Anthropometric measure and adipokine levels of a young undergraduate population with a usual diet
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Abstract

Background: obesity is the excessive accumulation of adipose tissue related to food intake and other factors. The aim of this study was to evaluate the intake of nutrients, anthropometric parameters, health indicators, adipokines and insulin levels in a population of young undergraduates.

Method: in this study, 378 young undergraduates were invited to participate. Due to the inclusion criteria and their own decision of participating, 90 attended the anthropometric, health indicators: waist circumference (WC), waist to hip ratio (WHR), waist to height ratio (WHR), and homeostatic model assessment-insulin resistance index (HOMA-IR) studies and completed the questionnaire of frequency of food intake; and 34 participants were selected to perform the determination of biochemical parameters, insulin and adipokines levels: leptin, IL-6, IL-8, tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and hepatocyte growth factor (HGF).

Results: according to WC, WHR and WHtR (women: 104 ± 20, 0.87 ± 0.08, 0.6 ± 0.13; men: 112 ± 10, 0.95 ± 0.09, 0.64 ± 0.06, respectively), obese population showed health, cardiovascular and metabolic risk. Overweight population showed cardiometabolic risk. In general, lipids intake was higher than 30%, being animal fat the most consumed. The levels of leptin (women: 17.2 ± 9.2, 28 ± 11.3, 36.8 ± 17.8; men: 4.3 ± 3.6, 9.5 ± 3.1, 24.8 ± 16.4) to lean overweight and obese, respectively) and insulin (women: 408 ± 182, 438 ± 187, 768 ± 590; men: 244 ± 88, 520 ± 256, 853 ± 590) increased along with body mass index (BMI), body fat percentage (BFP), visceral fat area (VFA), WC, WHR and WHtR. Lean (2.4 ± 1.3), overweight (2.2 ± 0.9) and obese (4.3 ± 1.1) women and overweight (2.8 ± 1.2) and obese (5.0 ± 3.1) men showed insulin resistance according to HOMA-IR. Significant correlation between leptin and HOMA-IR was found (p = 0.41). BMI, BFP, VFA, WC, and WHR positively correlated with leptin (p = 0.67, 0.75, 0.66, 0.60, 0.67, respectively) and insulin (p = 0.37, 0.40, 0.48, 0.49, 0.42, respectively), while WHR only with insulin (p = 0.43). No significant differences were found in the other adipokines.

Conclusion: the use of health indicators such VFA, WC, WHR, WHR and HOMA-IR are useful tools in the determination of health, cardiovascular and metabolic risk and are correlated with levels of leptin and insulin in the studied population.

Keywords: Anthropometric. Diet. Insulin. Leptin. Cardiometabolic risk.
INTRODUCTION

Obesity is considered to be a serious health problem affecting a significant proportion of people of all ages worldwide (1). The World Health Organization (WHO) defined obesity as an abnormal or excessive fat accumulation that may impair health (2). Epidemiological studies indicate a strong link between increased food intake and obesity (3); however, other factors, like genetics, the environment, socioeconomic and demographic factors, also contribute to the development of obesity (4). In addition, obesity is a state of chronic low grade of inflammation characterized by an excess of adipose tissue (5). Adipose tissue is a metabolic and endocrine organ, responsible for the storage of energy and the secretion of biologically active molecules (adipokines), including hormones (leptin), cytokines (IL-6 and TNF-α), chemokines (IL-8 and MCP-1), growth factors (nerve growth factor [NGF], HGF) and others (6). The main function of adipokines is to regulate appetite, energy balance, fat deposition, glucose homeostasis, lipid metabolism, immunity, insulin sensitivity, angiogenesis, inflammation processes, and blood pressure (7).

On the other hand, a high level of leptin and inflammatory markers (IL-1β, IL-6, TNF-α, IL-8 and MCP-1) in serum has been associated with obesity. Leptin is one of the principal adipokines that influence food intake, body weight and energy homeostasis. Leptin is synthesized and secreted by adipose tissue, enters the systemic circulation and crosses the blood-brain-barrier before being joined to the leptin receptor in the hypothalamus; this gives information about body energy stores and triggers the expression of anorexigenic neuropeptides to inhibit food intake and regulate energy homeostasis (8). The leptin concentration is variable and increased in relation to the increase in adipose tissue. However, studies show that leptin concentration in serum can be modified by macronutrient (fatty acids, carbohydrates and proteins) and micronutrient (minerals and vitamins) intakes (9).

