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Nutrition status and quantification of blood minerals by TXRF spectroscopy in vegetarian and non-vegetarian university students

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ABSTRACT. This study investigated whether university students following a vegetarian diet differed from non-vegetarian students in nutrient intake, biochemical, hematological and blood mineral profile of nutritionally relevant elements. In total, 107 students from a university, following either a non-vegetarian or a vegetarian diet for at least 1 year prior to the study, were recruited in two stages, setting up two experiments. Nutrient intake (experiment 1, n = 58), and biochemical and hematological parameters (experiment 2, n = 49) were evaluated. TXRF spectroscopy was used for determination of trace elements in whole blood. Vegetarians showed differences in nutrient intake, mainly higher consumption of unsaturated fatty acids and fiber. No significant differences in the biochemical and hematological parameters were found. The prevalence of abnormal parameters in a considerable number of vegetarians and non-vegetarians were found, mainly regarding high density lipoprotein (HDL-c) and total cholesterol (TC). TXRF spectroscopy proved to be a simple tool for determining nutrition-relevant elements (K, Fe, Cu and Zn) in blood samples. The high incidence of abnormal parameters, regardless of the dietary pattern, raises concern about the high prevalence of bad eating habits among young university students. Particularly for the vegetarian students, these results may partly counteract the beneficial lifestyle of a vegetarian diet evidenced by previous studies. It is important for students to be aware of its potential nutritional limitations. In this context, food and nutrition education programs in the academic context could contribute to set up autonomous and healthy subjects, regardless of the diet chosen.

Keywords: plant-based diet; eating habits; hematological analysis; nutrient intake.

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Introduction

According to the Academy of Nutrition and Dietetics (Melina, Craig & Levin, 2016) an "[...] appropriately planned vegetarian, including vegan, diets are healthful, nutritionally adequate, and may provide health benefits for the prevention and treatment of certain diseases", including cardiovascular diseases (Huang et al., 2012), diabetes (Kahleova & Pelikanova, 2015) and cancer (Key et al., 2014). These diets are appropriate for all stages of the life cycle, including pregnancy, lactation, infancy, childhood, adolescence, older adulthood, and for athletes. Moreover, they are more environmentally sustainable than diets rich in animal products because they use fewer natural resources and are associated with much less environmental damage (Melina, Craig, & Levin, 2016).

Frequently composed of grains, legumes, fruits and vegetables, vegetarian diets are related to the abundant intake of fibers, unsaturated fatty acids and antioxidants rather than saturated fatty acids and cholesterol (Foster, Chu, Petocz & Samman, 2013). This profile has been associated with some benefits, mainly associated with biochemical and hematological parameters. Wang et al. (2015), for example, presented a systematic review and meta-analysis providing evidence that vegetarian diets effectively lower blood concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), and non–high-density lipoprotein cholesterol (nHDL-c), and concluded that such diets could be a useful non-pharmaceutical means of managing dyslipidemia, especially hypercholesterolemia.

However, there are some nutritional concerns in a non-planned vegetarian diet, such as the risk of deficiencies in nutrients such as vitamin B12, vitamin D, unsaturated fatty acids n-3, and minerals such as calcium, iron, and zinc (Craig, 2010), whether by their low amounts in plant foods or by their lower bioavailability when compared to animal sources (Lee & Krawinkel, 2009).

A vegetarian group prone to these concerns is the academic students. Given the transition from school to the university, academics tend to follow an unhealthy diet, with frequent consumption of fast foods combined with the habit of skipping meals (Instituto Brasileiro de Geografia e Estatística [IBGE], 2011). This relationship is significant, especially when students migrate from their family environment, as evidenced by El Ansari, Stock, and Mikolajczyk (2012), in a study evaluating student eating behaviors from four different regions of Europe. The authors found that students who lived with their parents consumed larger amount of fruits and vegetables when compared to those who lived alone. Associated with other behaviors, such as physical inactivity, smoking and alcohol consumption, it may represent an increased nutritional risk and weight gain (Clarys et al., 2014).

