

Effect of Eating *Oreochromis aureus* on Biochemical Markers in Young People

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Abstract

Background: Although non communicable diseases have a multifactorial origin, changes in eating patterns seem to weigh more. Population groups that maintain their traditional eating patterns have a lower prevalence of these diseases.

Methods: Thirty-five students of the Universidad Autónoma de Tabasco, 22 women (62%, \bar{X} 18.58 years) and 13 men (37%, \bar{X} 18.76 years), were given 100 grams of *Oreochromis aureus* daily, five days a week, during eight weeks. Data on anthropometry, glucose, triglycerides, cholesterol, glycosylated hemoglobin, insulin and the HOMA-IR index were recorded before and after providing the fish.

Results: In the experimental group, significant differences were observed between the first and the second measurement of insulin ($p=0.004$) and of the HOMA-IR ($p=0.0001$). Although an increase in the amount of insulin was evident in the second value, it was not greater than the cutoff point plus one SD, according to reference values. A significant difference in after-fasting glucose values was recorded for the women, with a smaller second value ($p=0.003$), however this did not occur in the case of the men. A decrease in after-fasting glucose values ($p=0.015$) was recorded for the group that presented at least one anomaly, in contrast with the group that presented no anomaly in the initial evaluation ($p=0.078$). The group with no anomaly presented a slight increase in insulin ($p=0.074$), as well as an increase in the HOMA-IR ($p=0.019$), with an average in the reference intervals. A significant difference in the initial glucose ($p=0.006$) and HbA1c ($p=0.008$) values was recorded between the two groups.

Conclusions: The addition of 100 grams of *Oreochromis aureus* was accompanied by an increase in insulin values and a decrease in after-fasting glucose values, with no changes in glycosylated hemoglobin or lipids.

Keywords: Hypoinsulinemia; Type 2 diabetes mellitus; Functional food items; Tropical fish

Introduction

Non communicable diseases constitute the main health problem on a global scale, and as a group they are the main cause of death. It is estimated that they are responsible for 65% of worldwide deaths [1], a percentage that increases to 75% in Latin America [2]. Thus, type 2 diabetes mellitus, which in 1931 caused 1% of deaths in Mexico, caused 5.8% in 2001 [3], increased rapidly to 11.85% in 2007 [4] and was considered the first cause of death in 2012. Together with an increase in the prevalence of type 2 diabetes in Mexico 14% of the ≥ 20 year-old population [4] important reductions have been observed in the consumption of food items that used to be common in daily life.

The change from traditional diets to diets with greater proportions of modern industrialised food is the most important factor in explaining the pandemic [5]. In contrast, populations that maintain the habit of eating local ethnic cuisine dishes, like the Mediterranean [6,7], the Japanese [8,9] and the Mexican [10-12], have a smaller probability of developing non transmissible chronic diseases. This is the reason behind studies that analyse the protective effects of food items and dishes of traditional cuisines.

The traditional cuisine of Tabasco, in southeastern Mexico, includes fish, as the state of Tabasco is located on a flood plain subject to seasonal flooding. The two greatest rivers in Mexico are located in this region, as well as numerous lagoons inhabited by freshwater species. As a result of globalisation, many endemic species have been substituted by others that are more easily bred or have lower production costs. Such is the

case of fish in Tabasco, particularly where it is difficult to cultivate wild species in farms. Tilapia fish (*Oreochromis* spp) was introduced from Africa and adapted well to the climate. The consumption of this farmed fish has increased in importance, considering feeding security. It is the main aquatic product of Tabasco, only after oysters (*Crassostrea* spp), with 3487 tons recorded in 2011 [13]. For these reasons, the purpose of this study was to evaluate the effect of eating this fish on changes in biochemical markers.

Materials and Methods

Type of study

The study was experimental, analytical, longitudinal and prospective.

Study participants

Newly arrived students of the División Académica de Ciencias de la

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Salud, of the Universidad Juárez Autónoma de Tabasco (DACS-UJAT) that started class the last week of August 2013.

