

# Vegetarians at the University's restaurants: are they doing well?

*Vegetarianos nos restaurantes da universidade: eles estão se saindo bem?*

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## ABSTRACT

**Objective:** Vegetarians might be at nutritional risk due to their food preferences. The goal of this study was to compare nutritional status and food intake of vegetarians and omnivores that use restaurants at the Federal University of Paraná in Brazil. **Methods:** Clinical cross-sectional observational study performed between May 2014 and March 2015, assessing 84 vegetarians and 131 omnivores, adults, of both sexes. Anthropometric and body composition characteristics were evaluated on the total sample. In a subsample of 38 vegetarians and 63 omnivores, food intake of macronutrients, fibers, vitamin B12, vitamin D, calcium, iron and zinc were evaluated. Blood tests for vitamin B12, iron, and ferritin were performed in a subsample of 40 individuals of each group. **Results:** The studied groups presented similar anthropometric data and body composition, although both had individuals classified as at risk for cardiovascular diseases according to body mass index (BMI) (16.7% and 24.4% above 25 kg/m<sup>2</sup> among vegetarians and omnivores, respectively). The caloric intake did not differ as well, although the main sources of energy intake among vegetarians were carbohydrates as compared to omnivores that had lipids as the main source of energy in their meals. Vegetarians reached the recommended intake of fibers, and the omnivores, had a lower intake of this nutrient as expected. For vitamin B12 the prevalence of inadequate intake was 37.8% on the vegetarians group and 0.6% on the omnivores, and for calcium, 49% for both groups. Both presented vitamin D intake below the estimated average requirement. The intake of iron did not differ among groups, however, in the vegetarian group the inadequacy reached 50% for men and 100% for women; and in the omnivore group 93% for women. For zinc, the inadequacy risk was 100% for men and 90% for women in the vegetarian group and 25% of men and 4.5% for women on the omnivore group. Regarding the biochemical exams, the most evident deficiency was of serum vitamin B12 on vegetarians. **Conclusions:** The food choices among the investigated undergraduate vegetarians do not guarantee nutritional security as detected in this study. Except for calcium, the prevalence of inadequate intake of macro and micronutrients was higher among vegetarians as compared to omnivores, establishing a nutritional risk status to this group regarding to intake of sources of vitamin B12, vitamin D, iron and zinc.

## RESUMO

**Objetivo:** Os vegetarianos podem estar em risco nutricional devido às suas preferências alimentares. O objetivo deste estudo foi comparar o estado nutricional e a ingestão alimentar de vegetarianos e onívoros que utilizam restaurantes da Universidade Federal do Paraná, no Brasil. **Método:** Estudo clínico observacional de corte transversal realizado entre maio de 2014 e março de 2015, avaliando 84 vegetarianos e 131 onívoros, adultos, de ambos os sexos. As características antropométricas e de composição corporal foram avaliadas na amostra total. Em uma subamostra de 38 vegetarianos e 63 onívoros, a ingestão alimentar de macronutrientes, fibras, vitamina B12, vitamina D, cálcio, ferro e zinco foram avaliados. Exames de sangue para vitamina B12, ferro e ferritina foram realizados em uma subamostra de 40 indivíduos de cada grupo. **Resultados:** Os grupos estudados apresentaram dados antropométricos e composição corporal semelhantes, embora ambos apresentassem indivíduos classificados como de risco para doenças cardiovasculares de acordo com o índice de massa corporal (IMC) (16,7% e 24,4% acima de 25 kg/m<sup>2</sup> entre vegetarianos e onívoros, respectivamente). A ingestão calórica também não diferiu, embora as principais fontes de ingestão de energia entre os vegetarianos fossem carboidratos em comparação aos onívoros que tinham lipídios como principal fonte de energia em suas refeições. Os vegetarianos atingiram a ingestão recomendada de fibras, e os onívoros tiveram uma ingestão menor desse nutriente como esperado. Para a vitamina B12, a prevalência de ingestão inadequada foi de 37,8% no grupo dos vegetarianos e de 0,6% nos onívoros, e para o cálcio de 49% nos dois grupos. Ambos apresentaram ingestão de vitamina D abaixo do requisito médio estimado. A ingestão de ferro não diferiu entre os grupos, no entanto, no grupo vegetariano a inadequação chegou a 50% para os homens e 100% para as mulheres; e no grupo onívoro, 93% para as mulheres. Para o zinco, o risco de inadequação foi de 100% para homens e 90% para mulheres no grupo vegetariano e 25% de homens e 4,5% para mulheres no grupo onívoro. Em relação aos exames bioquímicos, a deficiência mais evidente foi de vitamina B12 sérica nos vegetarianos. **Conclusões:** As escolhas alimentares entre os vegetarianos de graduação investigados não garantem a segurança nutricional, como detectado neste estudo. Com exceção do cálcio, a prevalência de ingestão inadequada de macro e micronutrientes foi maior entre os vegetarianos em relação aos onívoros, estabelecendo um risco nutricional para esse grupo em relação à ingestão de fontes de vitamina B12, vitamina D, ferro e zinco.

## Keywords:

Vegetarianism. Nutritional Assessment. Food Intake. Body Composition.

## Unitermos:

Vegetarianismo. Avaliação Nutricional. Ingestão de Alimentos. Composição Corporal.

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## INTRODUCTION

Currently, the practice of vegetarianism has been increasing due to many reasons<sup>1-3</sup>. In 2012, 5% of the United States population was vegetarian and 1 to 2% was strictly vegetarian<sup>4</sup>. Although there are no scientific studies that indicate the quantity of vegetarians in Brazil, a public opinion research was carried out in 2012 and indicated a prevalence of 8% of vegetarians in the country<sup>5</sup>.

The number of publications regarding to the advantages and disadvantages of following a vegetarian diet have been increasing. However, many studies are still controversial, with some indicating, for example, that vegetarian diets are adequate in supplying certain nutrients, such as vitamin B12, vitamin D, calcium, iron and zinc<sup>6-12</sup>, while others inform the opposite<sup>9,13-21</sup>. This kind of controversy often occurs due to the outlining and methodology applied to the studies. In addition, vegetarians usually present different characteristics as compared to the general population, such as engagement on physical activity regular practice, alcohol and tobacco abstinence and lower weight levels<sup>7,22-26</sup>.