Numerous studies aimed at verifying the nutritional status of vegetarian and non-vegetarian subjects. These studies adopted different experimental approaches and analysis, including measurements of lipid profile and fasting blood glucose, and hematological parameters (Clarys et al., 2014; Zhang et al., 2013). Mineral blood profile was also proposed by some authors as an indicator of nutritional status (Canellas, Carvalho, Anjos & Lopes, 2012; Harrington, Young, Essader, Sumner & Levine, 2014). The determination of trace element levels in human blood is of interest for the biomedical area since several elements take part in all metabolic processes, and can be predictors for several pathological conditions (Canellas et al., 2012).

In this study, we propose the use of the total reflection X-ray fluorescence (TXRF) as a quantitative analysis of nutritionally relevant minerals, mainly iron (Fe), copper (Cu) and zinc (Zn). TXRF is a multielement technique widely used in the analysis of low concentrations in environmental, medical and biological samples. TXRF is a well-established analytical technique for the detection of major, minor and trace elements, especially suited for samples, whenever only a small specimen mass is available (Majewska et al., 2016).

Given the above and considering that university students may adopt unhealthy dietary habits, in this study, we investigated whether university students following a vegetarian diet differ from non-vegetarian students in nutrient intake (Experiment 1), biochemical, hematological and blood mineral profile of nutritionally relevant elements (Experiment 2).

Material and methods

Subjects

For this study, in total, 107 healthy adult female and male subjects between 18 and 35 years old, who was regularly enrolled in an undergraduate or graduate course at the Federal University of Lavras (Lavras, Minas Gerais, Brazil) and have been following either a vegetarian or non-vegetarian diet for at least 1 year prior to the study, were recruited in two stages, at different time intervals (Figure 1), setting up two experiments.

These experiments aimed at characterizing more broadly the university study population by evaluating nutrient intake (experiment 1) and biochemical and hematological parameters (experiment 2) at different times with different samples, without making, however, comparisons and correlations among these variables, but identifying possible differences between vegetarians and non-vegetarians regarding these variables alone.

In the first experiment, conducted from March 2013 to December 2014, we recruited 29 ovo-lacto vegetarians (12 women and 17 men) and 29 non-vegetarians (13 women and 16 men) to evaluate nutrient intake. These volunteers were instructed to fill a 3-day food record.

Then, from February 2016 to August 2016, we recruited 20 ovo-lacto vegetarians (15 women and 5 men) and 29 non-vegetarians (20 women and 9 men) to evaluate biochemical and hematological parameters, and blood minerals measurements. These volunteers were instructed to attend the laboratory of biochemical and hematological analyses associated with the university, where they were further instructed about proceedings to collect blood samples.

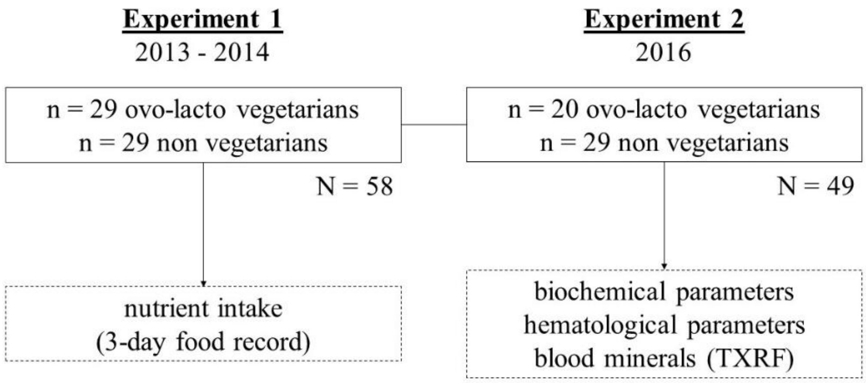

Figure 1. Recruitment steps of ovo-lacto-vegetarian and non-vegetarian university students, and analysis performed.
Source: From the authors.

There were no participants in common between the groups. In both recruitment stages, any subject who had chronic disease or who was overweight or obese was excluded. Signed written informed consent was obtained from each subject. The study has been approved by the Human Research Ethical Committee of the Federal University of Lavras, under protocol 21614413.3.0000.5148. Subjects were recruited at Federal University of Lavras campus using electronic and printed advertisements.

