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Relation of leptin in plasma with oxidative damage in indigenous tepehuán and mestizo populations from Durango

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Background: *Obesity is a multifactorial metabolic disorder that involves lipid peroxidation (LPX), activating the antioxidant systems to counteract cellular damage. Objective: To evaluate the correlation between the antioxidant capacity and LPX levels of leptin, in indigenous Tepehuán and Mestizo populations of the State of Durango.* **Methods:** *We conducted a nutritional clinical study and lipid profile to confirm the state of health of a group of 60 indigenous Tepehuán of Mezquital and 68 mestizos subjects of Durango city, aged between 18 to 59 years. We determined the concentrations of leptin, antioxidant capacity and LPX in fasting conditions on plasma of participants, comparing averages, minimum, maximum, and standard deviation through ANOVA and Kruskal-Wallis. For the correlation of variables, Pearson test was applied, getting the r value.* **Results:** *Leptin levels were lower in indigenous Tepehuán than mestizos independent of body mass index. Mestizo subjects and Tepehuán with overweight and obesity (OW/O) or both ethnic groups show a greater degree of LPX (3.39 ± 0.31, 2.72 ± 0.54 MDA µmol/l, respectively; p < 0.05); however, OW/O mestizos show more activation of its (0.37 ± 0.03 meq/trolox) than Tepehuán normal weight (NW) and OW/O (0.32 ± 0.01 meq/trolox). The correlation between antioxidant capacity and LPX in mixed OW/O was positive (r = 0.9; p < 0.001). There is a correlation between levels of leptin and the antioxidant capacity of Tepehuán subjects both NW and OW/O (r = 0.40; p < 0.05 and r = –0.66; p < 0.0001, respectively).* **Conclusion:** *Tepehuan groups with OW/O have less oxidative damage, while antioxidant mechanisms have a smaller activation than the top crosses of the same nutritional condition. The results suggest that antioxidant capacity has an implication on the regulation of leptin levels in Tepehuán subjects.* (Gac Med Mex. 2015;151:202-9)

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Introduction

Obesity is a complex, chronic metabolic condition that in Mexico, as in many other countries, has alarmingly increased. About 36 million of women and men older than 20 years are estimated to have overweight (OW)/obesity (O) in poor countries[1]. The degree of obesity is defined by the body mass index (BMI): it is severe if BMI ranges from 35 and 39.9 kg/m^2 and morbid if it is equal or higher than 40 kg/m^2. Obese patients are at risk of developing numerous complications including arterial hypertension, coronary heart disease, diabetes, sleep apnea and arthrosis[2]. There are three mechanisms that regulate body weight at the nervous central system level: first, a messenger protein

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(leptin) that indicates to the brain the amount of peripheral body fat; leptin is a protein produced in the adipose tissue that participates in food intake and energy expenditure regulation, which is why it is altered both in undernourished and obese subjects[3]. The second mechanism is crosstalk inside the brain and, finally, an executing system that includes, in addition to leptin, other adipocyte-secreted cytokines: adiponectin, resistin, interleukins 1 (IL-1) and 6 (IL-6), plasminogen activation inhibitor 1 (PAI-1)[4], angiotensinogen, adipsin, acylation stimulating protein (ASP) and visfatin. On the other hand, in the blood stream, leptin circulates bound to proteins that regulate its bioavailability, activity and degradation[5]. The actions of leptin in the hypothalamus are mediated by the expression and/or activity of a series of orexigenic and anorexigenic peptides[6].

