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Consumption of nutrients with antioxidant action and its relationship with lipid profile and oxidative stress in student users of a university restaurant

El consumo de nutrientes con acción antioxidante y su relación con el perfil lipídico y el estrés oxidativo en los estudiantes usuarios del restaurante de una universidad

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ABSTRACT

Introduction: The relationship between diet and health has aroused scientific interest, especially the consumption of antioxidant nutrients naturally present in foods, because of its action against the deleterious effects of free radicals in the body.

Objective: This study aimed to estimate the intake of antioxidant nutrients and its relationship with lipid profile and oxidative stress in student users of a university restaurant in comparison with non-users.

Methods: This cross-sectional study involved 145 university students divided into two groups: users of the university restaurant (group 1, n = 73) and non-users (group 2, n = 72). We measured body mass index and waist circumference, and estimated the intake of antioxidant micronutrients. Serum concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride levels, and plasma concentrations of malondialdehyde were determined.

Results: Intake of copper, zinc, selenium, and vitamin C were within the recommendations in both groups, and vitamins A and E were below the reference values. There was a correlation between the intake of vitamin C and triglycerides. Group 1 members had better dietary patterns in relation to antioxidant micronutrients, reflecting less atherogenic lipid profile and lower exposure to oxidative stress compared to group 2.

Key words: Students. Antioxidants. Dyslipidemia. Malondialdehyde.

RESUMEN

Introducción: la relación entre la dieta y la salud ha despertado interés científico, especialmente en lo referente al consumo de nutrientes antioxidantes presentes de forma natural en los alimentos, debido a su acción contra los efectos nocivos de los radicales libres en el cuerpo.

Objetivos: el objetivo de este estudio fue estimar la ingesta de nutrientes antioxidantes y su relación con el perfil lipídico y el estrés oxidativo en estudiantes usuarios de un restaurante universitario, en comparación con los no usuarios.

Métodos: este es un estudio transversal que incluye 145 estudiantes universitarios, divididos en dos grupos: los usuarios del restaurante universitario (grupo 1, n = 73) y no usuarios (grupo 2, n = 72). Fueron medidos el índice de masa corporal y la circunferencia de la cintura, y se estimó la ingesta de micronutrientes antioxidantes. Se determinaron las concentraciones séricas de colesterol total, colesterol unido a lipoproteínas de alta densidad, colesterol unido a lipoproteínas de baja densidad, los niveles de triglicéridos y las concentraciones plasmáticas de malondialdehído.

Resultados: la ingesta de cobre, zinc, selenio y la vitamina C se encontraba dentro de las recomendaciones de los dos grupos, y la de vitaminas A y E estaba por debajo de los valores

de referencia. Hubo una correlación entre el consumo de vitamina C y los triglicéridos. Miembros del grupo 1 mostraron mejores patrones dietéticos en relación con micronutrientes antioxidantes, lo que refleja menos perfil lipídico aterogénico y una menor exposición al estrés oxidativo en comparación con el grupo 2.

Palabras clave: Estudiantes. Antioxidantes. Dislipidemia. Malondialdehído.

INTRODUCTION

Guaranteeing a balanced diet for university students is a huge challenge, considering that this group is exposed to behavioural risk factors that undermine health and may contribute to the development of chronic non-communicable diseases (1,2). Changes in lifestyle lead to favouring the choice of food services outside the home, including food and nutrition units such as university restaurants, rather than family meals (3). The relationship between diet and health has aroused scientific interest, especially the consumption of antioxidant nutrients naturally present in foods, because of its action against the deleterious effects of free radicals in the body (4,5).

Epidemiological evidence has shown an inverse correlation between regular consumption of fruits and vegetables and the prevalence of chronic diseases, with the protective effect being attributed to antioxidant nutrients in these foods (6,7). In this sense, the intake of these compounds appears to be a strategy for preventing lipid peroxidation and atherosclerosis (8). Considering the dietary pattern of university students, the scientific interest in the metabolic role of dietary antioxidants in modulating cardiovascular risk factors, and the role of food and nutrition units in health promotion, this study aimed to estimate the intake of antioxidant nutrients and its relationship with lipid profile and oxidative stress in student users of a university restaurant, in comparison with non-users.

