

# Original/Obesidad Iron status and dietary intakes of iron in normal-weight and obese young Mexican women

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#### Abstract

*Introduction:* obesity is reported to be a predictor of iron deficiency. In Mexico, 45.5 % of women older than 20 years have obesity, and the prevalence of anemia is 10.2 % in women 20 to 29 years.

*Objective:* to investigate the relation between body mass index (BMI), percentage of body fat (% BF), dietary intakes and iron status of healthy normal-weight and obese young women.

*Methods:* a total of 86 women [normal-weight (n = 46) and obese (n = 40)] completed the study. Intakes were evaluated by an 8-day food-record. Anthropometrics and blood collection (hemoglobin, hematocrit, ferritin and transferrin) were done on the luteal phase of menstrual cycle; menstrual characteristics were also reported. Iron status was determined according to stages of iron depletion. T-test and Mann-Whitney U test were used to compare groups' variables. Pearson correlation was used to determine relationships between variables. An odds ratio (OR) analysis was used to measure the association of BMI, % BF and dietary intakes with iron status.

*Results:* biomarkers of iron were similar between groups. There was a positive correlation between % BF and ferritin (r = 0.222; p = 0.032). Similar intakes and menstrual periods may be the reason of similar iron status. BMI, % BF or dietary intakes were not independent contributors to stages of iron depletion.

*Conclusion:* guidance on dietary intakes is suggested for this population to avoid future iron deficiency complications.

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Key words: *Obesity. Body fat. Iron status. Dietary intake. Women.* 

#### RESERVAS DE HIERRO E INGESTA DIETÉTICA DE HIERRO EN MUJERES JÓVENES MEXICANAS CON NORMOPESO Y OBESIDAD

#### Resumen

*Introducción:* la obesidad se ha reportado como predictor de la deficiencia de hierro. En México, el 45,5% de las mujeres mayores de 20 años tienen obesidad, y la prevalencia de anemia es de 10,2% en mujeres de 20 a 29 años.

*Objetivo:* investigar la relación entre índice de masa corporal (IMC), porcentaje de grasa (%GC), ingesta dietética y reservas de hierro en mujeres jóvenes sanas con normopeso y obesidad.

*Métodos:* ochenta y seis mujeres [normopeso (n = 46) y obesidad (n = 40)] completaron el estudio. La ingesta fue evaluada por un diario de registro de 8 días. La antropometría y obtención de sangre (hemoglobina, hematocrito, ferritina y transferrina) se consiguieron en la fase lútea del ciclo menstrual; se reportaron las características de la menstruación. Las reservas de hierro se determinaron según etapas de depleción. Pruebas de T y Mann-Whitney U se usaron para comparar variables entre grupos. La correlación de Pearson se usó para determinar relaciones entre variables. La razón de momios se utilizó para medir la asociación de IMC, % GC e ingesta dietética con las reservas de hierro.

*Resultados:* los marcadores de hierro fueron similares entre grupos. Se encontró una relación positiva entre % GC y ferritina (r = 0,222; p = 0,032). La similitud en ingesta y periodos menstruales puede ser la razón de que existan reservas de hierro similares. El IMC, % GC e ingesta dietética no contribuyeron independientemente a las etapas de depleción de hierro.

*Conclusión:* se sugiere guía dietética para esta población con el fin de evitar complicaciones por deficiencia de hierro.

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## Introduction

Obesity and iron deficiency have become frequent conditions that are reported to be linked to each other<sup>1,2</sup>. It has been suggested a multifactorial etiology including inadequate bioavailable iron relative to body weight and diminished iron absorption found in subjects with excess of adiposity<sup>2</sup>. In Western societies, these conditions are prevalent among young and adult population. In Mexico, 45.5 % of women older than 20 years have obesity, and the prevalence of anemia is 10.2 % in women 20 to 29 years<sup>3</sup>. Women are at high risk of iron deficiency mainly due to menstruation losses and having a diet with low iron bioavailability<sup>4</sup> or unbalanced and inadequate micronutrient intake5. Effects of iron deficiency could be detrimental, for example anemia during pregnancy and lactation<sup>6,7</sup> or health concerns such as memory disorders<sup>8</sup>. There is a study that examined the relation between iron status and BMI in Mexican women, which reported obesity as a predictor of low iron status9; however, data utilized was from years 1998 and 1999. Besides this, other authors had studied similar conditions and their association in women of childbearing age; however, the ranges of age analyzed had been too wide from 13 to 51 years<sup>8-11</sup>. Therefore, the aim of this study was to investigate the relation between BMI, % BF and dietary intake of iron and iron status of normal-weight and obese Mexican women aged 18 to 30 years. We hypothesized that in young women, a higher BMI and % BF would relate to a low iron status.