Facing the fact that some nutrients may not be abundant in vegetarian diets it is necessary to monitor the dietary intake of individuals that adopt this food pattern, considering that they might be at nutritional risk. For this study we investigated undergraduate students that usually have their main meals in the restaurant located at the Federal University of Paraná (UFPR) in Brazil, evaluating their nutritional status and food intake in order to identify if they reach Food and Nutritional Security. The restaurants from higher education federal institutions in Brazil have an important mission of reaching such security, since the meals are subsidized by the Ministry of Education. The menu is planned by nutritionists and should guarantee nutritious meals to the students, independently of their culture or philosophic choices.

Considering the above, the objectives of this study are to compare the adequacy of the nutritional status and food intake of vegetarians and omnivores that are users of the restaurants located at the UFPR.

## METHODS

### Study outlining and subjects

This is a cross-sectional observational study approved by the Ethics Committee from the Health Sciences sector of the UFPR (CAAE protocol 25347414.6.0000.01020). Took part of the research adults individuals, practicing omnivorous or vegetarian diet (of any type) for at least six months and users of the University Restaurants (URs) at least three times a week. Exclusion criteria: individuals with diseases that limit food intake both quantitatively and qualitatively, using medicines that decrease vitamins and minerals absorption, using food

supplements or multivitamins, undergoing diet to lose weight or pregnant. The work has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and all participants were informed about the content of the research, as well as its objectives and procedures to gather data through a Free and Informed Consent Term, which was previously to the data collection, read and signed.

### Population study

Sample size calculus was performed through the StalCalc app of the Epiinfo 7.1.4 package, considering the prevalence of vegetarians with serum vitamin B12 deficiency in relation to the omnivores, observed on previous clinical studies. A confidence interval of 95% was used, power of 80%, 2.90 odds ratio for vegetarians with serum vitamin deficiency and effect size of and 28.57% to the exposed. As a result, a sample size of 84 vegetarians and 131 omnivores was obtained.

The study proposal was advertised at the restaurants in diverse campus in the university and individuals that desired to participate answered a questionnaire about their personal data and life style in order to evaluate their eligibility according to the mentioned inclusion and exclusion criteria. The eligible vegetarian individuals should not consume any kind of meat (including seafood) and derivatives over a six-month period prior the research.

### Demographic characteristics, anthropometric assessment and body composition

All participants were questioned about their basis characteristics (race, age, income level, undergraduate areas of study and current level of physical activity). The anthropometric assessment was performed by a trained researcher, including weight, height and waist circumference (WC), as the standard techniques<sup>27</sup>. The following cut-off points were used to classify WC: < 94 cm to men and < 80 cm to women – without risk of metabolic complications; ≥ 94 to 102 for men and ≥ 80 to 88 for women – substantial risk enhanced for metabolic concentrations<sup>28</sup>. Body mass index [BMI = weight (kg) / height (m<sup>2</sup>)] was also used and the subjects were classified according to the World Health Organization (WHO) recommended cut-off points<sup>28-30</sup>.

Body composition was evaluated through the electrical bioimpedance method, with the tetrapolar body composition analysis device RJL Systems®, model Quantum BIA 101Q. The procedures recommended by Kyle et al.<sup>31</sup> were adopted. Age, sex, race, weight, height, resistance, reactance were transferred to the bioimpedance software, calculating fat mass (FM) and free of fat mass (FFM), both in absolute values and body weight percentages.

The Lohman's<sup>32</sup> formulas were selected to calculate FFM. Percentage of FM was also calculated by the bioimpedance software through the equation  $\% \text{ FM} = [1 - (\text{FFM}/\text{Weight})] \times 100$ . Lohman's classification<sup>32</sup> was used to the standard percentage of body fat for men and women. Cut points for FM (%) were  $\leq 5\%$  or  $\geq 25\%$  for men and  $\leq 8\%$  or  $\geq 32\%$  for women – with risk of disorders associated to malnutrition or obesity, respectively; 6 to 24% for men and 9 to 31% for women – without risk<sup>32</sup>.

### Food intake and composition analysis

In a random subsample of 38 vegetarians and 63 omnivores, food intake was evaluated from a 24-hour Dietary Recall (24HR) of three days, including two week days and one weekend day, considering interchanged days. In this case, written and face-to-face instructions were provided to the subjects.

Food intake composition was calculated with the reference of Nutritional Composition of Consumed Food in Brazil, from the Brazilian Institute of Geography and Statistics (IBGE)<sup>33</sup>. For foods that were not found on that table, data of the Table of Chemical Composition of Foods of the Federal University of São Paulo (UNIFESP) were used. When preparations were not found in both tables, their recipes were calculated for further composition calculus. Standardized food servings elaborated by IBGE<sup>34</sup> were used and transformed in grams or milliliters.

### Estimated requirements of energy and nutrients

The estimated energy requirement (EER) was calculated to each participant from the Dietary Reference Intakes (DRIs) equations, proposed by the Food and Nutrition Board/Institute of Medicine (IOM), according to gender and life stage<sup>35</sup>. Coefficients of physical activity, proposed by the IOM<sup>35</sup> were utilized. Methods used to estimate inadequacy of macro and micronutrients intake were based on DRIs recommendations<sup>35-37</sup>.

To express the group energy intake, results were adjusted for body weight ( $\text{kg}/\text{m}^2$ ) and presented as average  $\pm$  standard deviation (SD). To determine the frequency of adequacy to the DRIs references, EER value was calculated for each participant and the percentage of cases was distributed as "below EER" when the average individual calorie consumption was found below 80% of its EER value, "adequate EER", when between 80 and 120%, and "above EER", when above 120%<sup>38</sup>.

Intake of proteins (Ptn), lipids (Lip) and carbohydrates (Cho) was also adjusted to body weight and expressed as g of nutrient/kg as well as in percentage of the individual total energy value (%TEV). The acceptable macronutrient distribution ranges (AMDR)<sup>35</sup> was used to identify the intake as "below AMDR", "above AMDR" or normal.

The estimated average requirement (EAR) was used as reference to evaluate the prevalence of inadequate intake of vitamin B12 on both groups. Iron and zinc were individually compared with the recommended dietary allowance (RDA) and the results were expressed in frequency of individuals with intake below or above RDA. For vitamin D, the average intake on both groups was compared to the EAR recommended value, since this nutrient showed asymmetric distribution. For fiber analysis the adequate intake (AI) value was used<sup>35</sup>.