MATERIALS AND METHODS

Using a cross-sectional design, we examined the data for 145 students aged 20-30 years at a public university in the city of Teresina, PI, in north-eastern Brazil. We included both male and female students who were users and non-users of the university restaurant from January to April 2013. Students with food allergies, intestinal malabsorption syndrome, and chronic diseases such as obesity, diabetes mellitus, hypertension, liver, cardiovascular and

renal diseases, and cancer, as well as students using anti-inflammatory medicine, were not included in the study. Pregnant students, lactating mothers, smokers, alcoholics, and competitive athletes were also excluded. The study was approved by the Ethics Committee of the Federal University of Piauí (protocol number: 12166013.0.0000.5214) and the participants provided informed consent.

The sample size was calculated as the number of students visiting the university restaurant's headquarters (Unit II) on a daily basis out of 1,425 university students. A 95% confidence interval margin of error of 7.3% was adopted and, based on the study by Freitas Jr. et al. (9) with university students, a 12% prevalence rate of dyslipidemia (or low levels of high-density lipoprotein HDL cholesterol) was used. The initial sample was estimated at 146 students, and the study was completed with 145 students divided into two groups based on use (group 1, n = 73) and non-use (group 2, n = 72) of the university restaurant. Group 1 included students using the service at least seven times per week for lunch or dinner, while group 2 included those students using university restaurant services up to two times per week for lunch or dinner.

Assessment of nutritional status

To assess nutritional status, body mass index (BMI) was calculated as body weight divided by the square of the height. Nutritional status was classified according to the guidelines of the World Health Organization (10). Waist circumference was measured using a flexible, inelastic tape around the natural waistline; the narrowest area between the chest and hips was used as a reference value (11).

Assessment of food intake

Information on the usual dietary intake of students was collected by applying a semi-quantitative food frequency questionnaire (FFQ), assessing the previous six months, adapted from the questionnaire of Slater et al. (12). The questionnaires included 91 items organized into 13 food groups: milk and dairy products, meat and eggs, foods like meat and sausage, cereals and pasta, legumes, breads and cakes, fruits, vegetables, roots and tubers, candy and sweets, oil and fat, drinks, and processed snacks. The response options quantified consumption ranging from never or rarely to seven times a day, week, or month.

The food selection and preparation forms of the FFQ were designed taking into consideration the food items that are standard to the university restaurant menu, foods sold in the university cafeterias, and the food items commonly found in breakfast and snacks. The amounts of energy and macro- and micro-nutrient antioxidants were calculated using DietSys software version 4.01 National Cancer Institute, USA, 1999. The nutrition information on energy, macronutrients, copper, zinc, and vitamins A, E, and C were included in the program, with preference to the data of the Brazilian Table of Food Composition (13), followed by the Nutritional Composition Table of Food Consumed in Brazil (14). The recommended amount of selenium was obtained from Ferreira et al. (15), and the Nutritional Composition Table of Food Consumed in Brazil (14). Those nutrients not found in the cited food sources were analysed using the USDA Food Search for Windows, version 1.0, SR23. After analysing diet composition, nutrients were adjusted for energy using the residual method (16).

The reference values used for macronutrient intake were those of the acceptable macronutrient distribution range (AMDR) (17), and cholesterol intake was compared with the recommendations of the I Guidelines on Consumption of Fats and Cardiovascular Health (≤ 300 mg/day) (18). In relation to the intake of antioxidant vitamins and minerals, ingestion of the adjusted values was compared with the estimated average requirement (EAR) (19,10).

Blood collection and separation of components

Venous blood samples (10 ml) were collected from participants following at least 12 hours of fasting. The collected blood was distributed into two tubes containing 5 ml blood each, one with ethylene diamine tetraacetic acid (EDTA) as an anticoagulant and the other without anticoagulant, to determine plasma malondialdehyde (MDA) and serum lipids, respectively. Blood was centrifuged at $1,831 \times g$ for 15 min at 4°C (Centrifugal CIENTEC® 4K15). Obtained serum and plasma were transferred to polypropylene microtubes; the former was used immediately for lipid profile analysis, while the latter was stored at -80°C until MDA analysis.