Experiment 1

Assessment of dietary intake

The 3-day food record was composed of three non-consecutive days during the week. The sampling period included 2 weekdays and one weekend day. The complete food records were returned to investigators where the dietary intake was calculated using software Diet Pro® (5.8, A.S. Sistemas, Viçosa, State of Minas Gerais, Brazil). For processed food not listed in the program's database, labels from the package were used. Intake of carbohydrates, lipids, proteins, cholesterol, monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), fiber, calcium, Fe, Zn and vitamin C were quantified. Food records were excluded if under- or over-reporting was apparent.

The adequacy percentage of micronutrient intake (Ca, Fe, Zn and vitamin C) was calculated in relation to the proportion of subjects who achieved the values of the Estimated Average Requirement (EAR). For fiber intake adequacy, the Adequate Intake (AI) was considered. Macronutrient adequacy was calculated considering the reference values proposed by Acceptable Macronutrient Distribution Range (Food and Agriculture Organization/World Health Organization [FAO/WHO], 2005). For MUFA, PUFA, SFA and cholesterol, WHO recommendations were used (Food and Agriculture Organization/World Health Organization [FAO/WHO], 2003). From these data, the ratio of saturated to unsaturated fatty acids was estimated. Energy intake adequacy considered the Estimated Energy Requirements (EER) (FAO/WHO, 2005).

Experiment 2

Biochemical and hematological measurements

Biochemical parameters examined were fasting blood glucose, total cholesterol (TC), low density lipoprotein (LDL-c), high density lipoprotein (HDL-c), very low-density lipoprotein (VLDL-c) and triacylglycerol (TAG).

Hematological measurements included counting of red blood cells (erythrocytes, hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (CMCH) and red cell distribution width (RDW)), and counting of white blood cell (leukocytes, lymphocytes, monocytes, eosinophils, basophils and platelets).

After a 12 h fast, blood samples were collected in Santa Cecilia laboratory, a private company from Lavras (State of Minas Gerais, Brazil), certified to ISO 9001, and measures were performed according to standardized methods adopted by the company.

Blood mineral measurement

Sample preparation

From samples collected in Santa Cecilia laboratory, a fraction of blood was separated to measure the mineral profile. Samples were collected into vacutainer tubes without additives (Greiner Bio-One International AG, VACUETTE). Immediately after collection, each blood sample was stored in a freezer at -18°C until the analysis.

Sample preparation followed the method proposed by Stosnach and Mages (2009) using whole blood, with adaptations. Briefly, at room temperature, a volume of 250 μL whole blood was taken, weighed and diluted with 1250 μL ultrapure water, from the Milli-Q water purification system (Millipore Systems Inc., Bedford, MA). An internal standard consisting of 10 μL Gallium solution (Gallium ICP standard traceable, Merck) was added (100 mg L^{-1}). Then, the solution was homogenized by shaking and a small aliquot of 10 μL was pipetted on a pre-cleaned ultra-pure quartz disk. After the deposition, the samples were left to dry very slowly under a laminar flow hood. Samples were analyzed in triplicate.

Total Reflection X-Ray Fluorescence analysis

All measurements presented were performed with the benchtop TXRF spectrometer 'S2 PICOFOX' (Bruker AXS Microanalysis, Berlin, Germany). The samples were analyzed applying a counting time of 1000 s.

Statistical analysis

Statistical analysis was carried out using R software version 3.0.2. Non-normally distributed variables were log-transformed prior to analysis. The food records were analyzed using the nutrition software Diet Pro® (5.8, A.S. Sistemas, Viçosa, MG, Brazil), which allows calculation of energy as well as the macro- and micronutrient intakes of each subject. One-way ANOVA was used to test differences in biochemical and hematological parameters as well as dietary intake between the groups. Normally distributed variables were compared using Student's t-test. Non-normally distributed variables (protein, PUFA and MUFA and biochemical and hematological parameters) were compared by Mann-Whitney test. The level of significance for all analysis was set at $p < 0.05$.

The prevalence of abnormal parameters in the study groups was calculated and was represented as the percentage of subjects with some variable outside the recommended ranges, according to reference values for each parameter.

Results

Experiment 1

Assessment of dietary intake

Table 1 lists energy, macronutrient and micronutrient intakes. Energy, lipids, SFA, calcium and vitamin C intake did not differ significantly between the groups ($p > 0.05$), but, although SFA intake was similar and represented less than 10% of the caloric intake as recommended by WHO, ratio of PUFA to SFA was higher in vegetarians (2.47 g) when compared to non-vegetarians (1.95) ($p < 0.05$). When stratified by gender, a significant difference was detected regarding consumption of Fe, which was higher in the group of vegetarian women ($p < 0.05$).

