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HOMA-IR Anomalies and Sugar Consumption in Young with Euglycemia

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Abstract: The aim of this study was to assess sugar consumption through a 24 h recall questionnaire, its quantification was performed with a Web-based application and then correlated with HOMA-IR. Seventy-four women and fifty-two men participated in the study, $\bar{x}$ 18 years old. Data were retrieved from the 24 h recall questionnaire and the amount of sugar in food and drinks was quantified. The quantities of sugar were assessed with the online application of www.fatsecret.com.mx. Likewise, we quantified glucose, triglycerides, total cholesterol, insulin, C peptide and glycosylated hemoglobin. Fifty-two (41.3%) participants presented biomarkers within desirable levels and 74 (58.7%) presented at least one value within the risk level. There were no significant differences in the average values of body mass index, waist-hip and waist-size indices between those with desirable values and those with anomalies. Among the young population with biomarkers within desirable values, 10% presented abnormal insulin and HOMA-IR values; besides, 25% of them had glycosylated hemoglobin values of ≥6.2%. There was a correlation between sugar consumption and HOMA-IR, ($p = 0.007$). In the young population with euglycemia, anomalies in HOMA-IR and glycosylated hemoglobin values can be found. Assessment of elevated dietary sugar consumption could predict anomalies in glycosylated hemoglobin and HOMA-IR.

Key words: Prediabetes, diabetes, sugar consumption, homeostatic evaluation index of insulin resistance

INTRODUCTION

Studies in children and adolescents have shown an increase in chronic non-communicable diseases such as type 2 diabetes (Pinhas and Zeitler, 2013; Cali and Caprio, 2008) or obesity-associated metabolic syndrome (Juarez *et al*., 2010; Cardenas *et al*., 2010). Hence, one of the main criteria to assess biomarkers for chronic non-communicable diseases has been the diagnosis of obesity. However, there are other data that reveal a lack of correlation between anthropometric indices and a possible diagnosis of type 2 diabetes (Munoz *et al*., 2013; Zvarova *et al*., 2013; Zhao *et al*., 2012; Irving *et al*., 2011; Sanchez *et al*., 2004).

Based on this information, it is necessary to establish methods for the diagnosis of risk factors that will allow determining those with the greatest possibilities of presenting anomalies in their biochemical markers and which laboratory tests could confirm this assumption. Given that fructose consumption, not the one found in fruits but the one used in modern industrialized food and drinks, is the main factor associated with increased probabilities of developing non-communicable diseases (Regnault *et al*., 2013; Lusting *et al*., 2012; Popkin, 2012), evaluation of its daily consumption could be indicative of the presence of anomalies in the biochemical markers.

The 24 h recall questionnaire allows evaluating consumption patterns of foods or of a particular nutrient. Although it is simple, it requires rigorous data collecting since relevant information can be omitted if variations in the products presentation or in the preparation of food are not taken into accounts, hence web-based resources are needed (Leijdekkers and Gay, 2013). Although, the best way to gather accurate data is to apply it three days in a week three times a year (Martin *et al*., 1993), consumption patterns could be assessed from a single questionnaire (Zamora *et al*., 2012). The use of the 24 h recall questionnaire has been useful to assess the association between those consumption patterns and the possibility of developing chronic non-transmissible diseases (Von Ruesten *et al*., 2013) or of dietary habits (Burriel *et al*., 2013), but not as a factor associated with anomalies in biochemical markers.

Objectives of the study: In this context, the objective of this research was to correlate the results of the questionnaire regarding food consumption patterns, specifically of the amount of fructose in both food and drinks measured with the app Fatsecret® in Spanish and the values of the biochemical markers.

MATERIALS AND METHODS

Study design: This study was performed as an analytical, cross-sectional exploratory study of correlation, with a non-probabilistic sample.

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Study population: Participants were freshman students of the Health Sciences Academic Division (HSAD) of the Autonomous Juarez University of Tabasco (Universidad Juarez Autonoma de Tabasco, UJAT), located in Southeast Mexico. Courses started at the end of August 2013. All freshman students at the UJAT are subjected to laboratory analyses as part of the enrollment process to the University.