Determination of serum lipids

Serum concentrations of total cholesterol, HDL-cholesterol, and triglycerides were determined using an enzymatic colorimetric method, in the automatic biochemical analyzer COBAS INTEGRA (Roche Diagnostics, Brazil) using Roche® kits. The low-density lipoprotein

(LDL) fraction was calculated using the formula of Friedewald et al. (1972), which is valid for triglyceride levels up to 400 mg/dl (21). For serum lipid evaluation, we used as a standard the reference values found in the IV Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis (21).

Determination of plasma MDA

The concentration of MDA in plasma was measured via high-performance liquid chromatography (HPLC), according to the method adapted from Rezaei et al. (22). Prior to processing, the samples were prepared using a calibration curve stock solution of MDA with 22 μ l of tetramethoxypropane and 10 ml of 1% sulfuric acid at concentrations of 0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, and 12.0 mM. In microtubes containing 100 μ l of plasma, 10 μ l of 4M sodium hydroxide were added. After vortexing, the samples were incubated in a VorTemp® apparatus at 60 °C for 30 minutes and then incubated on ice for five minutes. After cooling, 150 μ l of 1% sulfuric acid were added, and the solution was vortexed to precipitate proteins. Following incubating on ice for five minutes, the solution was centrifuged for ten minutes at 14,000 g, and 175 μ l of the supernatant was transferred to a new microtube, with the subsequent addition of 25 μ l of 2,4-dinitrophenylhydrazine (0.001 g/ml) and incubation at room temperature for 30 minutes, protected from light. The tube was then centrifuged at 14,000 rpm for five minutes, and 150 μ l of the supernatant were transferred to a vial, from which 100 μ l were used in HPLC.

Statistical analysis

Data were organized and analysed using SPSS 18.0 for Windows (SPSS Inc., Chicago, IL, USA). A descriptive analysis of the data by means and standard deviations was carried out. The Kolmogorov-Smirnov test was applied to verify the normality of the data, and the Levene test was used for measuring the homogeneity of variances. The Student's t-test and the Mann-Whitney U test were used to compare the averages of the parameters studied between groups 1 and 2. Associations between the variables were assessed using the Chi-squared test (χ^2). Pearson and Spearman tests were used for correlation analysis, adopting a significance level of 5.0%.

RESULTS

Average macronutrient consumption was within the acceptable distribution ranges, with no difference between both groups ($p > 0.05$). However, there was higher energy intake and monounsaturated fatty acid consumption, with reduced saturated fatty acid consumption, in the university restaurant users ($p < 0.05$) (Table I). Table I also shows that, in relation to the students' intake of nutrients with antioxidant action, the average intake of vitamin C, copper, zinc, and selenium was above the estimated recommended values for EAR, but vitamin A and E intake was lower. The intake of vitamin E and copper by the university restaurant users was higher compared to that of non-users ($p < 0.05$).

As shown in figure 1, more than 90% of the university students in both groups ate meat and eggs, cereal and pasta, and oils and fats daily. Members of group 1 consumed a similar percentage of fruits, vegetables, and sweets and treats, while non-users had higher consumption of dairy products. More than 70% of students in group 1 consumed legumes, and 60% of members of both groups consumed breads, cakes, and sugary drinks.

Table II shows the students' lipid profile results. Mean total cholesterol, LDL cholesterol, and HDL cholesterol were lower in group 1 compared to group 2 ($p < 0.05$).

Non-users of the university restaurant had significantly higher values of MDA, a marker for lipid peroxidation (0.81 ± 0.42 mmol/l; $p < 0.05$) (Fig. 2).

Table III shows the correlation between the intake of antioxidant micronutrients, lipid profile, and MDA in group 1. Our results show a significant negative correlation between dietary vitamin C and serum triglycerides ($r = -0.306$, $p < 0.05$).