### Biochemical assessment

During the recruitment of students to be part of this study, some chose not collect blood samples. For this reason, for this analysis, a convenience sample of 40 vegetarians and 40 omnivores, serum levels of vitamin B12, iron and ferritin were evaluated at the Hospital de Clínicas laboratory of the UFPR. An 8-hour fasting was requested for blood testing and, to interpret the results, standard references of the hospital's laboratory were used<sup>39</sup>. From these 40 participants of each group, 22 vegetarians and 29 omnivores returned the 24HR of three days, making possible to associate nutrient intake and biochemical results.

### Statistical analysis

The Statistical Package for the Social Science® (SPSS), version 20 for Windows® (SPSS Inc., Chicago, IL, USA) was used. Quantitative variables were analyzed by the unpaired t test or the Mann-Whitney test and qualitative variables by the Chi-Square test of the Fisher's Exact. Association among the quantitative variables was verified by the Pearson correlation coefficient or Spearman and among the categorical through the Chi-Square. Correlation coefficient (r) value was used to verify the positive or negative strength of association between variables. Odds ratio (OR) was determined through the Chi-Square test to establish the association between serum vitamin B12 deficiency and dietary intake among vegetarians, since it was the most expressive deficiency found in the results. Confidence interval (CI) of 95% was used for all analysis.

Since the analysis of the laboratory exams was performed in a subsample, at the end of the data collection, the statistical test power was recalculated for t vitamin B12 analysis. The G\*Power 3.1 program was used, obtaining a power 0.99.

## RESULTS

### Demographic characteristics

Both groups were similar regarding to sex, age, race and level of income, but were different regarding study area and level of physical activity (Table 1). Among the vegetarians,

**Table 1** – Demographic characteristics of vegetarians and omnivores.

Parameters	Vegetarians (n=84)	Omnivores (n=131)	p
<b>Sex</b>			
Male	32 (38.1%)	42 (32.1%)	$p=0.364^a$
Female	52 (61.9%)	89 (67.9%)	
<b>Age (Years)</b>	25.2 ( $\pm$ 6.4)	23.9 ( $\pm$ 6.9)	$p=0.082^b$
<b>Race</b>			
Caucasian	80 (95.2%)	116 (88.5%)	$p=0.092^a$
Non-Caucasian	4 (4.8%)	15 (11.5%)	
<b>Income Level</b>			
A	8 (9.5%)	19 (14.5%)	$p=0.282^a$
B	44 (52.4%)	77 (58.8%)	$p=0.356^a$
C/D/E	32 (38.1%)	35 (26.7%)	$p=0.079^a$
<b>Area of Study</b>			
Health and Medical Science	15 (17.9%)	44 (33.6%)	$p=0.012^{a*}$
Socially Applied Science	12 (14.3%)	23 (17.5%)	$p=0.623^a$
Human Sciences	20 (23.8%)	12 (9.2%)	$p=0.003^{a*}$
Computing and Engineering	10 (11.9%)	20 (15.2%)	$p=0.817^a$
Agronomic and Veterinary Science	7 (8.3%)	12 (9.2%)	$p=0.987^a$
Biological Science	12 (14.3%)	6 (4.6%)	$p=0.002^{a*}$
Languages and Arts	7 (8.3%)	8 (6.1%)	$p=0.532^a$
Mathematics and Natural Science	1 (1.2%)	6 (4.6%)	$p=0.251^c$
<b>Level of physical activity</b>			
Sedentary/Irregularly active	20 (23.8%)	54 (41.2%)	$p=0.009^{a*}$
Active/Very active	64 (76.2%)	77 (58.8%)	

Results correspond to the absolute numbers (n) and frequencies (%) for all variables, except age that corresponds to average  $\pm$  SD. Asterisks (\*) indicate significant differences ( $p<0.05$ ) from the <sup>a</sup>Chi-Square Test, <sup>b</sup>t Student Test and <sup>c</sup>Fisher's Exact Test. Among the non-Caucasian races black, yellow and brown races are included.

76.2% practiced physical activities actively or very actively and, among omnivores, 58.8%. Regarding to study area, most omnivores were taken courses at the medical science areas and vegetarians at the humanities science. The investigated vegetarians had adopted these dietary patterns for four years and six months in average (minimum six months, maximum twenty years).

#### Anthropometric and body composition characteristics

Vegetarians and omnivores presented similar anthropometric and body composition characteristics ( $p<0.05$ ) as shown on Table 2.

#### Food Intake

Caloric intake adjusted to body weight did not differ among groups ( $p=0.066$ ). Most part of both groups presented adequate energy ingestion from the individual EER evaluation (Table 3), however, intake below EER was more frequent among vegetarians and above EER among omnivores.

Ptn and Lip intake adjusted to body weight was higher among omnivores as compared to vegetarians ( $p=0.000$  and  $0.003$ , respectively). Cho intake did not differ between the studied groups ( $p=0.252$ ). However, when evaluating energy intake, the contribution of Cho was greater among vegetarians ( $p=0.000$ ). Considering the source of energy chosen regularly, an inversion between the groups was observed, being Cho the main choice of vegetarians and Lip the main choice of omnivores, with no difference on total energy intake.

Concerning the AMDR, a few omnivores ingested Ptn above or below the ideal, although the adjusted average for body weight was 1.5 grams, considered a hyperproteic diet. Among vegetarians, 31.6% of the participants ingested this nutrient below the expected. Compared to the vegetarians, a larger number of omnivores extrapolated the Lip intake. For Cho, 23.7% of vegetarians ingested the nutrient above the expected, and 38.1% of omnivores below (Table 3).

As expected, vegetarians had a higher dietetic fiber intake reaching the IOM recommendation, unlike the omnivores.