Study design

The clinical analysis laboratory carries out studies on the newly arrived students of the UJAT as part of the enrollment process to the University. Those that enrolled in the DACS-UJAT were invited to take part in this study, and 35 students accepted. As the Bachelor's courses on campus (Medicine, Nursing, Psychology, Odontology, Nutrition) have a greater number of women, 22 were women (62%, \bar{X} 18.58 years) and 13 were men (37%, \bar{X} 18.76 years). The study provided 100 grams of fish daily, the five days per week that the students were on campus. The fish was bought in a local fish farm to guarantee that the product was the same species (*O. aureus*) and that it satisfied the quality controls established in the norms [14]. There was a control group with students with the same characteristics, 13 women and 6 men, who was recruited at the same time.

The experimental phase (eight weeks) started on Tuesday 17th September and ended on Friday 8th November. The students were instructed to maintain their eating patterns and were told that the food provided was not to constitute an extra supply of calories. The fish was prepared in several ways, except fried or breaded, and fatty dressings like mayonnaise were avoided, following nutritional recommendations, as fatty acids in oils compete with those in the fish [15,16], apart from having more calories than food that is not fried and food without a dressing [17].

Anthropometry

Body mass indices were calculated following the indications established by the WHO [18] using a clinical scale (Básculas Nuevo León®, México) with capacity for 200 kg and a estadiometer. The scale was calibrated every day during the study. Body mass indice (BMI) were calculated with the mathematical expression: $\text{mass/height}^2 = \text{kg}/\text{m}^2$ and interpreted according to the PROY-NOM-043-SSA2-2011 for Mexico [19]. For women taller than 1.5 m and men taller than 1.6 m, a low weight is ≤ 18.49 , eutrophic is 18.5 - 24.99, overweight is 25 - 29.99 and obese is ≥ 30 . For women shorter than 1.49 m and men shorter than 1.59 m, a low weight is ≤ 18.49 , eutrophic is 18.5 - 22.99, overweight is 23 - 24.99 and obese is ≥ 25.9 .

Extensible millimetric fiber glass Vitamex® México measuring tapes, 1.80 m long and one cm wide, were used to obtain waist and hip measurements. The ideal hip measurements for the population of this geographical area are 80 cm for women and 90 cm for men [18]. The waist-hip index was calculated by dividing the waist perimeter by that of the hip, where values of 0.85 for women and 0.90 for men were considered the cutoff point [20]. The waist-height index was calculated as waist/height, with a value below 0.5 considered desirable [18].

Biochemical parameters

Blood samples were obtained from the students by the personnel staff of the clinical analysis laboratory of the UJAT after a 12-hour fast. Sterile equipment was used and blood was collected in Vacutainer® Serum flasks (Becton Dickinson, Franklin Lakes, NJ, USA). Glucose (GPA), triglycerides (TG) and total cholesterol (TC) were recorded following a dry analytical method using the automated equipment VITROS® 250 (Ortho-Clinical Diagnostics Johnson & Johnson, Rochester, NY, USA).

Glucose was evaluated following the criteria of the "NOM-015-SSA2-2010" [21] where ≤ 5.5 is desirable, 5.6 - 6.9 mmol/L represents

altered after-fasting glucose values (pre-diabetes) and ≥ 7 mmol/L indicates diabetes. Triglyceride and cholesterol levels were evaluated following the criteria of the "NOM-037-SSA2-2012" [22]. TG values ≤ 1.68 mmol/L are desirable, 1.69 - 2.25 mmol/L are borderline and ≥ 2.26 mmol/L are high. TC values ≤ 4.39 mmol/L are desirable, 4 - 4.3 are borderline low, 4.4 - 5.16 are borderline high and ≥ 5.17 are high.

Glycosylated hemoglobin

A Glycohemoglobin Pre-Fil reagent kit of StanbioLab® (Boerne, TX, USA) was used. The range of absorbance was calculated following the manufacturer's formula for each standard and sample. A conversion factor was used and the results are reported as HbA1c. Values between 4.2 and 6.2% are considered normal.

Insulin, C peptide and HOMA-IR

Insulin and C peptide were recorded with Architect 2000 equipment from Abbot Diagnostics® (Mississauga, Ontario, Ca). Insulin was considered normal at ≤ 19.3 $\mu\text{U}/\text{mL}$ [23]. Resistance to insulin was determined through the HOMA-IR index. This was calculated with the following equation: $(\text{after-fasting glucose in mg/dL}) (\text{after-fasting insulin in } \mu\text{U}/\text{mL})/405$ [24]. The cutoff value to determine resistance to insulin was 3.4, which corresponds to percentile 90 for a population of healthy under-age people [25,26].