DISCUSSION

This study evaluated the intake of macronutrients and micronutrients with antioxidant activity, lipid profile, and concentration of MDA as a marker of lipid peroxidation in university students. The analysis of food consumption showed normal macronutrient intake within recommended ranges (17) for both groups. However, for users of the university restaurant (group 1), there was higher caloric intake and consumption of monounsaturated fatty acids, with reduced consumption of saturated fatty acids. In this respect, we find that university meals provide adequate levels of macronutrients, highlighting this as the better standard of dietary lipid consumption, and suggesting that the university restaurant provides a nutritionally balanced diet with less atherogenic potential.

Overall, this study shows that the dietary patterns of the evaluated university students combine positive markers of diet quality (consumption of cereals, legumes, fruits, vegetables, meat, eggs, and milk) with negative markers (consumption of sweets and treats, drinks with added sugar, oils and fats). This pattern resembles the national food standard, according to the Consumer Expenditure Survey 2008-2009 14, but with more frequent and regular consumption of fruits and vegetables. The university restaurant evidently establishes healthy eating habits in the academic environment. In contrast, studies have shown that there is inadequate food consumption among college students (23,24).

Regarding the lipid profile markers analysed in this study, university restaurant users had reduced serum total cholesterol and LDL cholesterol levels. This can be explained by their high dietary intake of monounsaturated fatty acids and reduced intake of saturated fatty acids. Hence, university restaurant users are at a lower risk of cardiovascular disease. Moreover, the consumption of monounsaturated fatty acids did not influence the serum concentration of HDL cholesterol, although student users of the university restaurant had reduced levels of this lipid biomarker. It is suggested that reduced exercise among the assessed students (data not shown) may have contributed to these values found in the lipid fraction. Similarly, the study by Freitas Jr. et al. (9) examining university students identified reduced serum concentrations of HDL cholesterol in 12% of participants. They identified high serum triglyceride and total cholesterol levels in 23% and 9.7% of participants, respectively, in contrast to the results of this study.

This study showed that university students' antioxidant intake is above the EAR for copper, zinc, selenium, and vitamin C, and below the reference values for vitamins A and E intake, with group 1 members having a significantly higher intake of vitamin E and copper. In this respect, habitual vitamin C intake can be considered as a positive factor when assessing antioxidant consumption in the diets of students, especially if we take into account the reduced dietary amounts of vitamins A and E. This study showed a negative correlation between dietary vitamin C intake and serum triglycerides, demonstrating the independent role of this vitamin in preventing cardiovascular risk factors.

Several studies have been conducted to assess the intake of antioxidant nutrients, such as that of Agüero et al. (1), which evaluated the intake of vitamins and minerals of university students in Chile, noting average dietary intake above the recommendation for vitamins A and C, copper, and selenium, and low intake of vitamin E and zinc. Regarding the marker of

lipid peroxidation, the study showed reduced plasma MDA concentrations in student users of the university restaurant compared to non-users. This is likely due to the consumption of the antioxidant nutrients copper, zinc, selenium, and vitamin C within the recommended values, as well as by reduced serum total cholesterol and LDL cholesterol. Conversely, the study of Srivastava and Batra (25) identified high MDA concentrations in university students. One potential limitation of this study is the evaluation instrument used for measuring food consumption, the FFQ. Despite being adequate for the purposes of this study, the questionnaire does not allow the accurate estimation of habitual dietary intake because it relies on respondents' memory, and it does not contemplate intrapersonal variability, which compromises the analysis of dietary adequacy. We have tried to minimize potential measurement errors resulting from the use of the collection instrument in this study. Visual features (photographs) have been used to facilitate the reporting of food intake, in addition to consulting professionals and nutrition students who have been trained in applying the FFQ.

CONCLUSIONS

Users of the university restaurant consume levels of macronutrients within the recommended ranges, highlighting the quality of lipid intake. The antioxidant nutrients, copper, zinc, selenium, and vitamin C are consumed by both group 1 and group 2 students in accordance with the recommendations, and users of the university restaurant had better vitamin E and copper intake. Despite limitations, we tried to minimize potential measurement errors introduced by the questionnaire used to assess food intake. To this end, visual features have been used to facilitate reporting, in addition to consulting professionals and nutrition students who are trained in applying the FFQ. The lipid profile in the university restaurant users has fewer atherogenic characteristics, with the positive influence of vitamin C observed in reducing serum triglycerides. University restaurant users also had lower levels of MDA, thereby reducing their exposure to oxidative stress and their risk of cardiovascular disease.