Leptin plasma levels in persons with normal weight (NW) range from 1 to 15 ng/ml; conversely, levels of 30 ng/ml or even higher can be found in individuals with BMI higher than 30[7-9]. Plasma leptin is positively correlated with BMI and with total fat percentage in humans and animals. There is large variation in the leptin concentration among the general population, suggesting a multifactorial modulation of its secretion. Other factor that determines leptin values is sex: women show higher values than men, even after adjusting the values according to the BMI. Leptin increases the synthesis of IL-6 and tumor necrosis factor α in macrophages in addition to activating them[10]. Adipokines, including adiponectin, leptin, resistin and ghrelin, are adipocyte-produced circulating molecules that affect the use and expenditure of energy, with the resulting production of free radicals[11], involving the lipid peroxidation process, which is the conversion by oxidation of polyunsaturated fatty acids into the products known as MDA, a metabolite commonly used to assess oxidative damage[12]. The increase in reactive oxygen species (ROS), including MDA and conjugated dienes, is evident in obese individuals' adipose tissue[13]. Free radicals and other reactive species are produced in the body mainly as the result of oxygen consumption. Antioxidants (glutathione, vitamins A, E and C, selenium, zinc, etc.) and antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) exert synergistic effects on free radicals. In physiological conditions, antioxidants are produced in excess or at least in balance with ROS and free radicals[14]. Pathological excess of antioxidants is known as oxidative stress. These conditions are caused by a higher production of ROS or deficient antioxidants[14]. There is evidence indicating that oxidative stress plays a central role in the pathophysiology of obesity and its systemic effects on health, including metabolic syndrome[15], insulin resistance, type 2 diabetes, non-alcoholic fatty liver and atherosclerosis[16,17]. The relationship between leptin and variables that intervene in glucose homeostasis and obesity shows ethnic differences. A recent study that compared indigenous population from Peru with Caucasian population showed that indigenous populations had higher levels of insulin than Caucasians and lower levels of leptin[18]. Similarly, Chilean Indians also had higher levels of insulin and lower levels of leptin than Caucasians[19]. On the other hand, Mexican-American subjects showed higher levels of leptin when compared by sex and age with Anglosaxon-origin, non-Hispanic subjects[20]. These results observed in different ethnic groups suggest that susceptibility for the development of diseases related to the production of ROS is associated with the levels of leptin[21]. The study of Mexican populations or ethnic groups, such as the Tepehuan population, may possibly help to understand the likely mitochondrial mechanisms involved in the development of obesity[22] in the Mexican population. The purpose of the present work was to assess the degree of relationship between leptin plasma concentration and oxidative stress markers (antioxidant capacity [CA] and lipid peroxidation [LPX]) according to BMI in two Mexican populations, namely, Mestizo and Tepehuan indigenous populations.

Material and methods

The study was conducted following the principles of the Declaration of Helsinki by personnel trained on the Good Clinical Practice (GCP) and the International Harmonization Conference standards, according to the Mexican Official Standard (NOM – *Norma Oficial Mexicana)* regulations on research[23]. The protocol and the informed consent form were approved by the Ethics Committee of the Hospital General de Durango.

Type of study

Descriptive, comparative, cross-sectional, relationship-finding.

Study subjects

Population of subjects with NW, OW/O, clinically stable and without associated diseases, Mestizo subjects of the city of Durango and Tepehuan indigenous

individuals from the Mezquital municipality, in the State of Durango.

Clinical and nutritional status determination

Clinical and nutritional status was determined in order to classify the participants as subjects with NW or OW/O grade I[2], according to their BMI (weight/height^2)[24]; NW corresponds to a BMI ranging from 19 to 24.9 kg/m^2, whereas a BMI > 25 kg/m^2 is considered to be OW/O. Medical record was elaborated by means of history and physical examination, as well as with anthropometric data. Blood pressure was assessed with an aneroid sphygmomanometer adequately calibrated, after the patient was at rest for 10 min to minimize record errors. Arterial blood pressure was measured after 10 min rest in the supine position. Pulse pressure (PP) was obtained by substracting diastolic blood pressure (DBP) from sistolic blood pressure (SBP). Mean blood pressure (MBP) was calculated as the sum of SBP and PP, divided by 3. All data were recorded in an assessment and clinical history form.

Blood samples

Blood samples were obtained from the antecubital vein with the patient sitting in a straight position, in 8-h fasting conditions, between 07:00 and 09:00 h in the morning. Blood was collected in heparinized polypropylene tubes (two drops of heparin for 10 ml of blood), mixed by inversion and centrifuged at 3,000 rpm at 0 °C for 10 min. The resulting plasma was separated in two similar aliquots and stored frozen at –70 °C until its analysis.

Leptin quantification

Serum leptin was determined in duplicate using an immunoenzymatic spectrophotometric assay using a human leptin commercial kit (ELISA) (Millipore). The leptin assay had an intra-assay variability with a variation coefficient of < 4%.