# Methods

# Subjects and design

This was a prospective case-control study involving healthy young women that aimed to investigate the relation between BMI, % BF and dietary intake of iron and iron status. All participants recruited were undergraduate students living in the northeast of Mexico. Women with irregular menstrual cycles, pregnant or lactating, presenting hemorrhage, blood donors, or those who had taken iron supplements or drugs likely to modify iron status were not enrolled. Eligible participants were women 18 to 30 years of age, BMI 18.50-24.99 and  $\geq$  30.00, with regular menstrual cycles (having one menstrual cycle per month).

A total of 118 women informed consent; 10 women withdrew, 10 were taking supplements, 5 were BMI < 18.5, 6 had irregular menstrual cycles, and 1 was pregnant, thus the final analysis included n = 86, who completed their participation after two sessions; (1) recruitment and eligibility procedures and (2) anthropometric and blood collection, at the Nutritional Biochemistry Laboratory of the Center of Research in Nutrition and Public Health (CINSP, for its Spanish abbreviation) in

the School of Public Health and Nutrition (FaSPyN, for its Spanish abbreviation), University Autonomous of Nuevo Leon (UANL).

Subjects information was collected during the first session, including age, school's name, city of origin and smoking habits. A criterion questionnaire was applied to confirm the eligibility to participate. Once approving the eligibility, a second session was scheduled on day 20, considering day 1 as the first day of menstruation.

All procedures were reviewed and approved in 2012 by the Committee for Ethical Review of Research of FaSPyN, UANL. All subjects provided written informed consent before participating in this study.

# Anthropometric data

Anthropometric measurements and blood collection were conducted in the second session after a 12-hour fast, from 7:30 to 9:00 a.m., to prevent from diurnal variations of iron indicators<sup>12</sup>; This session was scheduled based on the menstrual cycle of each subject, on the late luteal phase (day 20), considering day 1 as the first day of menstruation, this in order to prevent from variations in iron status during the menstrual cycle<sup>13</sup>. If day 20 was a weekend day, days 18 to 22 were also appropriate.

Body weight and % BF were measured by a body composition monitor BC-545 (Tanita Corporation of America, Inc., USA). Height was measured with a stadiometer Seca 213 (Seca, USA). BMI was calculated as kg/m<sup>2</sup>. Subjects were grouped into normal or obese according to the World Health Organization (WHO) database of BMI<sup>14</sup>. Ranges of body fat for women aged 18 to 30 were considered as: 17.0 - 32.9 %, 33.0 - 38.9% and  $\geq 39.0$  %, adapted from the Body Fat Ranges for Standard Adults and Children (Tanita Corporation of America, Inc.) and Gallagher et al.  $(2000)^{15-16}$ .

# Dietary intakes

The dietary intake was evaluated by an 8-day food-record, in order to obtain a reliable iron intake<sup>17</sup>. The subjects were given instructions on how to record all foods and drinks they consumed during each day, the preparation method, and the portion sizes; especially on the recording of the different foods used in mixed dishes. Day one was set as the first day of the menstrual cycle for all participants to prevent variances of eating patterns<sup>18</sup>. The subjects were also instructed to continue their regular diet during the study protocol. A food record booklet was given to all subjects, who were telephoned twice during the recording timeframe to follow-up. After completion, the booklet was brought to the Laboratory and revised by trained interviewers. Intakes of energy, total iron, heme and non heme iron, and vitamin C were analyzed using the

Food Processor<sup>®</sup> (version 10.12.0, ESHA Research, Salem, Oregon, USA), updated by adding specific Mexican food products consumed by the participants. Intakes were compared to recommendations of intake for the Mexican population<sup>19,20</sup>.

### Menstrual characteristics and iron status

The age at menarche, duration of menstrual period (days) and the length of the menstrual cycle (days) was reported by subjects. Blood samples were drawn from an antecubital vein immediately after the an-thropometric measurements (late luteal phase). He-moglobin was determined in blood by the portable photometer HemoCue (Ängelholm, Sweden). He-matocrit was measured in blood by centrifugation at 10,000 rpm during 6 minutes in the AutoCrit Ultra 3 (BioSurplus, USA). Ferritin was determined in serum by spectrophotometry at 540 nm in the Evolution 300 UV (Thermo Scientific, USA). Transferrin was determined in serum at 540 nm in the A25 Autoanalyser (BioSystems, Spain).

