5 to 70.0 years. This suggests that age somehow interferes in plasma levels of lipocalin 2 in both men and women. A study on the gender difference in circulating levels of ghrelin conducted on an Arab population reported an association between circulating levels of ghrelin and the age of the subjects included in the study. It is worth mentioning that even when plasma ghrelin levels were higher in women than in men, women older

Table 2. Pearson correlation coefficient between lipocalin 2 and metabolic parameters

Variable	Lipocalin 2 plasma levels		
	Pearson r	p	
Age	0.3862	0.0056	<– 0.001
BMI	0.4020	0.02	<– 0.05
HOMA-IR	0.4780	0.001	<– 0.001
HDL	–0.4034	0.030	<– 0.05
TG	0.4811	0.01	<– 0.001

BMI: body mass index; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment index of insulin resistance; TG: triglycerides.

than 50 years showed a significant decrease in ghrelin levels in comparison with the ones observed in younger women[18]. Guo, et al. (2012)[19] demonstrated that in female mice deficient for lipocalin 2, serum estradiol levels as well as the expression and activity of estrogen receptor alpha were drastically reduced in various metabolic tissues. Also, lipocalin 2 (–/–) mice presented a decreased expression of the aromatase enzyme in the adipose tissues analyzed. Moreover, the reduction of these three parameters in lipocalin 2 knockout mice was more significant with the provision of a high-fat diet and with increasing age. Furthermore, the authors reported that lipocalin 2 (–/–) mice also showed a decreased expression of various transcription factors (PPARγ, LXRβ, and LDL-R) involved in lipid metabolism. This suggests that deficiency of lipocalin 2 promotes systemic alteration as well as a deregulation of lipid metabolism through a sex-specific estrogen-mediated action on adipose tissue, and that such systemic alteration and deregulation of lipid metabolism may increase with age. It has been reported that both the human and murine lipocalin 2 gene (*LCN2* and *Lcn2*, respectively) contain estrogen recognition sites within their promoter regions, which suggests that transcription of both genes could be regulated by either estrogen or its receptors[20]. According to the above, low circulating levels of this adipocytokine in the female group in our study may be attributed to reduced estrogen levels present in them, since most of the women involved in our study are middle-aged women at pre- or peri-menopausal stage. However, we believe that to elucidate the above, an assessment of lipocalin 2 plasma levels through the different decades that comprise the female reproductive life and its correlation with the corresponding estrogen levels would be useful. Despite the latter, the fact that the triglyceride/HDL ratio was higher in men than in women indicates that regardless of a lower lipocalin 2 concentration, estrogen still possesses a cardiometabolic protective action.

Taking this into account, we consider that further studies are needed in order to assess an association of the circulating levels of this adipocytokine with the ones of triglycerides and HDL specifically, stratifying age and BMI, in order to obtain a reference value for lipocalin 2 and for its association with the triglyceride/HDL ratio reliable enough to be used as a biomarker to determine the feasibility of developing cardiometabolic disorders. This is of considerable importance because recently it has been demonstrated that lipocalin 2 is implicated in vascular fibrosis by playing a key role in the aldosterone/mineralocorticoid receptor pathway[21].

Declaration of interest

The present study did not receive financial support. The authors report no conflict of interests.

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