Twenty four hour recall questionnaire: The recall questionnaire on food consumption in 24 h was applied from Tuesday to Friday, following the guidelines described in the "Manual of procedures for nutrition projects" (Shamah *et al*., 2006). Emphasis was placed on amounts and trademarks of the consumed products. Although there is a database on food composition at http://ndb.nal.usda.gov/, this does not depict products of the Mexican market. Therefore, to calculate the amount of sugar not coming from fruits we accessed the information provided at http://www.fatsecret.com.mx/ in Spanish.

Anthropometry: After the 125 participants agreed to it, we obtained their data on weight, height, waist and hip perimeters according to WHO guidelines (WHO, 2011). To determine the body mass index (BMI), a clinical weighing scale with a height meter was used (Basculas Nuevo Leon®, Mexico) with a 200 kg capacity. The scale was calibrated each day during the time of students' assessments. The BMI (Quetelet index) was calculated according to the equation: [weight/height^2 = kg/m^2] and interpreted according to the Mexican standard PROY-NOM-043-SSA2-2011. For the waist and hip perimeter measurements, we used non-extensible millimetric fiberglass tapes with a length of 1.80 m and a width of 1 cm (Vitamex®, Mexico). For the population of the geographical zone the desirable hip measurement for women is 80 cm and 90 cm for men (WHO, 2011). The waist-hip index (WHI) was calculated by dividing the waist perimeter by the hip perimeter, considering a value of 0.85 for women and 0.90 for men as cutoff values (He *et al*., 2009). The waist to height ratio (WHR) was calculated, for which a value of 0.5 is desirable (WHO, 2011).

Biochemical parameters: The 12 h fasting blood samples were obtained by the personnel of the clinical analyses laboratory of the UJAT. Sterile equipment was used and blood was collected with Vacutainer® Serum tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Glucose (GL) triglycerides (TG) and total cholesterol (TC) were measured in the serum using a dry analytical methodology with the automated equipment VITROS® 250 (Ortho-Clinical Diagnostics Johnson and Johnson, Rochester, NY, USA).

Glucose was evaluated according to criteria of the Mexican standard "NOM-015-SSA2-2010", which establishes values of $\leq$5.55 mmol/L as healthy, altered fasting glucose (prediabetes) values of 5.6 to 6.9 mmol/L and for diabetes, values $\geq$7 mmol/L. To assess the levels of TG and TC, criteria of the Mexican standard "NOM-037-SSA2-2012" were followed. TG was considered desirable at $\leq$1.68 mmol/L, borderline 1.69 to 2.25 mmol/L and high $\geq$2.26 mmol/L. Desirable TC values were $\leq$3.9 mmol/L, borderline low 4 to 4.39, borderline high 4.4 to 5.16 and high $\geq$5.17.

For glycosylated hemoglobin, the Glycohemoglobin Pre-Fil kit of Stanbio Lab® (Boerne, TX, USA) was used. For each standard and sample, the range of absorbance was calculated according to the formula provided by the manufacturer. The conversion factor was used and results were reported as A1c, a value between 4.2 and 6.2% was considered normal.

Insulin, C-peptide and HOMA-R: Insulin and C-peptide were determined in an Architect 2000 equipment from Abbot Diagnostics® (Mississauga, Ontario, Ca). Insulin resistance was measured with the homoeostasis model assessment index (HOMA-IR). This was calculated by means of the following equation: [(fasting glucose in mg/dL) (fasting insulin in U/mL)/405] (Keskin *et al*., 2005). As cutoff limit, a value of 3.4 was taken to determine the insulin resistance, which corresponds to the percentile 90 for a population younger than 20 years and otherwise healthy (Juarez *et al*., 2010; Garcia *et al*., 2007).

Statistical analysis: Data processing was accomplished with version 21.0 of the IBM SPSS Statistics Package for the Social Sciences (Chicago, IL, USA). Central tendency descriptive statistics and contingency tables were used for data analyses. The Kolmogorov-Smirnov test was used to analyze data distribution. Frequencies were estimated with 95% of confidence interval (95% CI) for each body measurement and biochemical marker. To determine the predictive value of each anthropometric index and of the questionnaire, chi-square tests were used, as well as probability of significant differences and the z score with Microsoft Office Excel® 2007, considering value in statistics tables $\leq$1.96 and $p \leq 0.05$ as significant.