Total antioxidant capacity in plasma

Total AC was determined using a colorimetric analysis with an antioxidant analysis kit (Cayman Chemical Company, USA). The assay was based on the capacity of antioxidant systems present in the patient's plasma to inhibit 2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) oxidation. The amount of produced $ABTS^+$ can be measured by reading the absorbance at 405-450 nm. Absorbance is inversely proportional to the concentration of antioxidants present in the subject's plasma. The capacity of blood's antioxidants to prevent $ABTS^*$ oxidation is compared with trolox (a water-soluble tocopherol analogue). The results were quantified as trolox meq activity per milliliter of plasma (meq/ml); usually, human plasma has an AC of 0.5-2.0 nM[25,26].

LPX measurements

Lipid peroxidation, or LPX, determination was based on the protocol specified by the TBARS assay kit (Cayman Chemical Company, USA). This method quantifies thiobarbituric acid reactive substances (TBARS), which are produced by lipid peroxidation and include MDA, which is the most abundant, stable and easily characterized. Maximuml absorbance of these species was at 532 nm and it was determined with a spectrophotometer model UV/VIS OD 650 (Beckman). The results were expressed in equivalent MDA µmols per plasma milliliter. Normal human plasma has typically a lipid peroxidation expressed in MDA of 1.86-3.94 (MDA µmol/l)[27].

Statistical analysis

Data of each parameter (age, blood pressure, BMI, leptin, MDA and meq/trolox) were organized in an Excel spreadsheet and then exported to the Graphic Instat platform version 7[28] of the software to obtain central tendency (means) and measures of dispersion (standard deviation). Then, data were examined with the Shapiro Wilk test of normality to define each variable's normality. Subsequently, data corresponding to the four subgroups were compared using a parametric (Student's t-test) or non-parametric test (Mann-Whitney) when the distribution was considered normal or not normal, respectively. Finally, distribution, mean, standard deviation and minimum and maximum and values for each parameter in both Tepehuan and Mestizo groups were compared according to nutritional status using a parametric (one-way ANOVA or Kruskal-Wallis) or non-parametric (Tukey-Kramer) test. On the other hand, the Pearson analysis was used to assess the correlation between leptin levels and BMI and biochemical parameters in both the Tepehuan and Mestizo groups acoording to nutritional status. P-values ≤ 0.05 were considered to be statistically significant. A Pearson correlation analysis was performed

to obtain the value of R. Differences with a p-value < 0.05 were considered statistically significant.

Results

Total study population was comprised by 128 individuals: 62 subjects with NW (32 Mestizos wlth BMI ranging from 19.3 to 24.8 kg/m^2 and 66 Tepehuan subjects with BMI ranging from 19.48 to 24.8 kg/m^2) and 66 subjects with OW/O (28 Mestizos with BMI ranging from 25.03 to 36.08 kg/m^2 and 38 Tepehuan subjects with BMI ranging from 24.9 to 31.1 kg/m^2) (Table 1).

When the plasma concentration values were compared between ethnic groups (ng/ml), statistically significant differences were found: values were lower in the Tepehuan subjects than in the Mestizo population (p < 0.02) (Fig. 1). In the Mestizo population, plasma leptin values were significantly higher in OW/O individuals (median: 20.45 ng/ml; range: 1.4-61.6 ng/ml) when compared with NW subjects (median: 7.66; range: 1.04-41.6 ng/ml; p = 0.0025; 95% confidence interval [CI]: 4.1-18.3). Similarly, plasma leptin concentrations were significantly higher in the indigenous Tepehuan population with OW/O (median: 6.5 ng/ml; range: 0.18-37.1 ng/ml) then in the subjects with NW (median: 2.9 ng/ml; range: 0.2-17.6 ng/ml; p = 0.03; 95% CI: 0.3-6.8). However, regardless of the BMI, leptin levels were lower in indigenous Tepehuan than in Mestizo subjects.

AC values in the studied groups showed a statistically significant difference (p = 0.003). Tepehuan subjects with OW/O showed lower AC (0.32 ± 0.08 meq/trolox) than Mestizos (0.37 ± 0.03 meq/trolox). However, among subjects with NW, there were no ethnicity-related differences (Fig. 2).