Statistical analyses

A Statistics Package for the Social Sciences edition 15.0 (SPSS, Chicago, IL, USA) was used to process the data. Data were analysed through central tendency descriptive statistics, together with contingency tables. A related samples test was used to compare the variables of the groups, according to the biochemical markers and the sex. Pearson's correlation test was also used to compare variables among groups and between sexes. A $p \leq 0.01$ was considered statistically significant.

Ethical considerations

The study was approved by the Comité de Investigación of the División de Ciencias de la Salud, registered in the Secretaría de Investigación of the Universidad de Tabasco, number 2011-1232. The study satisfied the Helsinki Declaration of the World Medical Association, and Mexico's norm for health research Ley General de Salud en Materia de Investigación para la Salud.

Results

The data recorded before and after providing the 100 gr of *Oreochromis aureus* are presented in Table 1. One of the objectives of the study was to maintain the media of the values recorded for the anthropometric indices, and these presented no differences. This was proved by the related samples tests for the body mass, waist-hip and waist-height indices, with the first obtaining a $t=-0.870$ and a $p=0.392$. Similar data were obtained for the other two indices.

The after-fasting glucose values identified seven students with an initial glucose value ≥ 5.6 mmol/L, and only one at the end of the study, with a $t=3.319$ and a $p=0.003$. Significant differences were recorded between the first and the second measurement of insulin with a $p=0.004$ and a HOMA-IR, $p=0.000$. Although an increase in the amount of insulin was evident in the second value, it was not greater than the cutoff point plus one SD, according to reference values. An analysis of the initial and final HbA1c values showed only a decrease in the width of the standard deviation ($p=0.964$), with an average at the cutoff point

	Sample (n = 35)		Control (n = 19)	
	Initial	Final	Initial	Final
Weight	65.15 (14.6)	65.59 (15.94)	67.71 (14.2)	66.36 (15.1)
Waist	83.13 (11.9)	83.48 (12.12)	83.09 (12.5)	83.27 (12.9)
Hip	100.05 (10.87)	99.89 (11.57)	99.57 (11.25)	100.01 (11.32)
BMI	24.86 (4.6)	25 (5.13)	25.2 (4.9)	25 (4.76)
WHil	0.83 (0.09)	0.84 (0.12)	0.83 (0.04)	0.83 (0.93)
WHel	51.5 (6.86)	51.7 (7)	51.6 (7.2)	51.8 (7.7)
Glucose	93.68 (9.7)	87.05 (7.7)*	88.6 (9.9)	91.08 (9.4)
Triglycerides	125.93 (59.9)	124.31 (63.95)	124.39 (66.9)	123.28 (72.3)
Cholesterol	177.51 (48.41)	171.86 (41.27)	170.38 (50.11)	176.66 (53.8)
HbA1c	6.38 (3.24)	6.13 (1.9)	6.35 (2.4)	6.32 (2.2)
Insulin	7.7 (5.19)	10.99 (5.19)*	7.8 (5.27)	7.6 (6.88)
HOMA-IR	1.74 (1.24)	3.12 (2.06)*	1.71 (1.33)	1.72 (2.2)

Table 1: Initial and final values. BMI = body mass index, WHil = waist-hip index, WHel = waist-height index, HbA1c = glycosylated hemoglobin. *Significant differences in the related samples test when $p \leq 0.01$.