Macronutrient intake, in general, was satisfactory in both groups. In relation to micronutrients, there was a trend of inadequacy, since EAR recommendations have been achieved for more than half of the volunteers only for vitamin C in both genders and groups, and Fe by men in both groups. The EAR recommendation for fiber was met by the highest percentage of vegetarian women (50%). The lowest percentage of Fe adequacy was observed in omnivorous women (12.5%), as presented in Table 2.

Experiment 2

Assessment of biochemical and hematological parameters

Biochemical profile data are presented in Table 3. No significant differences in fasting glucose, TC, TAG, HDL-c, LDL-c, VLDL-c and TAG:HDL-c were detected between the groups.

Mean fasting glucose of both groups was within the normal range, excepted for a minor percentage of subjects, who presented fasting glucose below the normal (Figure 1).

Table 1. Average daily energy, macronutrient and micronutrient intake of all subjects participating in the study – experiment 1.

Variable	Gender	Reference intake[a]	Diet type		p
			Vegetarian (29)	Non-vegetarian (29)	
Daily energy (Kcal)		2000 Kcal	1802.16 ± 466.48	1713.99 ± 422.02	0.669
Protein (%)	-	10 – 35	11.72 ± 2.11	17.62 ± 5.86	<0.001***
Carbohydrate (%)	-	45-65	56.86 ± 6.97	50.75 ± 7.12	<0.001***
Lipid (%)	-	20-35	31.41 ± 6.94	32 ± 5.87	0.735
Cholesterol (mg)	-	< 300	183.46 ± 219.31	431.7 ± 300.61	<0.001***
MUFA (g)	-	[b]	9.82 ± 3.5	9.06 ± 2.39	0.000***
PUFA (g)	-	6 - 10%	8.9 ± 2.20	7.63 ± 1.98	<0.001***
SFA (g)	-	Up to 10%	8.27 ± 3.03	9.28 ± 2.94	0.433
Fiber (g)	M	38.00	32.42 ± 18.63	27.02 ± 9.81	<0.001***
	F	25.00	25.83 ± 10.52	19.28 ± 5.97	<0.001***
Calcium (mg)	M	800.00	528.60 ± 253.52	598.93 ± 249.75	0.558
	F	800.00	529.93 ± 242.25	567.8 ± 255.60	0.716
Iron (mg)	M	6.00	9.89 ± 3.43	9.74 ± 2.72	0.901
	F	8.10	8.53 ± 2.41	6.55 ± 2.11	0.040*
Vitamin C (mg)	M	75.00	98.41 ± 68.42	89.89 ± 67.34	0.736
	F	60.00	81.49 ± 45.2	67.63 ± 45.16	0.429
Zinc (mg)	M	9.40	6.59 ± 2.36	9.62 ± 3.02	0.004**
	F	6.80	5.98 ± 1.57	7.02 ± 2.99	<0.001***

[a] Estimated Average Requirement (EAR) for micronutrients (calcium, iron, zinc and vitamin C); Adequate Intake (AI) for fiber; Acceptable Macronutrient Distribution Range (AMDR), OMS for MUFA, PUFA, SFA and cholesterol; Energy Requirements Estimated (EER) for energy intake. [b] Recommendation for MUFA = Total lipid - (PUFA + SFA + trans-FA). *significant difference at 5% by Student's t-test. **significant difference at 1% by Student's t-test. ***significant difference < 1% by Student's t-test.

Table 2. Percentage of academic vegetarians and non-vegetarians with adequate intake of nutrients according to the recommendations.

Variable	Gender	Adequacy (%)	
		Vegetarian (29)	Non-vegetarian (29)
Protein	-	72.4	93.1
Carbohydrate	-	93.1	82.7
Lipid	-	72.4	75.9
Cholesterol	-	82.7	44.8
MUFA	-	-	-
PUFA	-	69.0	69.0
SFA	-	82.7	62.1
Fiber	M	23.5	23.1
	F	50.0	25.0
Calcium	M	17.7	23.1
	F	16.7	6.30
Fe	M	94.1	92.3
	F	50.0	12.5
Vitamin C	M	58.8	61.5
	F	67.7	56.3
Zinc	M	11.8	38.5
	F	33.3	37.5

Source: From the authors.