When the values obtained for oxidative damage on lipids were analyzed, a statistically significant difference was found between groups (p < 0.001). Overall, subjects with OW/O showed higher degree of LPX (Mestizos: 3.39 ± 0.39 µmol/l; Tepehuan subjects: 2.72 ± 2.8 µmol/l), whereas in subjects with NW there was no statistically significant difference between etnic groups (Mestizos: 0.98 ± 0.4 µmol/l; Tepehuan subjects: 1.096 ± 1.5 µmol/l) (Fig. 2).

When the possible relationship between leptin levels and AC was assessed, a significant positive correlation was found in Tepehuan subjects with NW (R = 0.40; p = 0.04), whereas in Tepehuan subjects with OW/O the correlation was negative (R = -0.66; p = 0.0001) (Fig. 3). There was no correlation between leptin levels and LPX

Table 1. ANOVA to compare median, minimum and maximum values of demographic variables and biochemical parameters from the Tepehuan and mestizo subjects*

Variable	Normal values	NW (n = 62)		p-value	OW/O (n = 66)		p-value
		Tepehuan	Mestizo		Tepehuan	Mestizo	
N (males)		32	30		28	38	
Age (years)	18-48	32.0 (18-57)	21 (18-59)	> 0.05	40 (21-57)	25 (18-58)	> 0.05
BMI /kg/m2)	19-24.9 (NW); 25-29.9/ ≥ 30 (OW/O)	22.5 (19.48-24.8)	22.07 (19.3-24.8)	> 0.05	27.7 (24.9-31.1)	28.6 (25.03-36.08)	*< 0.05
Weight (kg)		59 (41.7-70)	61.3 (51.3-83)	> 0.05	76.5 (64.4-90)	85.7 (60.3-124)	***< 0.001
Height (m)		1.64 (1.49-1.69)	1.7 (1.5-1.8)	> 0.05	1.66 (1.5-1.7)	1.7 (1.5-1.95)	> 0.05
Mean blood pressure (mmHg)	70-110	81.66 (70-98)	90 (70-100)	> 0.05	83.3 (70-100)	90 (70-100)	> 0.05
Cholesterol (mg/dl)	< 200	146 (107-188)	180 (121-223)	> 0.05	155 (96-232)	165 (70-215)	> 0.05
HDL (mg/dl)	40-60	39 (25-74)	34 (11-57)	> 0.05	39 (25-74)	36 (6-71)	> 0.05
LDL (mg/dl)	70-130	83.5 (44-123)	107 (57-141)	> 0.05	89 (37-139)	97 (44-140)	> 0.05
VLDL (mg/dl)	2-30	20.5 (9-49)	37 (11-56)	> 0.05	38.5 (12-97)	19.5 (8-93)	*< 0.05
Triglycerides (mg/dl)	10-150	103 (44-243)	185 (60-279)	> 0.05	190 (62-525)	103 (42-464)	< 0.05

HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low-density lipoprotein.
*According to the one-way ANOVA and the Kruskal-Wallis test for between-groups comparison. P-value statistically significant: *< 0.05; very significant: **< 0.01; and highly significant: ***< 0.001.