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Table I. Mean values, standard deviations, and percentage-of-energy contribution of macronutrients and antioxidant micronutrients in the regular diet of students who were either users (group 1) or non-users (group 2) of the university restaurant

<i>Energy/macronutrients/antioxidant s Nutrients[#]</i>	<i>Group 1 (n = 73)</i>	<i>Group 2 (n = 72)</i>	<i>p</i>
Energy (kcal)	2,135.84 ± 681.78	2,006.30 ± 821.95	0.034*
Protein (%)	19.03	17.95	0.485
Lipids (%)	32.16	33.91	0.346
Carbohydrates (%)	61.40	59.13	0.517
Saturated fatty acids (%)	9.89	11.06	0.048*
Monounsaturated fatty acids (%)	13.32	10.05	0.000*
Polyunsaturated fatty acids (%)	8.95	8.64	0.558
Cholesterol (mg)	301.37 ± 93.83	297.24 ± 99.00	0.797
Vitamin A (µg)	345.00 ± 117.91	336.52 ± 110.03	0.655
Vitamin C (mg)	520.63 ± 757.74	661.345 ± 894.99	0.193
Vitamin E (mg)	6.74 ± 2.85	5.87 ± 2.43	0.004*
Copper (µg)	1,535.62 ± 507.25	1,365.69 ± 398.55	0.027*
Zinc (mg)	10.67 ± 2.09	9.91 ± 2.59	0.053
Selenium (µg)	51.22 ± 19.39	47.19 ± 14.59	0.251

Results expressed as mean ± standard deviation and percentage (%). [#]The gross intake of macronutrients and antioxidant micronutrients were adjusted for energy, with the exception of vitamin C. *p < 0.05 (Student's t-test or Mann-Whitney U test). Reference values: 10-35% protein, 20-35% lipid, 45-65% carbohydrates, ≤ 10% saturated fatty acid, and 5-10% polyunsaturated fatty acid (IOM, 2005). ≤ 300 mg/day of cholesterol (Santos et al., 2013), 625 g and 500 g for vitamin A for man and woman, respectively; 60-75 mg for vitamin C; 12 mg for vitamin E; 700 µg for copper; 9.4 mg and 6.8 mg for zinc for man and woman, respectively; 45 µg for selenium for man and women respectively (IOM, 2000; 2002).

Table II. Mean values and standard deviations of the serum total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides of university students who are users (group 1) or non-users (group 2) of the university restaurant.

<i>Parameters</i>	<i>Group 1</i> <i>(n = 73)</i>	<i>Group 2</i> <i>(n = 72)</i>	<i>p</i>
TC (mg/dl)	159.75 ± 32.49	176.38 ± 29.86	0.002*
LDL-c (mg/dl)	94.47 ± 26.00	103.73 ± 29.81	0.048*
HDL-c (mg/dl)	48.12 ± 13.5	54.22 ± 16.00	0.017*
TG (mg/dl)	93.14 ± 48.04	86.11 ± 36.37	0.690

Results expressed as mean ± standard deviation. * $p < 0.05$ (Student's t-test or Mann-Whitney U test). TC: Total cholesterol; LDL-c: Low-density lipoprotein cholesterol; HDL-c: High-density lipoprotein cholesterol; TG: Triglycerides.