Lipid profile was also similar between the groups and the mean values found for TC, TAG, LDL-c, VLDL-c and TAG:HDL-c were within the normal range, except for HDL-c. In fact, expressive percentage of individuals, both vegetarians and non-vegetarians, presented HDL-c outside the normality range (Figure 1).

In addition, a considerable number of individuals presented TC levels above the desirable range. It was noted that a higher percentage of individuals who presented TC above the desirable levels were non-vegetarians (28%), besides that, significant percentage of vegetarians (20%) also presented higher TC levels.

Hematological data (Table 3) indicated no significant differences in erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW between the groups. In the leukogram, no significant differences in leukocytes, segmented leukocytes, lymphocytes, monocytes, eosinophils and basophils were found between the groups. Platelet levels were significantly lower in vegetarians ($p < 0.05$). Nevertheless, the mean values observed for platelets, as well as for the other parameters, were within the normal range for both groups.

Table 3. Mean and standard deviation (SD) for fasting blood glucose, lipid profile and hematological parameters of vegetarian and non-vegetarian university students – experiment 2.

Parameter [a] (mg dL^{-1})	Vegetarian (n = 20)	Non-vegetarian (n = 29)	p [b]
	Mean (SD)		
Fasting glucose	78.75 (5.51)	78.34 (5.03)	0.714
TC	177.80 (29.80)	177.41 (36.28)	0.984
TAG	104.25 (61.39)	97.07 (46.43)	0.984
HDL-c	56.80 (14.60)	58.52 (12.62)	0.528
LDL-c	100.15 (19.08)	99.48 (27.62)	0.887
VLDL-c	20.85 (12.92)	19.41 (9.36)	0.943
TAG:HDL-c	2.00 (1.38)	1.72 (0.85)	0.707
Erythrogram			
Erythrocytes (1/mm^3)	4.64 (0.57)	4.78 (0.48)	0.179
Hemoglobin (g/dL)	13.40 (1.56)	14.10 (1.46)	0.077
Hematocrit (%)	40.10 (3.87)	41.94 (4.15)	0.078
MCV (fL)	86.86 (6.85)	87.92 (3.93)	0.855
MCH (pg)	29.04 (2.83)	29.57 (1.63)	0.416
MCHC (%)	33.37 (1.08)	33.62 (0.90)	0.489
RDW (%)	12.97 (1.41)	12.74 (0.57)	0.935
Leukogram			
Leukocytes (10^3/mm^3)	9.57 (0.57)	6.6 (1.29)	0.508
Segmented leukocytes (10^3/mm^3)	52.88 (1.56)	51.32 (9.67)	0.229
Lymphocytes (10^3/mm^3)	37.83 (8.50)	35.75 (7.21)	0.272
Monocytes (10^3/mm^3)	10.32 (13.19)	7.90 (2.12)	0.684
Eosinophils (10^3/mm^3)	4.94 (8.51)	2.86 (1.43)	0.927
Basophils (10^3/mm^3)	0.78 (0.84)	2.89 (1.39)	0.187
Platelets (10^3/mm^3)	231.86 (50.69)	267.05 (67.285)	0.046

[a] TC: total cholesterol; TAG: triacylglycerol; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; VLDL-c: very low-density lipoprotein; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: concentration of mean corpuscular hemoglobin; RDW: red cell distribution width. [b] Statistical significance was considered at $p < 0.05$. Source: From the authors.

Mean values for all parameters were within the normality range, excepted for a minor percentage of subjects, who presented parameters below the normality. However, a remarkable number of vegetarians (20%) presented hemoglobin levels below the normality range (Figure 1).

While a minor percentage of vegetarian and non-vegetarian subjects presented leukogram parameters below the normality, for eosinophils, however, the values were slightly higher. It was noted 20% vegetarians and 31% non-vegetarians presented levels below the normality range (Figure 2).