**Table 2** – Anthropometric and body composition characteristics of vegetarians and omnivores.

Parameters	Vegetarians (n=84)	Omnivores (n=131)	p
Weight (kg)	64.6 (± 12.9)	65.7 (± 13.5)	p=0.570 <sup>a</sup>
Height (m)	1.70 (± 0.09)	1.67 (± 0.09)	p=0.100 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	22.4 (± 3.7)	23.3 (± 4.2)	p=0.084 <sup>a</sup>
<b>BMI categorical</b>			
BMI < 18.5 kg/m <sup>2</sup>	7 (8.3%)	8 (6.1%)	p=0.850 <sup>b</sup>
BMI 18.5 to 24.9 kg/m <sup>2</sup>	63 (75.0%)	91 (69.5%)	p=0.380 <sup>b</sup>
BMI ≥ 25 kg/m <sup>2</sup>	14 (16.7%)	32 (24.4%)	p=0.115 <sup>b</sup>
<b>WC (cm)</b>			
Men	80.5 (58.5 – 105.5)	82.0 (63.5 – 108.2)	p=0.559 <sup>a</sup>
Women	75.0 (62.0 – 117.0)	74.0 (60.0 – 111.0)	p=0.753 <sup>c</sup>
<b>WC categorical</b>			p=0.811 <sup>b</sup>
With risk	25 (29.8%)	37 (28.2%)	
Without risk	59 (70.2%)	94 (71.0%)	
<b>FFM (kg)</b>	47.8 (± 8.5)	48.0 (± 9.4)	p=0.997 <sup>a</sup>
<b>FFM (%)</b>	74.9 (± 8.6)	73.3 (± 7.8)	p=0.262 <sup>a</sup>
<b>FM (kg)</b>	16.6 (± 8.2)	17.6 (± 8.0)	p=0.241 <sup>a</sup>
<b>FM (%)</b>			
Men	20.3 (7.5 – 38.6)	19.7 (7.6 – 37.8)	p=0.400 <sup>a</sup>
Women	26.7 (9.3 – 49.8)	28.9 (15.6 – 51.4)	p=0.620 <sup>c</sup>
<b>FM categorical (%)</b>			p=0.796 <sup>b</sup>
With risk	23 (27.4%)	38 (29.0%)	
Without risk	61 (72.6%)	93 (71.0%)	

Results correspond to the absolute frequencies (n) and relative (%) for categorical variables; median, minimal and maximum for WC (cm) and FM (%) of men and women. Results correspond to average ± SD for the other variables. No significant differences were found (p<0.05) from the <sup>a</sup>tStudent Test, <sup>b</sup>Chi-Square Test and <sup>c</sup> Mann-Whitney Test. BMI=Body Mass Index; WC=Waist Circumference; FFM=Free of Fat Mass; FM=Fat Mass.

Women of both groups reached better adequacies as compared to men (Table 3).

Taking EAR as reference (Table 4), despite both groups have reached the average intake of vitamin B12, there was 37.8% prevalence of inadequacy among vegetarians and 0.5% in omnivores (Figure 1). The vitamin D intake was below EAR for both groups (Table 4) and calcium prevalence of inadequacy was 49% for both groups (Figure 1).

Regarding iron intake, it was observed an inadequacy in 50% of the male vegetarians and none of the female reached the RDA for this nutrient. Omnivore men, on the other hand, reached RDA, meanwhile 93% of omnivore women presented intake below RDA. Inadequate intake of zinc was high in the vegetarian group, occurring in 100% of the men and 90% of the women. For omnivores that inadequacy was smaller, 25% of men and 4.5% of women (Table 4).

### Biochemical assessment

Average serum level of vitamin B12 was smaller in vegetarians, although within the reference value. Although

the average of both groups was normal, 30% of the vegetarians presented serum deficiency, showing a tendency to develop macrocytic anemia (Table 5). When the association between dichotomous vitamin B12 intake and blood levels was observed, a 16.71 OR (p=0.001; CI 95% and 2.05 – 136.08) was found.

Serum iron average was also smaller in vegetarians as compared to omnivores. Seven vegetarians and one omnivore showed results below the reference values, and two vegetarians and six omnivores presented increased serum values. Regarding ferritin, its average value was smaller on the vegetarian group, however none participants of both groups presented serum deficiency. Among omnivores, three participants showed increased levels of this variable.

No correlations were established between food intake and nutrient blood levels for the omnivores group. Only for the Ptn intake moderate correlation (r=0.451, p=0.035) was observed in relation to the serum iron among vegetarians (Table 6). Therefore, in order to understand the factors that might interfere on the laboratory results of the vegetarians,

**Table 3** – Energy, macronutrients and fiber intake adequacy of vegetarians and omnivores.

Parameters	Vegetarians (n=38)	Omnivores (n=63)	p
<b>Energy</b>			
Adjusted average intake (kcal/kg)	32.3 (± 10.9)	36.3 (± 10.1)	p=0.066 <sup>a</sup>
EER average (kcal)	2114.7 (1863.7-4049.1)	2156.7 (1453.0-3339.8)	p=0.961 <sup>b</sup>
<b>Energy intake adequacy</b>			
Below EER	13 (34.2%)	15 (23.8%)	-
Above EER	5 (13.2%)	16 (25.4%)	-
<b>Proteins</b>			
Adjusted average intake (g/kg)	1.0 (0.4-1.8)	1,5 (0.9-3.2)	p=0.000 <sup>a†</sup>
Average intake (%TEV)	12.5 (8.2-17.6)	17.7 (11.7-26.8)	p=0.000 <sup>a†</sup>
AMDR (%TEV)	10 to 35	10 to 35	-
Below AMDR (n, %)	12 (31.6%)	1 (1.6%)	-
Above AMDR (n, %)	-	1 (1.6%)	-
<b>Lipids</b>			
Adjusted average intake (g/kg)	1.02 (0.38-1.83)	1.27 (0.57-2.09)	p=0.003 <sup>b†</sup>
Average intake (%TEV)	28.2 (12.4-43.1)	33.2 (21.3-45.0)	p=0.000 <sup>a†</sup>
AMDR (%TEV)	20 to 35%	20 to 35%	-
Below AMDR (n, %)	9 (23.7%)	3 (4.8%)	-
Above AMDR (n, %)	12 (31.6%)	28 (44.4%)	-
<b>Carbohydrates</b>			
Adjusted average intake (g/kg)	4,9 (± 1.7)	4.5 (± 1.3)	p=0.252 <sup>a</sup>
Average intake (%VET)	61.3 (± 6.8)	49.9 (± 6.3)	p=0.000 <sup>a†</sup>
AMDR (%VET)	45 to 65%	45 to 65%	-
Below AMDR (n, %)	10 (26.3%)	24 (38.1%)	-
Above AMDR (n, %)	9 (23.7%)	9 (14.3%)	-
<b>Total fiber</b>			
Average intake (g/1000 kcal)	15.7 (± 4.1)	9.9 (± 3.2)	p=0.000 <sup>a†</sup>
IOM Recommendation (g/1000 kcal)	14	14	-
AI men (g)	38	38	-
* Below AI (n, %)	5 (62.5%)	18 (90.0%)	-
AI women (g)	25	25	-
* Below AI (n, %)	12 (40.0%)	31 (72.1%)	-