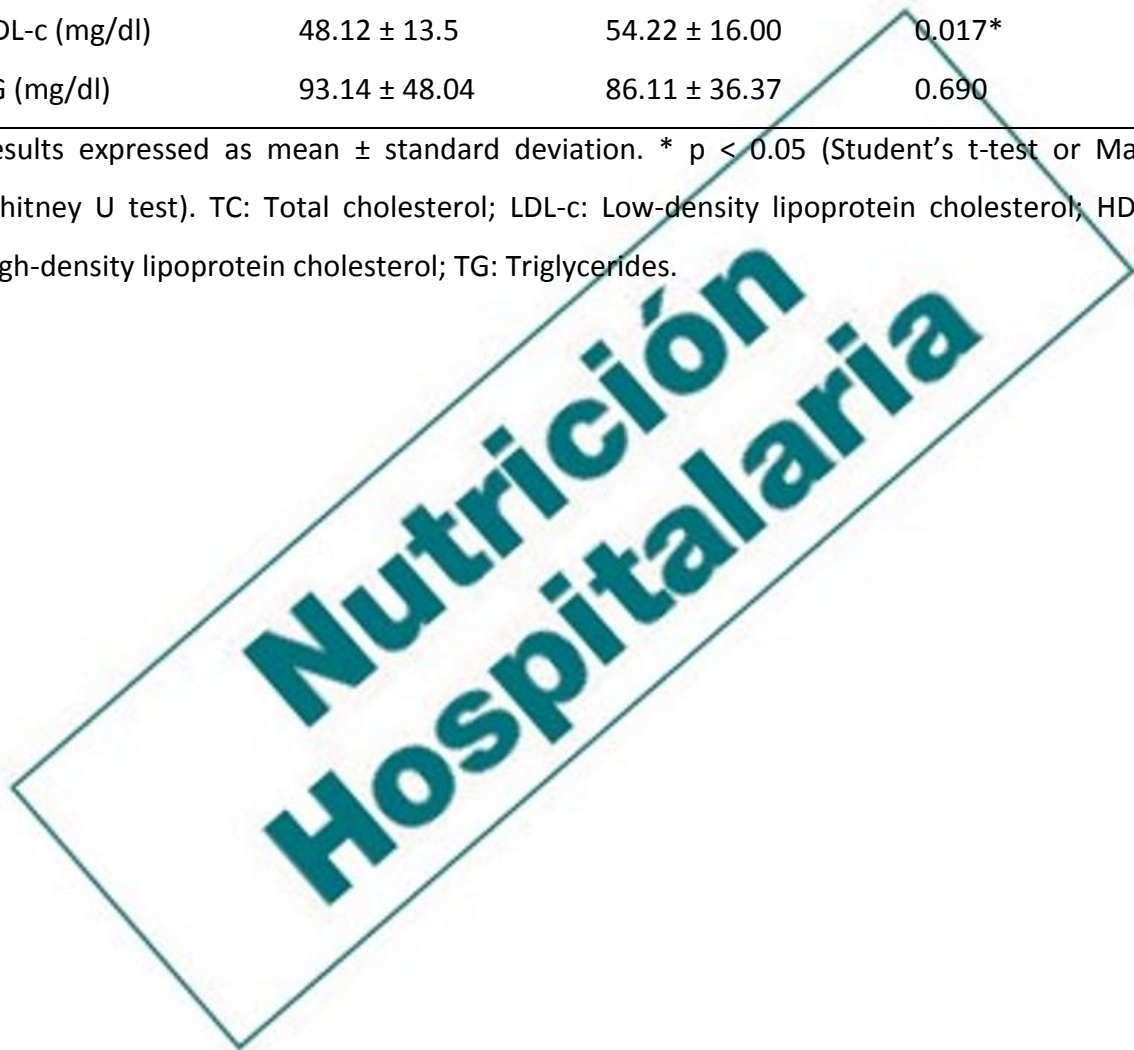


Table III. Correlation (r) between dietary intake of antioxidant micronutrients and MDA lipid profile in university students using the university restaurant

Parameters	TC	LDL-c	HDL-c	TG	MDA
Vitamin A	-0.156	-0.174	-0.070	-0.133	0.025
Vitamin E	-0.103	-0.091	0.037	-0.175	0.017
Vitamin C	-0.099	-0.085	0.060	-0.306*	-0.076
Copper	-0.007	0.059	0.006	0.028	0.077
Zinc	0.060	-0.058	0.075	0.074	0.060
Selenium	-0.069	-0.145	0.058	-0.038	0.046
MDA	-0.002	0.105	-0.080	-0.158	

*p < 0.05 (Pearson or Spearman correlation). TC: Total cholesterol; LDL-c: Low-density lipoprotein cholesterol; HDL-c: High-density lipoprotein cholesterol; TG: Triglycerides; MDA: Malondialdehyde.