IL-6 and TNF-α are pro-inflammatory markers that are related to obesity. IL-6 influences body weight, inhibits the lipase lipoprotein, and increases glucose uptake and insulin sensitivity. TNF-α contributes to adipose tissue regulation and the infiltration of M1 macrophages, suppresses adiponectin expression, reduces fatty acid oxidation in hepatocytes, and induces apoptosis and insulin resistance (6). IL-8 and MCP-1 are two chemokines that seem to be increased in obese subjects (10). IL-8 has a major impact on the inflammation and obesity complications, is overexpressed in visceral adipose tissue and is a marker of oxidative stress (10,11). Also, MCP-1 regulates the migration and infiltration of monocytes, natural killer cells and memory T lymphocytes (12), and a higher expression of MCP-1 was detected in adipose tissue of obese patients (13).

Nutrients intakes have a direct influence on weight gain and, consequently, on BMI, body fat, and other anthropometric parameters and impacts on the levels of adipokines. The aim of this study was to evaluate the intake of nutrients, anthropometric parameters, health indicators, adipokines and insulin levels in a population of young undergraduates with a usual diet.

MATERIAL AND METHODS

SUBJECTS

In this study, 378 young undergraduate adults between 18 and 22 years old from Metropolitan Autonomous University - Campus Xochimilco in Mexico City were invited to participate. The exclusion criteria were alcoholism, smoking, pregnancy, menstrual period, use of antibiotics, narcotics, lactation, diarrhea and vegetarianism or veganism. Due to these criteria, 117 young people were excluded; the rest of the students were invited to participate, however, 171 failed to attend and only 90 attended the anthropometric study and completed the questionnaire regarding frequency of food intake. To perform the determination of biochemical parameters and adipokines, the population had to be paired by age, sex and BMI; therefore, 34 participants were selected to take blood samples from, of which 17 were women and 17 were men. These individuals were separated into three groups: six lean, five overweight and six obese. The project was approved by the local Committee of Ethics according to the 1964 Declaration of Helsinki and its later amendments, and has been performed in accordance with ethical standards. All participants signed an informed consent prior to the study.

ANTHROPOMETRIC ANALYSIS

Body mass index (BMI) was measured according to the WHO criteria (lean ≤ 25 kg/m², overweight 25-30 kg/m² and obese ≤ 30 kg/m²) (2). Waist and hip circumference were determined to calculate the waist to hip ratio (WHR) and waist to height ratio (WHtR); the reference values used of both ratios were the ones cited on references 14 and 15. Body fat percentage (BFP) and visceral fat area (VFA) were determined by segmental multifrequency bioelectrical impedance (SMF-BIA) analysis using an Inbody 720 Biospace, Seoul, Korea.

DIETARY ANALYSIS

Dietary information was recorded and evaluated by previously trained nutritionists using a semi-quantitative food questionnaire (FFQ). The questionnaire was used to determine the total energy intake, proportion of protein, carbohydrates, lipids, minerals and vitamins in the diet of all participants during the previous year, as in other studios (16). The estimation and interpretation of nutrient intake was determined using the SNUIT software and the tables of composition of energy and nutrients, developed and validated by the National Institute of Public Health (INSP) (17). The results were compared with the ones recommended to Mexican population (18).
BIOCHEMICAL ANALYSIS

A fasting blood sample was collected after 12-hour fasting. Serum was obtained by centrifugation at 2,000-3,500 rpm for five minutes and stored at -20 °C until analysis. Biochemical analysis included glucose, triglycerides, total cholesterol and high-density lipoproteins; all biomolecules were determined by automated Biochemical Analyzer Ikem (Kontrollab, Mexico), and results were compared with the reference (19). Very low-density lipoprotein (vLDL cholesterol) and low-density lipoprotein (LDL cholesterol) were calculated according to Friedewald et al. (20).