Iron status was determined according to the stages of iron depletion, defined as: (1) iron storage depletion (ISD), (2) iron deficient erythropoiesis (IDE), and (3) iron deficiency anemia (IDA). ISD was defined as normal hemoglobin (12-16 g/dL), hematocrit (37-47 %), serum transferrin (200-360 mg/dL) and low serum ferritin (< 12  $\mu$ g/L). IDE was defined as normal hemoglobin and hematocrit and high serum transferrin (> 360 mg/dL). IDA was defined as low hemoglobin (< 12 g/ dL), hematocrit (< 37 %) and high serum transferrin (> 360 mg/dL)<sup>21-23</sup>.

# Statistical analyses

To test normality assumptions, the data were evaluated by Shapiro-Wilk, and Kolmogorov-Smirnov tests, indicating normality for all variables, except for dietary intakes. The continuous variables are shown as frequency, percentage or means  $\pm$  standard deviation (SD); dietary intakes are shown as median, and 25th and 75th percentiles. An independent T-test was used to show differences on normal variables between normal and obese groups; and a Mann-Whitney U test to determine differences between groups on nonnormal variables. Nonnormal variables were transformed to normality for further statistical analysis. One-tailed Pearson correlation was used to determine relationships between BMI, % BF and dietary intakes and biomarkers of iron status. An odds ratio (OR) analysis was used to measure the association of ranges of BMI. % BF and dietary intakes of iron with the stages of iron depletion of subjects. All statistical analyses were performed with the SPSS statistical software for Windows (version 15.0; SPSS Inc, USA). Significance was set at *p* < 0.05.

# Results

# Subjects characteristics

Women included in this study were divided into normal-weight (n = 46) and obese (n = 40) groups based on the WHO database of BMI (2014). Women in the obese group (21.25 ± 2.71 y) were older than those in the normal-weight group (19.90 ± 1.53 y) (p = 0.017). Subjects were predominantly living in Monterrey city or its metropolitan area (87 %). Of participants, 11 (12.8 %) reported smoking (data not shown).

# Anthropometrics

Weight, BMI and % BF were different between normal vs. obese groups (p < 0.001), while height was similar (p = 0.339). The mean BMI were  $20.89 \pm$ 1.29 and  $34.37 \pm 4.89 \text{ kg/m}^2$ , for the normal and obese groups, respectively. The mean % BF of the obese group was almost the double of the normal-weight group (41.86 vs. 21.09 %; p < 0.001) (Table I).

# Dietary intakes

Dietary intakes are depicted in Table II. There was no statistical difference between groups on the dietary intakes reported for total energy, total iron, heme and non heme iron, iron density or vitamin C; however, median intakes are somewhat higher in the obese group. In contrast, total energy based on body weight (kcal/kg/d) resulted statistically lower in the obese group (p < 0.001). Median total iron intakes were 14.50 and 16.02 mg/d for normal-weight and obese women, respectively, while the recommendation for Mexican women is 21 mg/d of total iron<sup>20</sup>. Of the 86 women, only 16.3 % had iron intakes according to the recommendation or above and 41.9 % had intakes below 15 mg/d (data not shown). In addition, median intakes of vitamin C were lower than the recommendation (75 mg/d) in both groups<sup>19</sup>.Of participants, 55.8 % had an intake of vitamin C below the recommendation (data not shown).

Table I       Anthropometrics				
	Normal (n = 46)	<i>Obese</i> (n = 40)	p-value	
Height (m)	$1.58 \pm 0.06$	$1.60\pm0.05$	0.339	
Weight (kg)	$52.47 \pm 4.23$	$90.58 \pm 11.97$	< 0.001*	
BMI (kg/m <sup>2</sup> )	$20.89 \pm 1.29$	$34.37 \pm 4.89$	<0.001*	
Body fat (%)	$21.09 \pm 4.42$	$41.86 \pm 4.92$	<0.001*	

\* Significance at p < 0.05.

Table II       Dietary intakes of participants <sup>a</sup>					
	Normal (n = 46)	$Obese \\ (n = 40)$	p-value		
Total energy (kcal/d)	1572.19 (1350.81, 1866.34)	1642.45 (1240.60, 2155.40)	0.776		
Total energy (kcal/kg/d)	30.21 (24.99, 36.05)	18.52 (13.19, 23.57)	<0.001*		
Iron total (mg/d)	14.50 (11.70, 20.24)	16.02 (12.10, 20.12)	0.711		
Heme iron (mg/d)	2.14 (1.04, 3.86)	2.48 (0.98, 3.57)	0.648		
Non heme iron (mg/d)	12.94 (10.56, 17.50)	13.00 (10.08, 16.98)	0.701		
Iron density (mg/1000 kcal)	6.07 (5.40, 7.41)	7.09 (5.01, 9.83)	0.261		
Vitamin C (mg/d)	42.81 (27.70, 72.11)	55.88 (30.60, 82.66)	0.357		

<sup>a</sup> Expressed as median (25<sup>th</sup>,75<sup>th</sup> percentiles).