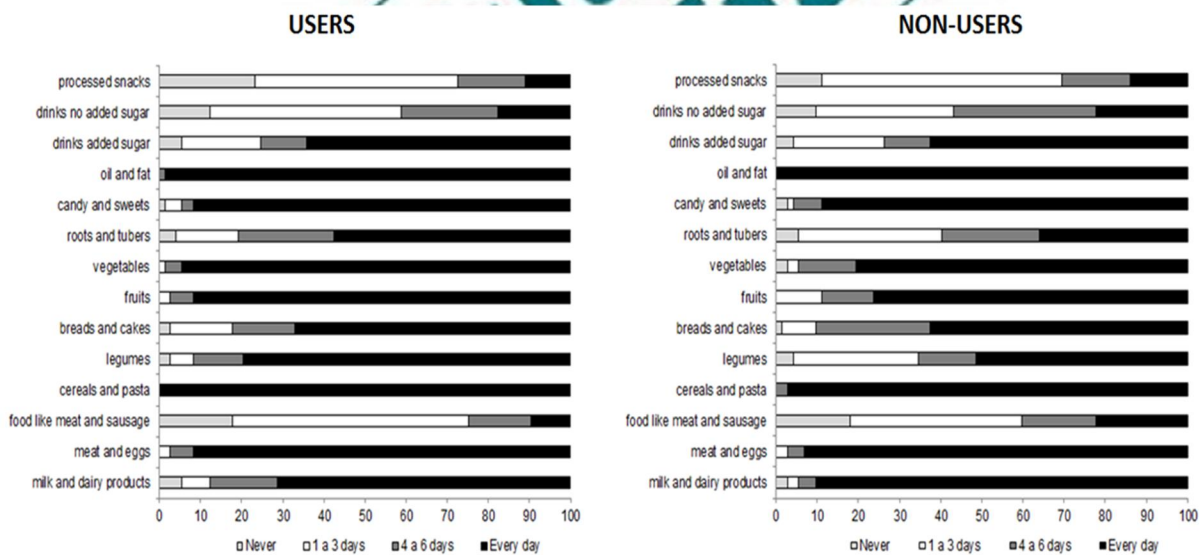


Figure 1. Weekly food consumption of college students who are users or non-users of the university restaurant.

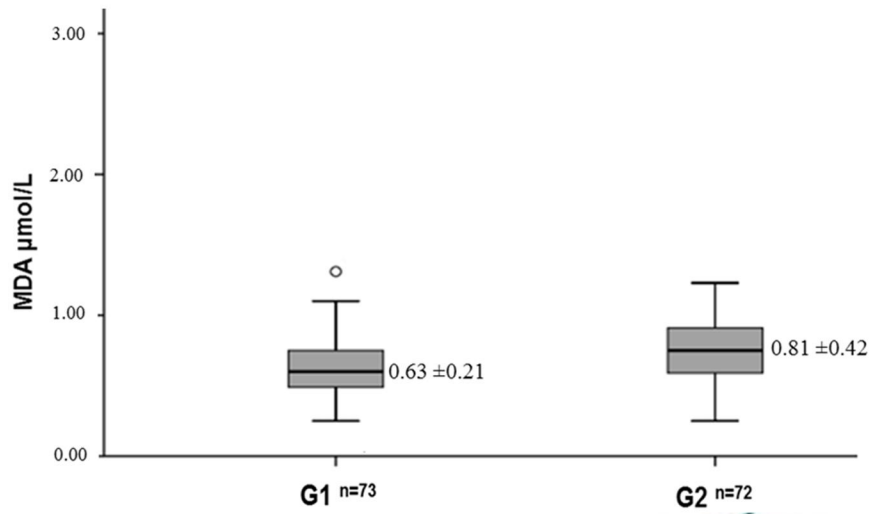


Figure 2. Plasma concentrations of MDA of group 1 (G1) and group 2 (G2) students. G1, group 1: Users of the university restaurant; G2, group 2: Non-users of the university restaurant. Mann-Whitney U test ($p = 0.02$) G1 versus G2.

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