Ethical considerations: According to the guidelines of the UJAT, the project was evaluated in two aspects. The methodology by the Research Committee and by the Ethics Committee to warrant fulfillment of the World Medical Association Declaration and the provisions of the Mexican General Health Law for Health Research. Once reviewed and accepted the study was registered as No. 2013-0051 of the Secretariat of Research, Graduate Studies and Vinculation of the UJAT. All students were invited to participate anonymously in the study, once they accepted, the signed the informed consent form. They were also told that they could withdraw from filling out the questionnaire whenever they wished to. Each questionnaire was ascribed a registry number to avoid identification of the students.

RESULTS

The 856 freshman students enrolled at the HSAD presented values of fasting glucose of $\bar{x}$ 4.76 mmol/L (SD 0.49, CI 95% 4.73-4.8); triglycerides, $\bar{x}$ 1.24 mmol/L (SD 0.72, CI 95% 1.19-1.29); total cholesterol, $\bar{x}$ 4.49 mmol/L (SD 0.93, CI 95% 4.43-4.56). Of these, 6.6% presented GL values of $\geq$5.6 mmol/L considered prediabetes, 18.6% presented TG of $\geq$1.68 mmol/L and 18% had TC of $\geq$5.1 mmol/L. The Kolmogorov-Smirnov test revealed that glucose and cholesterol data were distributed in asymmetric curves, with the mean closer to the upper limit. The triglycerides curve was more asymmetric, with the mean very close to the right of the upper limit (Fig. 1).

All students, regardless of their results, were invited to participate in the next phase of the study. This was accepted by 126 students, 85 women (67%, $\bar{x}$ 18.58 years) and 41 men (33%, $\bar{x}$ 18.76 years), this is because women predominate in the curricula of medicine, nursing, psychology, odontology and nutrition. Of the 125, 52 (41%) had normal values of GL, TG and TC and 74 (59%) had at least one value in the risk level. The ANOVA test among the participants, regarding the whole original HSAD population, revealed F (Fisher-Snedecor) values of 49 for GL, 21 for TG and 0.16 for TC. When compared to the critical value of 3.85, it was inferred that the means of the whole population and the sample were similar for GL and TG, but not for TC.

Correlations among glucose, triglycerides and cholesterol: The Pearson test was applied to the students with anomalies. Correlations were found between glucose and triglycerides ($p = 0.002$), cholesterol and triglycerides ($p = 0.008$) and between glucose and cholesterol ($p = 0.02$).

Anthropometric parameters and biochemical markers: The averages of the anthropometric indices lied towards the upper limit rather than in the mid-range of the reference intervals. The Pearson correlation test did not reveal significant differences in the average values of BMI between those showing markers within desirable levels and those with anomalies. The same was found for the waist-hip index and waist to height ratio. The lack of differences was also found when comparing between genders.

Insulin resistance and fasting glucose: Of the 74 (59%) students with at least one biochemical anomaly, 38 had fasting glucose values of $\geq$5.6 mmol/L and 14 had cholesterol values of $\geq$a 4.4 mmol/L. In those 52 (41%) with glucose, triglycerides and cholesterol values within desirable levels, insulin was of $\bar{x}$ 8.63 (SD 4.38) and HOMA-IR was of $\bar{x}$ 1.95 (SD 1.19). The amplitude of the standard deviations (SD) in the insulin and HOMA-IR values was because 10% of these students presented anomalies in these markers. In 23% of them, HbA1c was of $\bar{x}$ 6.2%. In the 74 students with at least one anomaly, insulin values were of $\bar{x}$ 11.16 (SD 8.78) and HOMA-IR $\bar{x}$ 2.63 (SD 2.22). In this group, the proportion of HbA1c $\geq$6.2% was of 34% and HOMA-IR values of $\geq$3.5 were found in 19% of these students.

When analyzing the possibility of presenting HOMA-IR anomalies according to whether they presented elevated values of fasting glucose, triglycerides and cholesterol or desirable values, the χ^2 value was 1.66. This value is quite distant from the critical value of 2.7 with one degree of freedom, indicating that there were no differences in the possibility of presenting insulin resistance between those with anomalies and those without them.