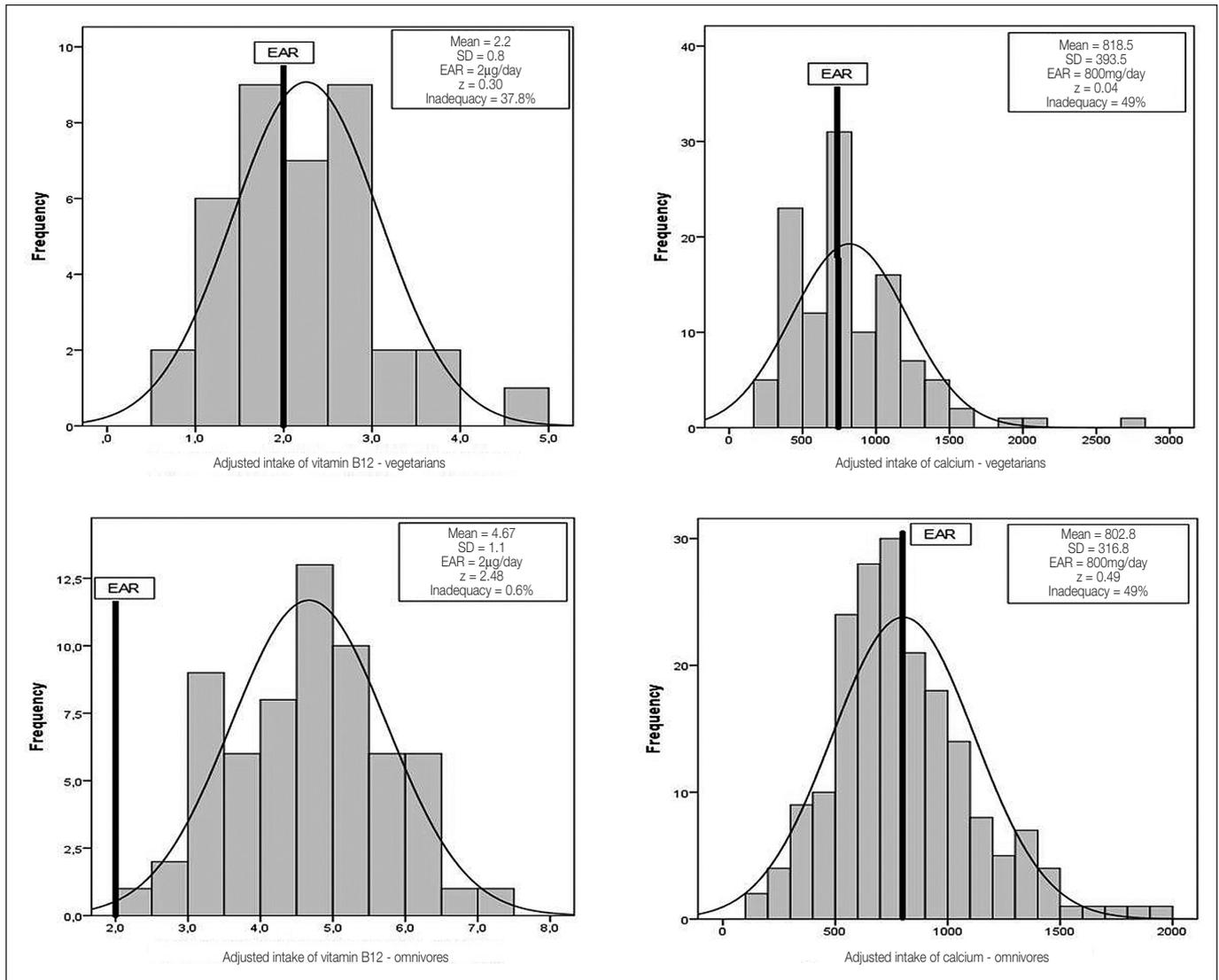
Results correspond to absolute numbers (n) and frequencies (%) for energy below and above EER; Ptn, Lip and Cho below and above AMDR and fiber below AI. Results correspond to average ± SD for the average energy (kcal and kcal/kg) and average intake (in g, g/kg and %TEV) of Cho and total fiber; median and minimum and maximum values for EER averages of groups and average intake (in g, g/kg and %TEV) of Ptn and Lip, Ptn (in g) and Lip (in g/kg). AI values were expressed as median. (\*) indicates significant differences (p<0.05) from at Student and †Mann Whitney Test. † Energy adequacy intake was individually evaluated, through the following classification: below (<80%), adequate (80 to 120%) or above (>120%) of the individual EER. EER=Estimate Energy Requirement; TEV=Total Energy Value; AMDR=Acceptable Macronutrient Distribution Ranges; IOM=Institute of Medicine; AI=Adequate Ingestion.

the association of biochemical exams with the length of the vegetarian dietary practice was analyzed. No significant correlation was found between vegetarianism time and serum iron ( $r=0.355$ ,  $p=0.105$ ) and serum ferritin ( $r=-0,069$ ,  $p=0,761$ ), although a moderate negative correlation with serum vitamin B12 occurred ( $r=0.455$ ,  $p=0.033$ ), showing that the longer the vegetarianism length, the lower the vitamin levels in the blood. According to linear regression model,

vegetarianism length could explain serum vitamin B12 in 20.7% and for every month of this diet a reduction of 0.720 ng/mL of serum vitamin B12 occurred ( $p<0.033$ )

## DISCUSSION

In this study people that had a vegetarian diet for at least six months were chosen. Vegetarianism length of time average



**Figure 1** - Prevalence of intake inadequacy of vitamin B12 (A) and calcium (B) in vegetarians and omnivores. Bars correspond to the frequency distribution of vitamin B12 (Figure A) and calcium (Figure B) intake, accordingly to the three-day RA analysis. Gauss curve, that overlaps the bars, represents the normal distribution of the data. The bold vertical line represents the EAR for vitamin B12 (2µg/day) (Figure A) and for calcium (800mg/day) (Figure B). ANOVA Test was applied for intake adjustment. EAR=Estimated Average Requirement; z=z score.

was four years and six months, with some people reaching 20 years, that was considered enough time to analyze the importance of this feeding practice for the studied group. Participated of this study 68 vegetarians that eat eggs and dairy products and eight strictly vegetarians, both treated, on this study, as “vegetarians”. In this study were evaluated, in total, 84 vegetarians and 138 omnivores.