\* Significance at p < 0.05.

#### Menstrual characteristics and iron status

Menstrual characteristics and iron status indicators are presented in Table III. The age at menarche was different between normal and obese groups (12.82 vs. 12.09 y; p = 0.035); obese women were younger than normal weight at the age at menarche. The duration of the menstrual period (days) and the length of the menstrual cycle (days) were similar for both groups (p = 0.177) and (p = 0.703), respectively. Biochemical indicators of iron status were statistically similar for both groups. Although, the mean of serum ferritin was higher in the obese group (39.51 vs. 32.37  $\mu g/L$ ; p = 0.105) and the mean of serum transferrin was lower in the obese group (302.06 vs. 314.25 mg/ dL; p = 0.378).

The frequency of subjects in stages 2 and 3 was 19.7 % (data not shown). The stage 2 or iron deficient erythropoiesis (IDE) was predominant (15.1 %) in comparison to stage 1 (0.0 %) and stage 3 (4.6 %). A total of 9 women in the normal weight group (19.6 %) and 4 women in the obese group (10.0 %) presented IDE, while 2 subjects in the normal weight group (4.3

%) and 2 in the obese group (5.0 %) presented iron deficiency anemia or stage 3.

# Influence of BMI, % BF, and dietary intake of iron on iron status

A significant, positive correlation was found between % BF and serum ferritin (r = 0.222; p = 0.032) (**Fig. 1**). No significant relationship was detected between BMI or dietary intake of iron and iron status. According to the odds ratio analysis, in this group of young women (n = 86), none of the ranges of % BF contributed to any stage of iron deficiency (**Table IV**), neither those of BMI or dietary intakes.

### Discussion

This prospective case-control study provide evidence on the relation between BMI, the % BF and dietary intake of iron and iron status in healthy young women, living in the northeast of Mexico. Iron status

Table IIIMenstrual characteristics and biomarkers of iron status <sup>a</sup>					
	Normal (n = 46)	<i>Obese</i> (n = 40)	p - value		
Age at menarche (y)	$12.82 \pm 1.43$	$12.09 \pm 1.39$	0.035*		
Duration of menstrual period (d)	$5.20 \pm 1.04$	$4.83 \pm 1.18$	0.177		
Length of menstrual cycle (d)	$28.50 \pm 1.81$	$28.67 \pm 2.08$	0.703		
Hemoglobin (g/dL)	$13.58 \pm 0.94$	$13.25 \pm 0.82$	0.136		
Hematocrit (%)	$41.92 \pm 2.40$	$41.25 \pm 2.55$	0.264		
Ferritin (µg/L)	$32.37 \pm 17.80$	$39.51 \pm 18.58$	0.105		
Transferrin (mg/dL)	$314.25 \pm 61.93$	$302.06 \pm 50.89$	0.378		

<sup>a</sup>Expressed as median ± standard deviation.

\* Significance at p < 0.05.



Fig. 1.—Positive relationship between serum ferritin  $(\mu g/L)$  and percentage (%) of body fat in young Mexican women in both normal-weight and obese groups (n, 86). (r = 0.222; p = 0.032).

Table IV		
Odds ratios and 95% confidence interval for ranges		
of percentage of body fat which relate to iron status in		
young women $(n = 86)$		

Percentage of body fat <sup>a</sup>	Stage 2 IDE	Stage 3 IDA
17.0 - 32.9 %	8.207 (0.953 – 70.671)	1.250 (0.306 – 5.009)
33.0 - 38.9 %	0.982 (0.106 – 9.067)	2.245 (0.387 – 13.013)
≥ 39.0%	0.0	0.647 (0.123 – 3.406)

<sup>a</sup> Adapted for women ages 18 to 30 y from Tanita Corporation of America, Inc. and Gallagher et al. 2000.

\* Significance at p < 0.05.

in a young female population should be considered as of relevance in order to decrease the risk of anemia in terms of possible future gestations and lactation<sup>6,7</sup> and other health concerns as university students such as memory<sup>8</sup>. Biomarkers of iron status were not statistically different between normal-weight vs. obese groups as others had previously reported<sup>24</sup>; however, serum ferritin levels were somewhat higher in obese than in normal-weight women (39.51 vs. 32.37  $\mu$ g/L; p = 0.105).