Twenty four hour recall and anthropometry: Although the 24 h recall questionnaire does not inquire about specific products, when applying the questionnaires, we collected data on trademarks and presentations by applying http://www.fatsecret.com.mx aimed at determining the amount of sugar not coming from fruits. The tests to analyze the relation between the anthropometric indices and the consumption of sugar did not reveal significant differences ($p<0.05$).

Twenty four hour recall and insulin resistance: Averages of table sugar consumption, like high fructose syrup, was high in both those with desirable values in the markers and those with anomalies: $\bar{x}$ 113.79 g (SD 80.04) and $\bar{x}$ 114.3 g (SD 64.9), respectively. The Pearson test in students with desirable values of fasting glucose, triglycerides and cholesterol revealed a statistical value of 0.547 with $p = 0.007$ for the correlation between sugar consumption and HOMA-IR. In this group of participants with desirable levels, there was also a correlation between sugar consumption and the insulin value, 0.475 and $p = 0.022$, as well as between HOMA-IR and triglycerides, 0.420 and $p = 0.046$.

DISCUSSION

The results of glucose, triglycerides, cholesterol and HOMA-IR measurements obtained in the freshman students at the UJAT were correlated with the amount of extra sugar in their food and drinks. Although being part of the Latino ethnic group is already a risk factor for type 2 diabetes mellitus, this study shows evidence of the possibility of developing anomalies in the biochemical markers regardless of the body mass, which has already been shown for this population (Munoz *et al*., 2013). These data suggest that changes in food

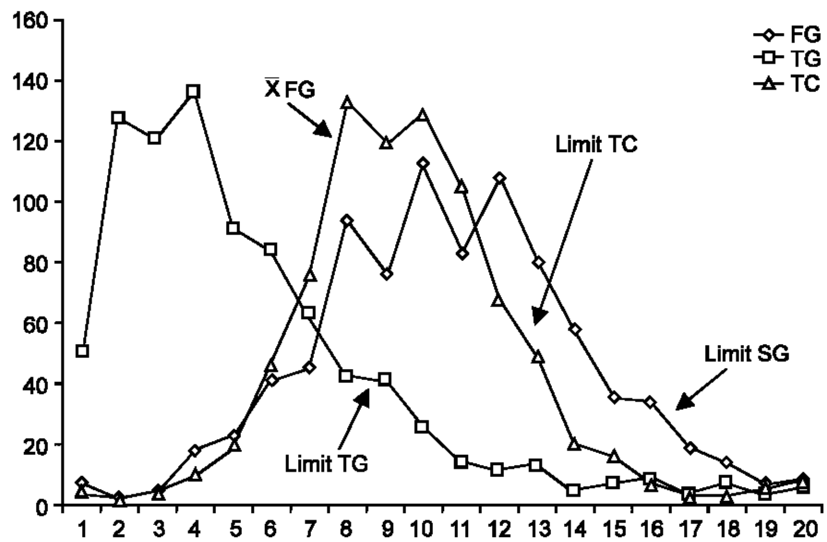

Fig. 1: Distribution curves of glucose, triglycerides and cholesterol of the whole HSAD population. Kolmogorov-Smirnov test results. In the vertical axis, the frequency. The numbers in the horizontal axis correspond to the intervals. FG = fasting glucose, TG = triglycerides, TC = total cholesterol. The mid value within the reference values for glucose (4.71 mmol/L) is found at interval 10, the cut-off limit for prediabetes (5.6 mmol/L) lies at interval 16. The cut-off limit for triglycerides (1.68 mmol/L) lies at interval 8; the high value (2.25 mmol/L), at interval 14. The cut-off limit for cholesterol (5.1 mmol/L) lies at interval 12. N = 856

Table 1: Average values of the participants' indicators

	Desirable	Anomalies
Indicator	---------------------- $\bar{x}$ (SD)	----------------------
BMI women	23.73 (4.28)	24.36 (4.3)
BMI men	20.02 (3.55)	28.39 (3.35)
WHI women	0.81 (0.09)	0.81 (0.06)
WHI men	0.83 (0.1)	0.9 (0.04)
WHR women	49.45 (6.65)	50.94 (5.27)
WHR men	50.9 (6.0)	56.88 (2.96)
Glucose	4.74 (0.49)	5.41 (0.61)
Triglycerides	87 (4.38)	186 (90.67)
Cholesterol	157 (22.2)	194.35 (40.59)
HbA1c	4.9 (2.4)	7.1 (3.7)
Insulin	8.63 (4.38)	11.16 (8.78)
HOMA-IR	1.95 (1.19)	2.63 (2.22)