The fact that vegetarians are more physically active in comparison to omnivores agree with the results of other studies<sup>7,23-26</sup> and one could suggest that their body composition would be more favorable as compared to omnivores. However, weight, BMI, WC, FM and FFM did not differ between the groups, demonstrating that in spite of greater physical activity, body composition of vegetarians was not different from the omnivores and both groups are affected by the nutritional transition, independently of their food practices.

To our knowledge, this is the first time that the identification of study area was registered in this type of study. Although many reasons could be mentioned regarding to the food practices chosen by individuals, it is interesting to note that most vegetarians chose the human sciences and omnivores health science as the fields of study.

Energy intake below EER was observed mainly among vegetarians and above EER among omnivores, with an inversion of energy sources among groups, being Cho the main source of energy among vegetarians and Lip the main source among omnivores. This is explained, once vegetarians replace food of animal sources for vegetables<sup>10,40,41</sup>. Despite this inversion, caloric intake between groups did not differ, as well as the anthropometric characteristics, demonstrating that the source of energy intake did not interfere on body composition.

**Table 4** – Vitamins and minerals intake adequacy intake of vegetarians and omnivores.

Energy/Nutrient	Vegetarians (n=38)	Omnivores (n=63)	p
<b>Vitamin B12</b>			
Average intake (µg)	2.2 (± 0.8)	4.7 (± 1.1)	p=0.000 <sup>a†</sup>
EAR (µg)	2	2	-
Inadequacy (%)	37.8%	0.6%	-
<b>Vitamin D (µg)</b>			
Average (µg)	1.55 (0 – 12.1)	3.71 (0.9 – 24.5)	p=0.001 <sup>a†</sup>
EAR (µg)	10	10	-
<b>Calcium</b>			
Average intake (mg)	818.5 (± 393.5)	802.8 (± 316.8)	p=0.704 <sup>a</sup>
EAR (mg)	800	800	-
Inadequacy (%)	49	49	-
<b>Iron</b>			
Average intake (mg)	14.5 (± 3.9)	15.2 (± 5.4)	p=0.706 <sup>a</sup>
RDA for men (mg)	14.4	8	-
* Below RDA (n, %)	4 (50.0%)	-	-
RDA for women (mg)	32.4	18	-
* Below RDA (n, %)	30 (100%)	40 (93.0%)	-
<b>Zinc</b>			
Average intake (mg)	14.6 (6.5 – 24.1)	13.9 (7.0 – 34.9)	p=0.000 <sup>b†</sup>
RDA for men (mg)	16.5	11	-
* Below RDA (n, %)	8 (100%)	2 (25%)	-
RDA for women (mg)	12	8	-
* Below RDA (n, %)	27 (90.0%)	2 (4.5%)	-

Results correspond to the absolute numbers (n) and frequencies (%) for iron and zinc below RDA; average ± SD for the average ingestion of vitamin B12, calcium and iron variables; median and minimum and maximum values for the median ingestion of vitamin D and zinc. Asterisks (\*) indicate significant differences (p<0.05) from at Student Test and <sup>a</sup>Mann Whitney Test. †Frequencies between parenthesis for iron and zinc were calculated in relation to the total number of participants of each sex in each group. EAR=Estimated Average Requirement; RDA=Recommended Dietary Allowance.

The omnivores diet was hyperproteic and extrapolated EAR (of 0.66 g/kg/day for men and women from 19 to 30 years), due to high meat intake and its processed products<sup>12,40,41</sup>. Among vegetarians, Ptn intake was adequate, although 31.6% of the participants presented ingestion below AMDR. There is a concern in the literature regarding the essential amino acids content in vegetarian diets, especially the strict ones<sup>42</sup>.

However, researches have demonstrated that when the recommended energy level is reached there is a vast intake of Ptn from vegetal sources, being possible to reach the need for these amino acids<sup>4,41</sup>. In addition, it is important to highlight the intake of eggs, milk and derivatives provides all essential amino acids<sup>35</sup> and the largest part of the vegetarians in this study was composed by vegetarians that eat eggs and dairy products. Moreover, a mix of rice and beans is part of Brazilian food culture which provides a profile of essential amino acids close to adequate<sup>43</sup>.

Considering AMDR, the intake of Lip was below expected

among vegetarians that might represent health risks for these individuals, since the absorption of some vitamins could be limited. Among omnivores, the high frequency of individuals with Lip intake above recommended may also represent a risk, predisposing to obesity, some types of cancer, insulin resistance, among others<sup>35</sup>.

The high content of Cho provided by vegetarian diets was already documented on the literature, especially complex Cho, rich in fibers<sup>41</sup>. In this study, it was observed that the vegetarians reached the fiber intake recommendation, provided by a larger amount of fruits, vegetables, grains, seeds, among other wholesome foods<sup>9,41,44-50</sup>. Among the positive effects of this food source is the satiety effect that may have direct consequences on the food intake and weight control<sup>46-49,51-53</sup>. Nevertheless, no anthropometric measures that indicated advantage of the vegetarian choice in comparison to the omnivore were found in the present work. Although omnivores had consumed a small fiber amount, it cannot be affirmed that the frequency of intake among both

**Table 5** – Distribution of serum vitamin B12, iron and ferritin among vegetarians and omnivores.

Laboratory Exams	Vegetarians (♂ n=13 / ♀ n=27)	Omnivores (♂ n=21 / ♀ n=19)	p	Reference Values
Vitamin B12 (pg/mL)	249.4 (± 90.4)	412.8 (± 164.2)	p=0.000 <sup>a</sup>	187 - 883
Vitamin B12 deficiency	12 (30%)	1 (2.5%)	p=0.001 <sup>b</sup>	-
Iron (µg/dL)	98.37 (± 97.9)	123.53 (± 119.05)	p=0.007 <sup>a</sup>	♂ 65 - 175 ♀ 50 - 170
<b>Iron deficiency</b>				
Men	1 (7.7%)	1 (4.8%)	p=1.000 <sup>c</sup>	-
Women	06 (22.2%)	-	p=0.032 <sup>c</sup>	-
<b>Increased iron</b>				
Men	-	5 (23.8%)	p=0.132 <sup>c</sup>	-
Women	2 (7.4%)	1 (5.3%)	p=1.000 <sup>c</sup>	-
<b>Ferritin (ng/mL) *</b>	50.01 (± 50.75)	118.64 (± 129.22)	p=0.000 <sup>a</sup>	♂ 21.81 – 274.66 ♀ 4.63 – 204.0
<b>Increased ferritin</b>				
Men	-	3 (14,2%)	p=0.245 <sup>c</sup>	-
Women	-	-	-	-

Results correspond to average ± SD. Asterisks (\*) indicate significant differences (p<0.05) for <sup>a</sup>t Student Test, <sup>b</sup>Chi-Square Teste and <sup>c</sup>Fisher's Exact Test. Iron and ferritin variables were classified for men and women, for they have different cut-off points. ♂=Men; ♀=Women.