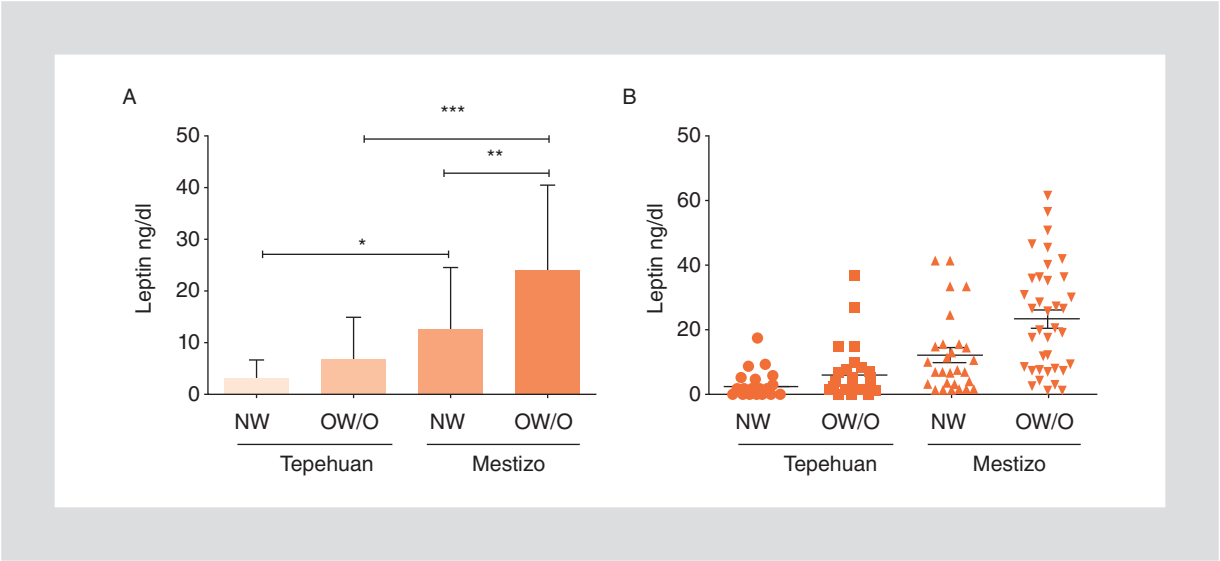

Figure 1. *Comparison of leptin circulating levels in Tepehuan and Mestizo subjects by nutritional status.* **A:** *bar chart according to the one-way ANOVA and the Tukey-Kramer comparison test (p-value: * < 0.05; ** < 0.01; *** < 0.001).* **B:** *scatter plot.*