The results of this study indicate that BMI was not related to neither iron status indicators or to stages of iron depletion in young women. It has been reported that obesity was a significant independent predictor of iron deficiency in Mexican adult women (18 - 50)y) (OR =  $1.92 (95\% \text{ CI: } 1.23 - 3.01)^9$ . In contrast, a study in Colombian women (13 - 49 y) reported that overweight and obesity were associated with a lower likelihood of anemia [OR = 0.8 (95% CI 0.7, 0.9) and OR = 0.8 (95% CI 0.6, 1.0), respectively, in comparison to normal-weight<sup>11</sup>. Chambers et al (2006) suggests the inconsistency of results due to the use of different biomarkers of iron status<sup>25</sup>. In our study, the percentage of body fat was a positive correlate with serum ferritin (r = 0.222; p = 0.032), similar to other study results where serum ferritin of older Japanese adults was correlated with visceral fat (r = 0.254, p < 0.0001) and subcutaneous fat  $(r = 0.231, p < 0.0005)^{26}$ . As the % BF was correlated with serum ferritin, we considered an odds ratio (OR) analysis to measure how different ranges of % BF may contribute to stages of iron depletion; however, this results (Table IV) showed no statistical significance, neither those of BMI or dietary intake of iron, indicating that this variables may not be independent contributors to stages of iron depletion as previously suggested<sup>10</sup>, thus other variables such as iron menstrual loss1 should be of relevance to consider in this type of research.

In this study, although median dietary intakes were below the recommendation for Mexican women for iron and vitamin C, both normal-weight and obese participants had similar median intakes of total iron, heme, non heme-iron and vitamin C, 14.50 vs. 16.02 mg/d (p = 0.711), 2.14 vs. 2.48 mg/d (p = 0.648), 12.94 vs. 13.00 mg/d (p = 0.701) and 42.81vs. 55.88 mg/d (p = 0.357), respectively, and no women was taking

vitamin or mineral supplements. In addition, small or absent body iron stores have been reported in young women with menstruation of 5 days or longer, intense menstrual bleeding or using intrauterine devices (IUD) without hormones<sup>4</sup>. In our subjects, the duration of the menstrual period was similar between normal-weight vs. obese groups, 5.20 vs. 4.83 d (p = 0.177). Although menstrual flow was not measured, both similar iron intake and menstrual periods may be the reason of similar results between groups in terms of biomarkers of iron status and frequencies in stages of iron depletion. As iron status may result impaired when blood is drawn during menses<sup>13</sup> we considered the late luteal phase (day 20) of the menstrual cycle as the most adequate to determine iron status.

Our study has several limitations. Subjects were recruited in terms of BMI rather than in percentage of body fat; although, there was a clear difference in BMI  $(18.50 - 24.99 \text{ vs.} \ge 30.00 \text{ kg/m}^2)$  (p < 0.001). Besides, the % BF was not overlapped between normal-weight vs. obese women (p < 0.001); however, for further research it is suggested to have measures of body fat distribution. The use of an 8-day food record as the method to determine the intake of iron has the limitation that respondents may have reactivity as a result of the recording itself<sup>27</sup>. To reduce error in this study, a trained interviewer instructed all subjects to follow a regular diet. Besides, subjects were telephoned twice in the 8 days to verify the diet recording process and remind participants to continue with their regular diets. Ferritin is a biomarker that may be affected by chronic inflammation process caused by the adipose tissue<sup>28,29</sup> in both our normal weight and obese women, thus we also considered hemoglobin, hematocrit and transferrin as indicators to define iron status of participants.

It is also recommended for further research to determine biomarkers of inflammation or antioxidant status that may relate to iron status to consider the status of chronic inflammation while women present high percentages of body fat. It should also be noted that our subject population were healthy young women living in the northeast of Mexico, and thus results may not be representative for the Mexican population or for young women with preexisting iron-related disorders or other mental disorders such as altered eating behaviours, impulsiveness and depression, especially in those with obesity<sup>30</sup>.

### Conclusion

In conclusion, this study showed a positive relationship between serum ferritin and the % BF in young healthy normal-weight and obese women with similar dietary intakes and duration of menstrual periods. Despite this relationship, our hypothesis could not be supported in the study group. In addition, ranges of % BF were not independent contributors to stages of iron depletion. The use of several biomarkers for assessing iron status of obese subjects should be reconsidered in further research due to inconsistency of results. Guidance on dietary intakes is suggested for this population in order to prevent iron deficiency and its complications.

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