**Table 6** – Association between nutrient intake and laboratory exams in vegetarians and omnivores.

		Protein Intake (g/kg)	Vitamin B12 Intake (µg)	Iron Intake (mg)
<b>Vitamin B12 (pg/mL)<sup>a</sup></b>	Vegetarians	r=0.333 p=0.087	r=0.264 v=0.236	-
	Omnivores	r=0.191 p=0.321	r=0.156 p=0.419	
<b>Iron (µg/dL)<sup>a</sup></b>	Vegetarians	r=0.451* p=0.035	-	r=0.134 p=0.552
	Omnivores	r=-0.116 p=0.549		r=0.077 p=0.690
<b>Ferritin (ng/mL)<sup>b</sup></b>	Vegetarians	r=0.310 p=0.160	-	r=0.101 p=0.654
	Omnivores	r=0.013 p=0.947		r=0.143 p=0.477

Results correspond to the correlation coefficient (r) that represents the positive or negative association strength between laboratory exams and intake of some of the nutrients shown on the table, based on a three-day RA analysis. Asterisks (\*) indicate significant associations (p<0.05) from <sup>a</sup>Pearson Correlation, <sup>b</sup>Spearman Correlation tests.

men and women of this group is synonymous of inadequacy, considering that the reference for this nutrient is the AI.

High prevalence of low vitamin B12 intake, which may lead to the serum deficit of this nutrient in a few months, represents a risk for vegetarians, considering that the clinical symptoms may take years to surface. Chronic deficiency may cause serious and irreversible neuropsychiatric problems,

besides gastrointestinal, hematological symptoms, megaloblastic anemia and increased homocysteine, a situation that increases the risk of total and cardiovascular mortality, stroke and chronic heart failure<sup>54-59</sup>. The literature documents this vitamin B12 deficiency both for vegetarian diets that eat eggs and dairy products and for strict vegetarians<sup>15,17</sup>, corroborating with our findings.

Biochemical assessment indicated that vegetarians presented blood levels 40% lower than the omnivores ones and 30% of the vegetarians was already with an established deficiency. Among the vegetarians that were examined, 38 included eggs and dairy products on their diet and only two were strictly vegetarians, demonstrating that even with the consumption of eggs and dairy, serum deficiency of this vitamin persisted. Some authors consider that, despite the content of vitamin B12 in these foods, they are not good sources, explaining the results that we found<sup>60-62</sup>.

There was no association between vitamin B12 intake and blood levels, which might be explained by the hepatic reserve capacity. The small number of individuals investigated should be taken into consideration as well, in addition to the limitations of the 24HR method. On the other hand, when data were looked at as dichotomous variables, a high OR related to vegetarian diet and vitamin B12 serum deficiency was found. Vegetarianism length of time was also negatively related to this vitamin status, indicating that for every month of this diet choice occurred a 0.720 ng/mL decrease of the vitamin in blood. This is the first time this approach was taken to analyze vegetarian diets.

In this study, the cut-off point for serum vitamin B12 deficiency was very restrict, smaller than 187 pg/mL. In the literature, it has been demonstrated that values beneath 406 pg/mL are already associated with its metabolic deficiency<sup>63</sup>, therefore, the frequency of deficient individuals might have been underestimated in our study. Herrmann & Geisel<sup>64</sup> considered as the ideal cut-off point for vitamin B12 should be 490 pg/mL, which would make all vegetarians and 30 omnivores of our study deficient. Facing the low intake of vitamin B12, the American Dietetic Association recommends its supplementation through fortified foods and/or supplements in order to increase its availability to the organism<sup>3,11,17,65,66</sup>.

Another concern regarding nutrient deficiency was related to the average intake of vitamin D, low on both groups, but especially on vegetarians. Crowe et al.<sup>13</sup> found values of vitamin D intake lower than in the present study when assessing vegetarians that eat eggs and dairies (1.2  $\mu\text{g}$ , n=417), strictly vegetarians (0.7  $\mu\text{g}$ , n=87), semi-vegetarians (2.2  $\mu\text{g}$ , n=208), and omnivores (3.1  $\mu\text{g}$ , n=1359). In the present study, laboratory exams were not performed to test for 25-hydroxivitamin D, however it has been reported that vegetarians present smaller concentrations<sup>13</sup>, which might occur due to the low intake of this nutrient and due to low exposition to sunlight ultraviolet B rays<sup>36,67</sup>. It is recommended that individuals, in general, especially strictly vegetarians, assess their serum 25-hydroxivitamin D levels and, in case of deficiency, supplement vitamin D and/or consume fortified foods, in addition to increasing sun light exposition<sup>4</sup>.

Both groups presented prevalence of calcium intake inadequacy of 49%. This result corroborates with the deficient intake of this nutrient currently observed in the general populations, not only among vegetarians or practitioners of other type of restrictive diets. A study of Ezmirly et al.<sup>19</sup> evaluated 16 strictly vegetarians and 16 omnivores and identified that both did not reach calcium recommendation according to RDA. In the Shridhar et al.<sup>12</sup> study, although vegetarians consumed, in average, more calcium than omnivores (980 and 946.5 mg/day, respectively,  $p < 0.001$ ), 12.5% of omnivores presented inadequacy in relation to RDA, meanwhile this inadequacy was presented in 16.8% of vegetarians.

This data is important if the nutrient bioavailability is taking into consideration once the content of fiber in vegetarian diets is very high. Calcium deficiency, along with vitamin D deficiency, may lead to osteomalacia, osteoporosis, increase bone fractures risk and systemic arterial hypertension<sup>35,67,68</sup>. According to Appleby et al.<sup>69</sup> when the intake of calcium is smaller than 525 mg/day, the risk of bone fracture increases 30%.

Observing the individual intake of calcium among the omnivores participants in this study, nine (five women and four man), showed intake below 525 mg a day, indicating an increased risk osteoporosis and bone fractures. Among vegetarians there were eleven (ten women and one man) showing intake below this cut point. It has been demonstrated that the risk of fractures due to low intake of calcium is even higher in women, especially after the menopause, due to the sudden reduction of estrogen<sup>70</sup>. Although we studied young women, it is important to consider that with the increase of age the maintenance of the present food intake pattern might increase the predisposition to the risk<sup>71</sup>.