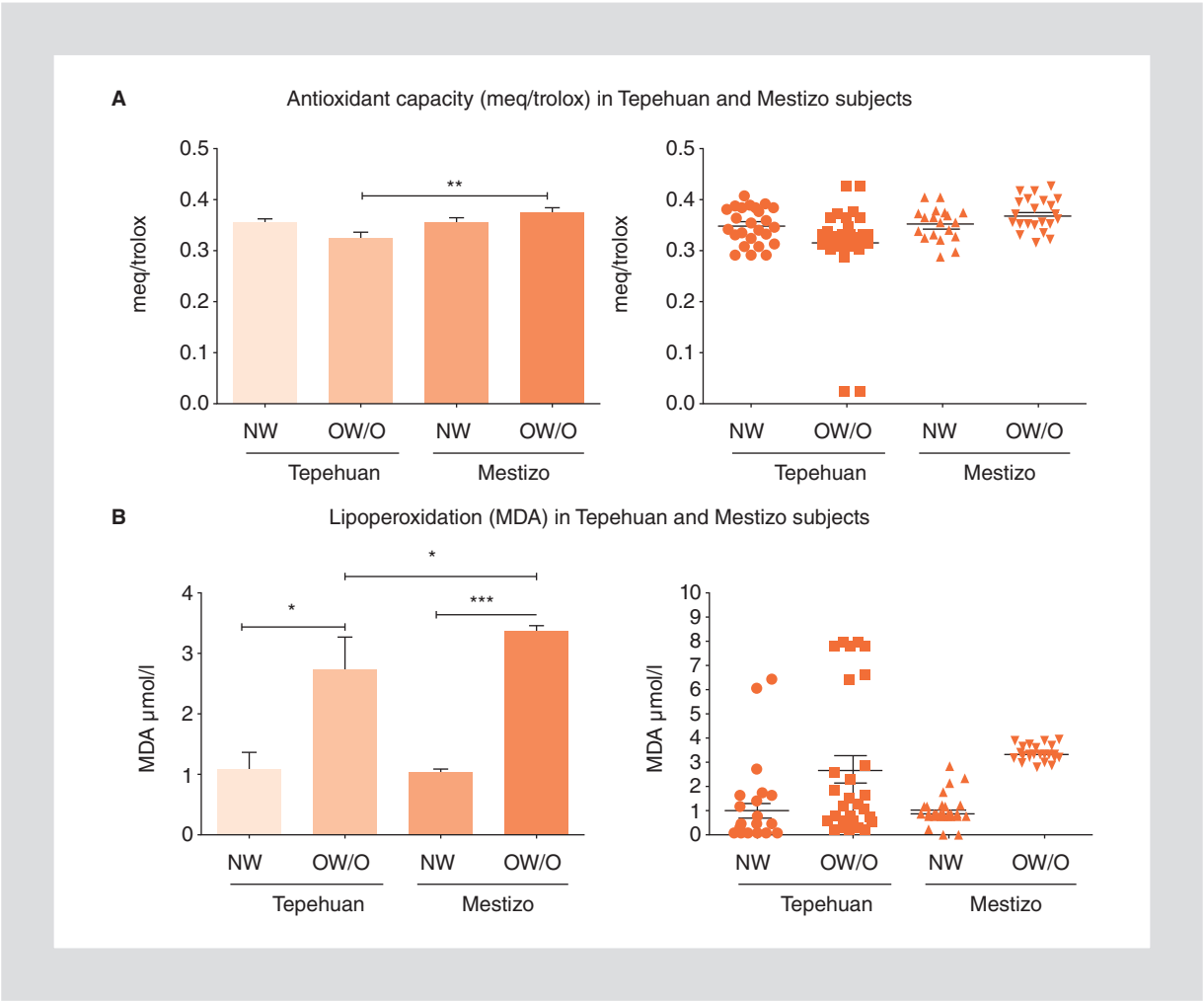

Figure 2. *Comparison of LPX and AC in Tepehuan and Mestizo subjects by nutritional status.* **A:** *bar chart according to the one-way ANOVA and the Tukey-Kramer comparison test (p-value: * < 0.05; ** < 0.01; *** < 0.001).* **B:** *scatter plot.*