	Women (n = 22) (SD)		Men (n = 13) (SD)	
	Initial	Final	Initial	Final
Weight	60.79 (13.23)	60.85 (14.06)	78.88 (9.63)	80.50 (12.31)
Waist	79.86 (11.22)	80.13 (11.14)	93.42 (7.5)	94 (8.98)
Hip	98.38 (11.22)	97.5 (11.10)	105.28 (8.3)	107.42 (10.3)
BMI	23.86 (4.68)	23.87 (4.98)	28 (3.21)	28.55 (4.05)
WHil	0.81 (0.09)	0.82 (0.13)	0.88 (0.06)	0.87 (0.04)
WHel	50.13 (6.97)	50.33 (7.05)	55.81 (4.56)	56.13 (5.16)
Glucose	93.00 (10.18)*	85.77 (7.62)*	95.87 (8.74)	91.07 (7.35)
Triglycerides	125.18 (66.8)	112.18 (64.50)	128.28 (33.40)	162.42 (47.85)
Cholesterol	177.09 (46.81)	168.31 (37.42)	178.85 (57.11)	183 (53.43)
HbA1c	6.24 (3.43)	6.28 (2.07)	6.84 (2.76)	5.68 (1.68)
Insulin	7.65 (5.5)*	10.60 (6.64)*	7.85 (4.47)	12.22 (2.47)
HOMA-IR	1.77 (1.32)*	3.23 (2.35)*	1.63 (1.07)	2.75 (0.6)

BMI = body mass index, WHil = waist-hip index, WHel = waist-height index, HbA1c = glycosylated hemoglobin. * = Significant differences in the related samples test when $p \leq 0.01$.

Table 2: Comparison between women and men.

of 6.2%. Triglycerides and total cholesterol presented no differences.

With respect to the sex (Table 2), the after-fasting glucose values differed significantly in the women, with a decrease in the second measurement ($p=0.003$), in contrast with the men. Although the women's insulin values increased only slightly ($p=0.029$), the HOMA-IR values were significant ($p=0.003$). No differences were recorded for the men's insulin values ($p=0.067$) and the HOMA-IR ($p=0.036$), nor for the HbA1c ($p=0.042$). Pearson's correlation test indicated a significant association between the initial insulin values and the HOMA-IR in the women ($p=0.0001$), as well as between the final insulin values and the HOMA-IR in the men ($p=0.001$).

A comparison of the anthropometric indices of the students that presented anomalies and of those that did not indicated no significant differences in the media of either their pre-treatment ($p=0.390$) or post-treatment ($p=0.186$) BMI. Similar data were recorded for the other indices (Table 3). A significant difference was recorded between the initial glucose ($p=0.006$) and initial HbA1c ($p=0.008$) values of the two groups. A decrease in after-fasting glucose values ($p=0.015$) was recorded for the group that presented at least one anomaly, in contrast with the group that presented no anomaly in the initial evaluation ($p=0.078$). The group with no anomaly presented a slight increase in insulin ($p=0.074$), as well as an increase in the HOMA-IR

($p=0.019$), with an average in the reference intervals. In those that had no anomalies in the initial evaluation, the value of insulin increased slightly ($p=0.027$) and the HOMA-IR increased significantly ($p=0.01$), also with an average within the reference intervals. Pearson's test determined a significant correlation between the initial insulin and the HOMA-IR ($p=0.0001$) and between the final insulin and glucose ($p=0.004$) in those that presented at least one anomaly.

Discussion

Studies on fish diets and diabetes are controversial. Although several studies have observed a protective effect after eating fish [27-29], others have found inconsistent data [30-32] or have observed an increased risk from eating fish rich in omega 3 fatty acids [33]. As epidemiological studies analyse data for populations with a wide variety of feeding patterns, customs and geographical areas, this study was carried out to evaluate the effect of a specific fish in a tropical area, on glucose metabolism.

The selection of students according to the presence or absence of anomalies in the biochemical markers, rather than considering anthropometric indicators, was based on data related to the lack of association between body volume, or even the circumference of the waist, and the possibility of presenting anomalous glucose values [34,35]. As may be seen in Tables 2 and 3, there were no differences