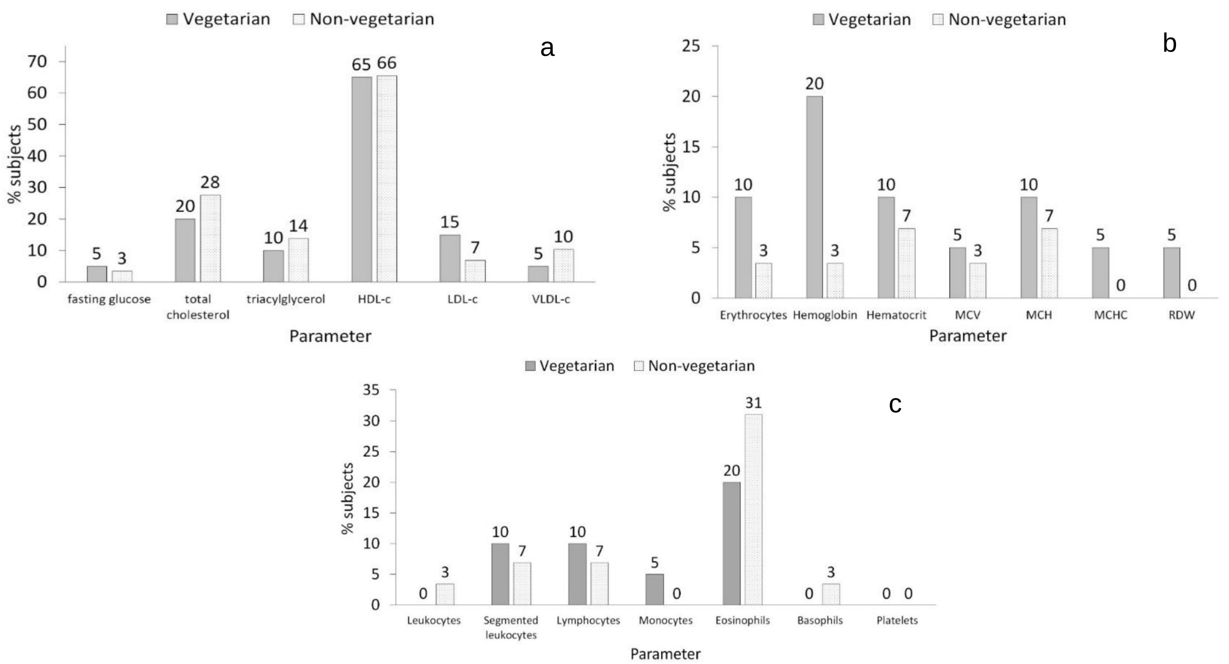

Figure 2. Prevalence of abnormal parameters in fasting glucose and lipid profile (a), erythrogram (b) and leukogram (c) in vegetarian and non-vegetarian groups – experiment 2. Source: From the authors.

Blood mineral measurement

Figure 3 illustrates a typical X-ray fluorescence spectrum of a blood sample using TXRF. It was possible to detect the presence of 9 elements: chlorine (Cl), potassium (K), iron (Fe), copper (Cu), zinc (Zn), bromine (Br), rubidium (Rb), niobium (Nb) and antimony (Sb).

Table 4 presents the quantitative results found between the groups for the nutritionally relevant minerals K, Fe, Cu and Zn evaluated in this study. No significant differences were detected between vegetarian and non-vegetarian groups.