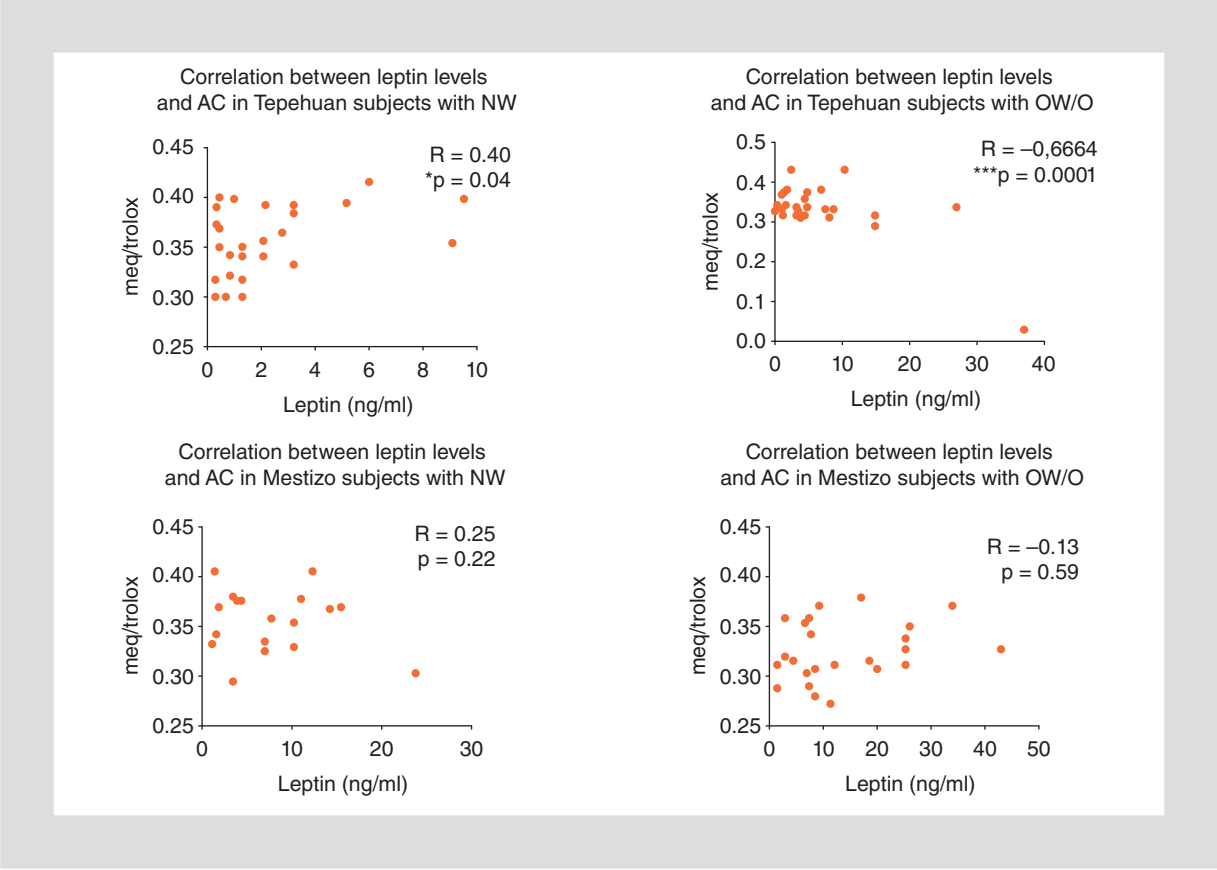

Figure 3. *Correlation analysis between leptin levels and AC (meq/trolox) in indigenous Tepehuan and Mestizo subjects. *R: Pearson's correlation index; p-value < 0.05 statistically significant; * p < 0.05; ***p < 0.0001.*

in the studied groups. Additionally, the correlation between AC and LPX was positive and statistically significant in Mestizos with OW/O (R = 0.993; p < 0.001) (Fig. 4).

Discussion

Essentially produced by adipocytes, leptin informs the brain about the body fatty deposits status[29]. In situations where intake exceeds energetic expenditure, an increase in the levels of leptin is produced, which causes an intake inhibition and an increase in energy expenditure. Conversely, in situations of negative energetic balance, such as fasting, a drop in leptin levels is produced, which leads to increased intake and a decrease in energy expenditure as a compensatory mechanism[30]. Therefore, leptin may be the key to cope with situations of stress when there is shortage of food or famine. Its primary role is thought not to be obesity avoidance, but death in case of shortage of food. In our results, standard deviations were very broad, which reflects the large leptin concentrations diversity in Mestizo subjects. The same happened when both Mestizo groups were compared. In this context, Tepehuan indigenous subjects are in an energy expenditure control physiological status that allows for them to maintain leptin levels under the ranges established for other populations[7-9,31], thus preventing a pathological inflammatory state that is likely to trigger chronic conditions. However, the Mestizo population with OW/O shows leptin levels above the normal range, which suggests a pathological state of energy balance, with a trend towards a chronic inflammatory state. There is a small percentage of the obese population wlth normal plasma leptin levels[32] and even other with reduced levels of this hormone (partial deficiency). Tepehuan indigenous subjects with NW and obesity show lower levels of leptin than Mestizos, suggesting that the Tepehuan ethnic group has low, but not absent leptin production.

ROS increase leads to polyunsaturated fatty acids breakdown and MDA formation in the cell membrane. This process is known as lipid peroxidation[3]. MDA is a natural product of lipid peroxidation and a direct