	With anomalies (n = 19) (SD)		Without anomalies (n =16) (SD)	
	with anomalies	Final	Initial	Final
Weight	66.37 (15.65)	66.4 (16.75)	64.16 (14.13)	64.93 (15.77)
Waist	84.76 (11.57)	85.38 (10.68)	81.81 (12.37)	81.93 (13.3)
Hip	102.69 (9.8)	100.92 (14.75)	97.90 (11.53)	99.06 (8.61)
BMI	25.42 (4.88)	25.41 (5.29)	24.40 (4.6)	24.67 (5.14)
WHil	0.82 (0.06)	0.85 (0.14)	0.83 (0.1)	0.82 (0.09)
WHel	52.65 (6.14)	53.1 (5.9)	50.57 (7.4)	50.62 (7.79)
Glucose	99.46 (9.73) ^{bc}	89.42 (6.33) ^b	89 (7.1) ^c	85.12 (8.4)
Triglycerides	170.15 (62.32)	155.46 (65.99)	90 (22.7)	99 (51.23)
Cholesterol	195.46 (64.23)	189.69 (6.33)	162.93 (23.82)	157.37 (27.11)
HbA1c	7.9 (3.01) ^c	6.45 (2.11)	5.15 (2.96) ^c	5.88 (1.88)
Insulin	7.96 (7.12)	11.56 (6.11)	7.49 (3.1) ^b	10.52 (5.89) ^b
HOMA-IR	1.96 (1.71) ^b	3.62 (2.08) ^b	1.56 (0.67) ^a	2.75 (2.06) ^a

BMI = body mass index, WHil = waist-hip index, WHel = waist-height index, HbA1c = glycosylated hemoglobin. ^a = within-group differences and ^b = among-group differences when $p \leq 0.01$. ^c = within-group differences when $p \leq 0.05$.

Table 3: Comparison among students of the sample regarding the presence of anomalies.

in the averages of the body mass, waist-hip and waist-height indices between women and men, nor between those that presented anomalies and those that did not at the first evaluation. It seems that, rather than obesity originating the anomalies, obesity occurs together with the anomalies.

The data recorded after eating *Oreochromus aureus* indicated that after-fasting glucose levels decreased and insulin increased. The relatively low insulin values recorded in this sample, both in people with anomalies in biochemical markers and in those without anomalies, increased at the end of the experiment. These differences were significant in the experimental group in general ($p=0.004$) and explain the decrease in the after-fasting glucose values (Table 1). However, the increase in the value of insulin was not greater than the cutoff point. This may respond to an improvement in the function of the pancreas, as hypoinsulinemia follows a decrease in the function of the beta cell and constitutes a starting point for type 2 diabetes mellitus [36].

High sustained levels of blood glucose interfere with the release of insulin, the repair of the mass of beta cells, and the translocation of membrane proteins after a silencing of genes [37]. Thus, it is possible that the low values of insulin at the beginning responded to a high consumption of sugar in food and beverages, as the HbA1c values (6.38 ± 3.24) were near or above the cutoff point in both the students with and without anomalies (Table 3) and no important changes occurred at the end of the study ($p=0.73$), though the standard deviation value decreased at the end (6.13 ± 1.9).

Eating fish is an aspect that does not can be masked, so it is likely producing a Hawthorne effect. Accordingly, since the subjects that are part of an experiment known change their behavior, it was possible to expect that their pattern of food consumption change. The participants could have consumed more traditional foods as part of its incorporation to the study. Traditional foods of Mesoamerica such as corn tortillas have proven metabolic effects benefices at studios in animals [38]. However, such a modification would be obvious also sample glycosylated hemoglobin values, which does not occur [39].

The main contribution of this study regards the use of a fish that is produced in fish farms in tropical regions. This is important considering that people in temperate countries eat fish with a greater content of eicosanopentaenoic fatty acid, and people in less developed tropical countries may now find available a low cost functional food item. Proof

that eating *Oreochromis aureus* is associated with an improvement in glucose metabolism contributes to establishing better eating habits in order to prevent non transmissible chronic diseases associated with food and the damage they generate.

This study was limited in that it did not determine key proteins [40], the expression of genes involved in the processes of production and release of insulin, or anomalies that translate into peripheral resistance, such as the effect on glucose transporters. These are questions to be answered by experiments focused on explaining how this food item modifies glucose and insulin values.

Conclusions

Thirty-five young people were provided with 100 grams of *Oreochromus aureus*, five days per week during eight weeks. The students presented no differences between the initial and final values of body mass and glycosylated hemoglobin. This agrees with their maintaining their eating patterns during the experiment. As the students presented differences between the initial and final values of after-fasting glucose and insulin, it is very probable that eating fish was the factor that produced the changes in the glucose and insulin values.

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