ADIPOKINES ANALYSIS

Serum samples were used to determine leptin, TNF α, IL-6, IL-8, MCP-1, HGF, and insulin. All components were assayed with the MILLIPLEX MAP Human Adipokine Magnetic Bead Panel 2 (Millipore, USA) Endocrine Multiplex Assay kit and analyzed with the MAGPIX® (Luminex, USA).

STATISTICAL ANALYSIS

Descriptive analyses were performed for all variables. Significant differences in anthropometric data, blood, adipokines and dietary analysis among lean, overweight and obese individuals were determined by Kruskal-Wallis (p ≤ 0.05) and the test of multiple comparisons of Dunn using GraphPad Prism software version 5.01 (GraphPad Software, Inc., San Diego, CA).

The correlations between adipokine levels, anthropometric parameters, and nutrients were evaluated in the whole population without stratification. All data were normalized by raising them to the square, before the Pearson’s analysis of correlation was performed (p ≤ 0.05).

RESULTS

The average body composition of the groups studied is shown in Table I. No significant differences were found in height for each sex (159.6 ± 14.8 women and 171.2 ± 6.2 men). As expected, the BMI was different (p ≤ 0.05) between lean and obese women and men. In addition, WC, WHR, WHtR, BFP and VFA increased as BMI increased (Table I). According to WC, obese women and men showed a risk of cardiovascular diseases due to the higher values shown.

Body fat percentage (BFP) was also different between lean and overweight and lean and obese women (p ≤ 0.05); in men, differences (p ≤ 0.05) were found between lean and obese individuals. Overweight and obese participants of both sexes showed higher BFP than the recommendation, which confirms the nutritional status of the individuals (Table I) since the BMI does not consider the amount of fat in the body. Overweight and obese women and men showed higher VFA than the recommendation (lnbody 720 user manual) which also agreed with WC. Furthermore, WC showed the same behavior as BMI (Table I).

The WHR was significantly different between lean and obese women and men. Furthermore, obese women and men showed higher values than recommended, which indicates a risk of cardiometabolic diseases. On the other hand, no significant difference was found in WHtR overweight and obese individuals of both sex compared to lean individuals (Table I). Additionally, overweight and obese individuals of both sexes showed a risk of developing cardiovascular diseases.

In the case of HOMA-IR, both sexes showed higher values than the recommendations, except for the lean men group.

The total energy intake was not significantly different between lean, overweight and obese individuals (Table II). No significant differences were found between the groups and each macro-nutrient. However, when the percentage intake was compared

| Table I. Body composition and health indicators of the studied population |
|-----------------|-----------------|-----------------|
| Lean n = 6      | Overweight n = 5| Obese n = 6     |
| Weight (kg)     | 54 ± 6†         | 70 ± 8*         | 94 ± 21*        |
| BMI (kg/m²)     | 22 ± 2†         | 28 ± 1*         | 35 ± 6*         |
| BFP (%)         | 31 ± 6†         | 39 ± 3*         | 47 ± 4*         |
| VFA (cm²)       | 53 ± 12†        | 89 ± 12*        | 149 ± 51*       |
| WC (cm)         | 74 ± 6†         | 86 ± 5*         | 104 ± 20*       |
| WHR             | 0.80 ± 0.01†    | 0.83 ± 0.04†    | 0.87 ± 0.08*    |
| WHtR            | 0.46 ± 0.01†    | 0.5 ± 0.05*     | 0.6 ± 0.13*     |
| HOMA-IR         | 2.4 ± 1.3†      | 2.2 ± 0.9†      | 4.3 ± 1.1†      |