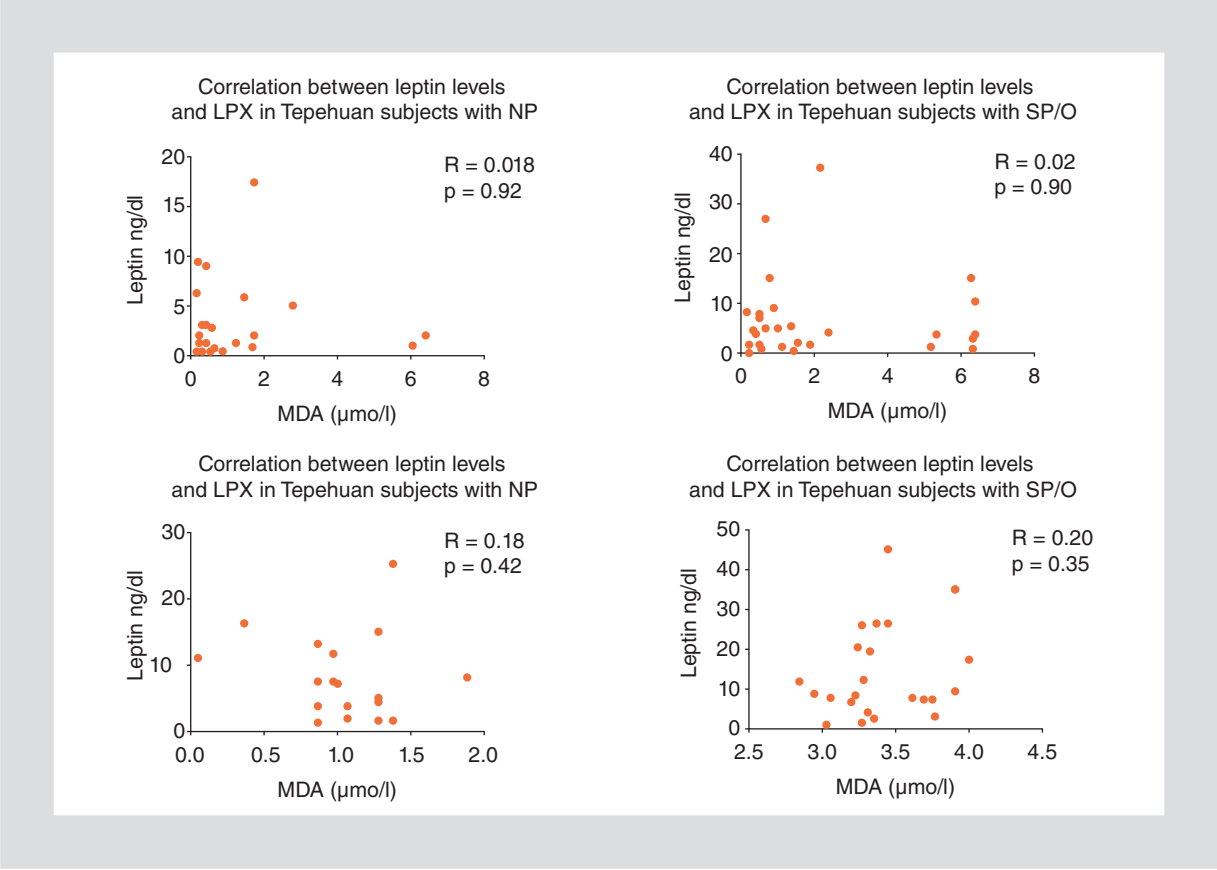

Figure 4. *Correlation analysis between leptin levels and LPX (MDA µmol/l) in indigenous Tepehuan and Mestizo subjects. *R: Pearson's correlation index; p-value < 0.05 statistically significant.*

marker of oxidative stress[34]. MDA values found in Tepehuan subjects with OW/O were markedly lower than those in Mestizo subjects with the same nutritional status; there, it is possible that this difference was driven by physical activity, lifestyle and culture of the different ethnic groups. According to our results, there was a significant increase in plasma MDA in the Mestizo OW/O group, suggesting that there is a pathological state in the oxidative and compensatory antioxidant processes that could entail an increase in LPX and a decrease in antioxidant enzymatic activity.

Bosnak et al.[35] suggest that antioxidant enzimes activities wear out as a compensatory mean to protect against dangerous effects of increased ROS, indicated by the increasing level of MDA in undernourished children, together with negative correlations between GSH activity and MDA, between catalase and MDA and between superoxide dismutase and MDA, showing that the magnitude of initial oxidative stress has exceeded the compensatory capacity of antioxidants. In our study, subjects with OW/O suffered more oxidative damage, which is why active AC was higher in these groups; however, OW/O Mestizo subjects showed more AC activation than Tepehuan indigenous subjects with OW/O, which suggests that, since Tepehuan suffered less oxidative damage, their antioxidant mechanisms were consumed in order to prevent ROS-generated damage, whereas Mestizos had to generate more AC, which was not enough to avoid direct oxidative damage. These alterations could be attributed to insufficient micronutrient (zinc, selenium, copper, etc.) and antioxidant vitamins (E, C and A) intake[36]. Among other possible social and economical reasons, the SP/O phenomenon reached Tepehuan populations later than it did to Mestizos and possibly this explains a different adaptive response between these groups[37]. These results suggest that, in Tepehuan indigenous subjects, AC is implicated in leptin levels regulation.

In view of all this, we suggest that life expectancy and quality of these populations can be increased by reducing the degree of oxidative phenomena[38]. This could be accomplished by improving healthy dietary habits and incresing antioxidant defenses[39-40].

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