SD: Standard deviation. BMI: Body mass index. WHI: Waist-hip index. WHR: waist-height ratio. Glucose, triglycerides and cholesterol in mmol/L. HbA1c in %. Insulin in µU/mL. n = 126

consumption patterns (Perichart *et al.*, 2010; Ventura *et al.*, 2008), rather than overweight, must be considered as a risk factor for diabetes.

The average consumption of sugar added to food and drinks was similar to that of Latino adolescents (Ventura *et al.*, 2008). However, in contrast to that study in which an increase in the cardiovascular risk was found in those with a BMI of $\geq$ to the 85th percentile, in the data reported herein we found anomalies in the biomarkers with a BMI previously considered as "normal" or "healthy". Although half of the participants had triglyceride and cholesterol values above the desirable levels, we did not find an association between them and the consumption of extra sugar as has been reported in other studies (Welsh *et al.*, 2011; Kell *et al.*, 2014).

Fasting glucose values in the population of 856 freshman students was found to be above the cut-off limits for normal values (4.76 mmol/L) in 51.9% of them, which agrees with the slowly ascending fasting glucose data in the general population observed worldwide. This has occurred more rapidly in those countries that modified recently there feeding patterns, as is the case of Mexico (Danaei *et al.*, 2011). Although most triglycerides values are below the limit between desirable and intermediate, its asymmetric curve is also prolonged to the right (Fig. 1).

The anomalies found in the participants in regard to elevated glycosylated hemoglobin and insulin, as well as the diagnosis of insulin resistance assessed through HOMA-IR, provide the foundations for the need of risk factor indicators with a greater capacity of association with biochemical markers. This is even more relevant because the diagnosis of type 2 diabetes mellitus is made without association to anthropometric indicators (Munoz *et al.*, 2013; Zvarova *et al.*, 2013; Zhao *et al.*, 2012), which are the basis to assess the metabolic profile of an otherwise healthy population. Although other studies have not found an association between sugar consumption and HOMA-IR (Wang *et al.*, 2014), it has not been taken into account the difference between the carbohydrates from fruits and vegetables that contain prebiotics, like resistant starches and inulin

and the sugar added to food and drinks. Our results are more consistent with those emphasizing the differences among types of food and not only as a function of macronutrients (Lau *et al.*, 2005; Stanhope *et al.*, 2011). The strength of the study lies in the similarity of the 125 participants and 856 freshman students. The results from the measurement of the biomarkers obtained in this study, as well as those obtained in youngsters from the same geographical area (Munoz *et al.*, 2013) reveal the urgent need to design new strategies to identify the people with risk factors for type 2 diabetes mellitus.
Since we are dealing with a non-probabilistic sample, the results cannot be extrapolated to the general population; however, we show that identifying the products with industrialized sugar content is related with the probability of finding anomalies in the biochemical markers, particularly with those not usually determined like HbA1c, insulin and HOMA-IR. On the other hand, the results provide the rationale for a wider epidemiological study. The study also sets forth the use of fitness applications, like Fatsecret®, as an area of opportunity in the school system at all levels, as these are elements that contribute to the knowledge in health sciences. Although the filling out of the 24 h recall questionnaire is simple, it must be taken into account that an intentional search of the consumption of products with a high content of industrialized sugars is necessary, not just of starches and fats. It is necessary to manage data with applications (APPS) for mobile devices or through the Internet.

Conclusion: The fast increase in the prevalence of type 2 diabetes mellitus can be reversed by analyzing biomarkers in the young population. Although useful, routine assessments of fasting glucose, triglycerides and cholesterol can be insufficient to detect anomalies in glucose metabolism. The amount of consumed sugar added to food and drinks, measured with the 24 h recall questionnaire, can be an indicator of anomalies in the insulin effect.

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