BMI: body mass index; BFP: body fat percent (normal range for men is 15.5% and for woman 23.5%); VFA: visceral fat area (normal range: < 100 cm²; over: 100–150 cm²; extremely over: > 150 cm²) (Inbody 720 user manual); WC: waist circumference (> 112 cm and ≥ 88 cm indicate high risk regarding men and women, respectively) (17); WHR: waist-hip ratio (> 0.90 in women and > 0.95 in men indicate high risk of cardiometabolic disease) (17); WHtR: waist to height ratio (> 0.5 indicates centralized obesity associated with cardiometabolic risk) (18); HOMA-IR: insulin resistance index (> 2.37 indicates insulin resistance) (19). Different symbols (*) mean difference (p ≤ 0.05) between groups of the same gender. The symbol † indicates that these values were high according to normal ranges for the Mexican population.
ANTHROPOMETRIC MEASURE AND ADIPOKINE LEVELS OF A YOUNG UNDERGRADUATE POPULATION WITH A USUAL DIET

with the Mexican population recommendations, it was observed that the intake of carbohydrates and proteins in both sexes agreed with the recommendations. In the case of lipids, it was found that all groups consumed more than 30%, which is higher than the recommendation (Table II). Additionally, both male and female groups consumed more animal fat than vegetable oil (data not shown); nevertheless, no significant differences were found. In addition, the intake of saturated fat was higher than the recommendation. The mono and poly-unsaturated fat agreed with the recommendation.

The biochemical values obtained in all groups were within the recommended range, in agreement with the reference values (data not show).

Significant differences in leptin levels between lean and obese women and men. The levels of leptin and insulin increased along with BFP (Table I and Fig. 1A and B).

On the other hand, no significant differences were found in the levels of inflammatory markers IL-6, IL-8, MCP-1 and TNF-α (Fig. 2A and 2B) and HGF (Fig. 2C) between overweight and obese women and men compared to lean individuals.

The correlation analysis between leptin and insulin levels with anthropometric parameters and health indicators is shown in table III. The other adipokines did not show any correlations.

### DISCUSSION

BMI is an indicator used to set the nutritional condition; however, this does not differentiate between lean and fat tissue since it does not consider body fat distribution, which is an important limitation due to obesity being more closely related to visceral adiposity than overall adiposity (21). There are other indicators

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**Table II.** Percentage of energy intake of each nutrient and recommendations for Mexican population

|                         | Women          | Men            | Recommendation (20) |
|-------------------------|----------------|----------------|---------------------|
|                         | Lean Overweight Obese | Lean Overweight Obese |  |
| Total energy intake     | 2,274 ± 1,499 2,066 ± 935 1,886 ± 447 | 2,231 ± 662 2,668 ± 2,080 1,885 ± 761 |  |
| Carbohydrates (%)       | 48 ± 7.5 49 ± 8 48 ± 8 | 49 ± 8 44 ± 8 45 ± 8 | 50-60 |
| Proteins (%)            | 15 ± 2 15 ± 2 14 ± 0.9 | 14 ± 3 14 ± 2 17 ± 3 | 10-15 |
| Lipids (%)              | 36 ± 6 36 ± 6 36 ± 4 | 36 ± 6 41 ± 11 39 ± 6 | 20-30 |
| Fat (%)                 |                 |                |                     |
| Saturated              | 11.4 ± 2.0 11.9 ± 2.0 11.3 ± 1.8 | 11.5 ± 2.7 12.0 ± 2.2 12.3 ± 1.8 | < 10 |
| Monounsaturated        | 15.1 ± 2.7 15.6 ± 2.5 15.1 ± 3.0 | 14.8 ± 2.9 17.3 ± 3.3 14.8 ± 2.7 | 15-20 |
| Polyunsaturated        | 6.9 ± 1.7 6.6 ± 1.6 7.6 ± 1.3 | 7.1 ± 2.7 8.6 ± 3.6 6.4 ± 1.7 | 6-11 |

**Figure 1.**

Leptin (A) and insulin (B) levels in serum of studied population. *Difference p ≤ 0.01.
which consider body fat distribution, such as WC and VFA, and the relations WHR and WHtR, which have also been associated with overweight and obesity (14,15). WHR has been also correlated with coronary heart disease, adult-onset diabetes mellitus and stroke (22). In this study, WC was significantly different between lean regarding overweight and obese women and men; in addition, the obese population showed health risks in accordance with Mexican recommendations (14) (Table I). Regarding WHR, obese women and men showed a risk of developing metabolic complications. In the case of WHtR, overweight and obese women and men showed a high cardiometabolic risk (15) (Table I).