Hunt<sup>72</sup> had already reported that the recommended iron intake for vegetarian women in fertile age is difficult to be reached. This implies that vegetarian women might be in frequent need of drug supplementation with its possible consequences such as oxidative stress, constipation, nausea, vomiting and diarrhea<sup>35,42</sup>. Other alternatives to improve iron absorption should be applied such as intake of organic acids concomitantly with non-heme iron sources and the use of techniques to reduce the phytate content of the foods, such as the immersion of legumes, grains and seeds in water and germination of vegetables. In addition, non-heme iron sources should not be consumed with polyphenols and calcium frequently<sup>73-75</sup>.

Differently of the findings in the present study, there are studies showing that vegetarians eat more iron than omnivores. Calkin et al.<sup>76</sup>, for example, found the following average iron intake from a three-day RA:  $18 \pm 1.6$  mg/day in strictly vegetarians;  $14.2 \pm 0.8$  mg/day in vegetarians that eat eggs and dairy;  $14.4 \pm 0.9$  mg/day in Adventist omnivores and  $16.1 \pm 1.1$  nmg/day in non-Adventist omnivores.

However, the evaluation of total intake of iron without considering its chemical form does not indicate the bioavailability of this mineral, which would be worse among vegetarians considering that vegetables present the less absorbable non-heme iron structure<sup>16</sup>, explained by the presence of chelating substances in the food<sup>20,35,77,78</sup>.

Therefore, vegetarians, including the ones that eat eggs and dairy, might present depletion of iron in the body. For that type of vegetarians, it would be ideal to assess the heme iron and non-heme iron intake in the diet separately<sup>16</sup>. This analysis was not performed in this study, once the food composition tables do not display these nutrients separately. This bias aspect was surpassed by the fact that RDA for vegetarians was considered in this study, as mentioned above.

When assessing serum levels of iron, a deficiency in the vegetarian group was observed, possibly indicating anemia. However, when analyzing iron in plasma, only the amount of iron in transit, connected to transferrin, is considered, which does not characterize the iron status in the body. It was also observed that a number of participants of both groups presented iron levels above the reference values. To complement the analysis serum ferritin was also studied, which is a more specific biochemical test to assess iron reserves in the absence of infection<sup>79</sup>.

Serum ferritin deficiency was not observed in both groups, however, it was observed that their values were approximately 60% lower among vegetarians as compared to omnivores. Through individual evaluation, none of the participants presented serum deficiency. Our findings are similar to the trend observed by Leonard et al.<sup>16</sup> that found high levels (40%) of this deficiency in 31 vegetarian women (twenty-two semi-vegetarian and nine vegetarians) in comparison with 76 omnivores.

A few cases, three out of 40 of excess of serum ferritin were detected among omnivores. In one case, it is possible to suspect of hemochromatosis, a genetic alteration that makes the organism absorb an above normal amount of iron, without its proper elimination<sup>80</sup>. It is also possible that high levels of ferritin are related to inflammatory response. Moreover, they might have some lesion in the liver, which made this organ increase the ferritin production and, with malnutrition, the cells migrated to the blood<sup>79,81</sup>. Nevertheless, it is not possible to make any reference about that on this study. These individuals were excluded from the evaluation. This excess may also occur due to excessive supplementation<sup>82</sup>, however, this hypothesis can be excluded, since one of the exclusion criteria of this study was the use of any kind of supplementation.

Studies demonstrate that the iron intake is related to the ferritin status<sup>16,83</sup>, however, in this study, this association was not observed. The correlation analysis showed that the serum iron concentration and ferritin are not associated with the consumption of this nutrient, which may have occurred due

to the small sample size or with the fact that the serum iron level vary throughout the day.

For zinc, both intake and blood levels may be deficient in vegetarians, with greater prevalence among strict vegetarians, females and those individuals from developing countries<sup>12,14,18,19,84-86</sup>. As a consequence, impotence, hair loss, lesions on the eyes and skin, diarrhea and impaired appetite may occur<sup>35,85</sup>. In this study the high inadequacy of zinc intake among vegetarians occurred probably because the sources of this nutrient are mainly found in foods of animal origin. Some vegetable products are source of this nutrient; however, its bioavailability is reduced due to the presence of phytic acid, fibers, oxalates, tannins and polyphenols<sup>72,77,78,85,87</sup>. Therefore, a higher inadequate intake of zinc, another problem for the vegetarians is its low absorption.

Deficiency of zinc is difficult to be estimated in the organism. Since its reduction in the plasma or in the serum might reflect a redistribution of the nutrient in the organism, and not necessarily a deficiency, therefore, this evaluation was not performed in this study<sup>88</sup>. In general, to avoid zinc deficiency it is recommended that vegetarians increase the intake of good sources of the nutrient and utilize strategies to reduce the content of phytates and other inhibitors of its absorption, even if there are no clinical symptoms of deficiency. Other strategies are the use of fortified foods or small doses of supplements, when properly evaluated and needed<sup>18</sup>.

Among the limitations of this study is the small number of strictly vegetarians selected, which prevent the possibility of evaluating this group separately. As for the food intake analysis, the use of 24HR has a few disadvantages as follow: the demand of high level of motivation and collaboration among participants to describe their food intake in details, the knowledge about diverse servings size, the time consuming process to register all the food items during the day and the lower adhesion of males to the method<sup>89</sup>. In addition to these limitations, centesimal food composition tables may present flaws or might be outdated, as well as the softwares elaborated from these tables<sup>7</sup>. To minimize these problems an updated database was used. Moreover, nutrients were calculated by trained nutritionist that possesses more understanding about the theme, reducing the calculation biases.

## CONCLUSIONS

Body composition did not differ among vegetarians and omnivores assessed in this study, indicating that the adopted diet pattern without adequate adjustments do not guarantee a healthy nutritional status. Indeed, energy intake did not present difference among the groups, although the main source of energy was Cho for the vegetarians and Lip for the omnivores. Vegetarians did present a diet richer in fiber; however more restrict in vitamin B12, vitamin D, iron and zinc as compared

to omnivores. Calcium intake did not differ among groups. Vegetarians presented important serum vitamin B12 deficiency and may be at nutritional risk.

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