In this work, to study the body composition of the studied population, BFP and VFA were used; they were also used as indicators of fat distribution. In general, the obese population showed the highest values of BFP and VFA (Table I), which could increase the health and metabolic risks (Inbody 720 user manual).

As there were no significant differences between the overweight and lean and obese groups, we considered the overweight groups to denote an intermediate state between lean and obese states. Therefore, the overweight condition must be considered as an independent group when obesity is studied. In addition, this behavior could indicate that there is a high possibility that their nutritional condition will soon be transformed to obese if they do not change their nutritional habits and lifestyle.

In this study, insulin resistance was also calculated according to HOMA-IR. A value of HOMA-IR of 4.3 and 5.0 was found for obese women and men, respectively, which is higher than the reference for the young Mexican population (23). Also, lean and overweight women and overweight men showed the same behavior. These studies also indicate that fat mass and insulin levels could be the major determinants of serum leptin levels in adults. In addition, this study showed a significant correlation between leptin and HOMA-IR ($r^2 = 0.41$) (Table III). Zuo et al. (24) reported an association between HOMA-IR and serum leptin level; the authors suggest that the leptin level could be used as an indicator of insulin resistance and that leptin level contributes to identify a metabolic risk.

Leptin levels were different ($p < 0.01$) according to sex. Women showed higher levels of leptin than men, possibly due to her body composition since they have more BFP (Table I and Fig. 1A). In addition, leptin levels increase according to BMI, VFA, WC, WHR and WHtR; however, the obese population did not show any significant differences between sexes. Therefore, it seems that leptin levels could be related to the fat content and its distribution in the body (Table III). These results were like those of Hellström

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**Figure 2.**

Adipokine levels in serum of studied population. A. Inflammatory markers levels of women. B. Inflammatory markers levels of men. IL-6, IL-8, MCP-1 and TNF-α. C. Hepatic grow factor levels of women and men.
et al. (25), who explained that a higher proportion of adipose tissue in women increased the production of leptin per unit mass of adipose tissue in comparison to men.

On the other hand, the levels of insulin were high in obese women and men (761.4 and 852.7 pg/ml, respectively); these values were higher than those recommended by reference ranges of the Endocrine Self-Assessment Program (56-564 pg/ml) (26). As in the case of leptin, the levels of insulin increased along with BMI, VFA, WC, WHR and WHtR.

For the Mexican population, there are no levels of references reported for inflammatory markers; however, our results (Fig. 2A-C) agreed with those of Kleiner et al. (2013) (27) and Kim et al. (2011) (28), who reported similar levels in young healthy individuals to those aged 18 years and above. No significant differences were found in IL-6, IL-8, MCP-1 and TNF-α and HGF between lean, overweight and obese women and men, probably due to the healthy state of the studied population since their biochemical parameters were in normal range. In addition, no correlation was found between these and any anthropometric parameters, possibly due to the size of the studied population. It is important to highlight that obesity is an inflammatory cellular stage and that the elevation of inflammatory markers (IL-6, TNF-α) is age-associated (26); hence, it is important to study these markers of obesity during youth.

In conclusion, the use of health indicators such VFA, WC, WHR, WHtR and HOMA-IR is a useful tool in the determination of health risk and these indicators are correlated with the levels of leptin and insulin in the studied population, which could probably be due to BFP and the high intake of fat from food. If the nutritional habits of study groups are not modified, these levels could remain and, in the future, lead to a decrease in the healthy state.

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**Table III. Correlation between leptin and insulin with anthropometric parameters and health indicators**

|          | Leptin | Insulin |
|----------|--------|---------|
| Weight   | 0.60*  | 0.45*   |
| BMI      | 0.67*  | 0.37*   |
| BFP      | 0.75*  | 0.40*   |
| VFA      | 0.66*  | 0.48*   |
| WC       | 0.60*  | 0.49*   |
| WHR      | ---    | 0.43*   |
| WHtR     | 0.67*  | 0.42*   |
| HOMA-IR  | 0.41*  | ---     |

*Differences p ≤ 0.05. BMI: body mass index; BFP: body fat percent; VFA: visceral fat area; WC: waist circumference; WHR: waist-hip ratio; WHtR: waist to height ratio; HOMA-IR: insulin resistance index.*
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