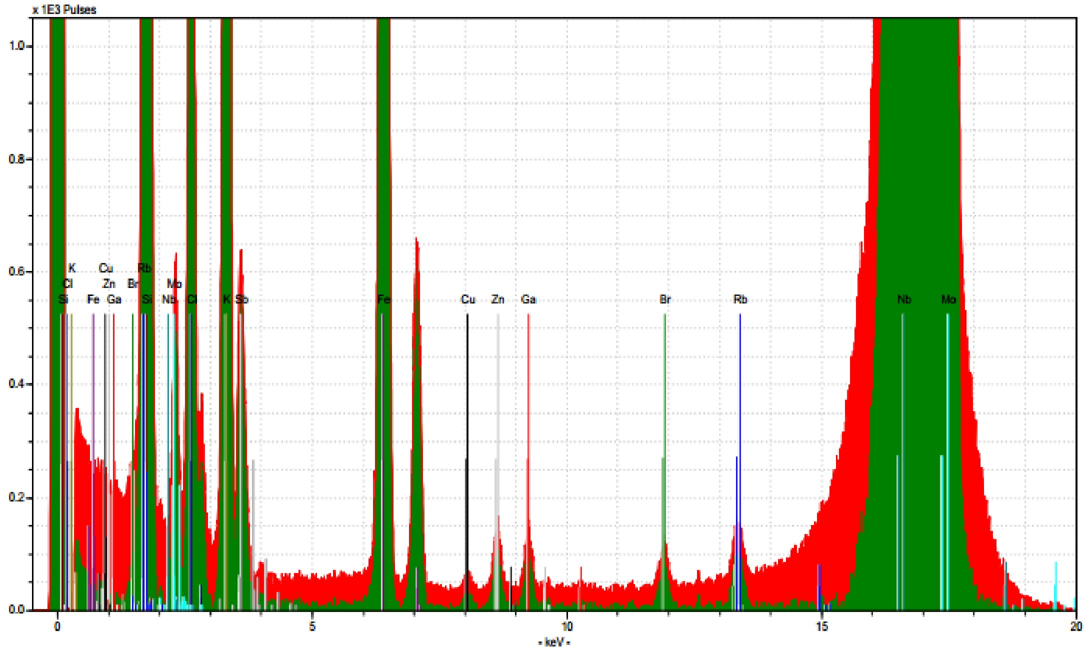

Figure 3. X-ray fluorescence spectrum of a blood sample using TXRF.

Source: From the authors.

Table 4. Mean and standard deviation for essential minerals in whole blood of vegetarian and non-vegetarian university students – experiment 2.

Parameter (mg dL^{-1})	Vegetarian (n = 20)	Non-vegetarian (n = 29)	p[a]
	Mean (SD)		
K	198.27 (30.35)	196.20 (70.74)	0.895
Fe	37.49 (9.76)	40.14 (1.56)	0.662
Cu	0.09 (0.04)	0.08 (0.04)	0.387
Zn	0.47 (0.12)	0.49 (0.12)	0.745

[a] Statistical significance was considered at $p < 0.05$.

Source: From the authors.

Discussion

In the present study, vegetarian students showed differences in nutrient intake when compared to non-vegetarians, mainly due to the higher consumption of unsaturated fatty acids and fiber. Lower intake of cholesterol was also observed in the vegetarian group, while SFA did not differ between the groups. No significant differences in the biochemical and hematological parameters were found between the groups, except for platelets count, which was significantly lower in vegetarians. The prevalence of abnormal parameters in a considerable number of vegetarian and non-vegetarian subjects were verified, mainly regarding HDL-c and TC.

The higher consumption of MUFA and PUFA corroborates previous studies (Hawk, Englehardt & Small, 2012; Kristensen et al., 2015) as well as the higher ratio of unsaturated to saturated fatty acids (Majewska et al., 2016) and fiber intake (Vinagre, Vinagre, Pozzi, Zacari & Maranhao, 2014). However, no influence of this dietary pattern on plasma concentrations of total cholesterol were noted between the groups, contradicting studies that evidence this tendency when food of animal origin is restricted (Santos et al., 2013; Vinagre et al., 2014; Wang et al., 2015).

In fact, it is well established SFA, as well as trans fatty acids, are the dietary lipid components that most influence blood cholesterol, while cholesterol has minor effects on this parameter (Zhang et al., 2013). The similar intake of SFA between the groups may explain these results, because the studied vegetarians consumed animal products such as milk and eggs.

Findings regarding lipid profile, mainly HDL-c, in vegetarian groups are controversial. While some authors found no differences between vegetarians and non-vegetarians (Cedo et al., 2015; Santos et al., 2013; Vinagre et al., 2014) another study showed a significantly lower value of this parameter in vegetarians (Huang et al., 2012). Wang et al. (2015) in a systematic review and meta-analysis showed that vegetarian diets, compared with omnivorous diets, improve therapeutic targets for cardiovascular disease risk reduction, including LDL-c. There were also relative reductions in HDL-c, TC, with no effect on TAG.

However, as no significant difference between the groups was detected in the present study, it can be assumed that a considerable number of students evaluated herein have inadequate dietary intake, regardless of the absence of meat in the diet. This tendency corroborates other studies that found high prevalence of malnutrition and cardiovascular risk factors among young university students (Delgado Floody, Alarcon Hormazabal & Caamano Navarrete, 2015), and particularly for the vegetarian students, these results may partly counteract the beneficial lifestyle of a vegetarian diet evidenced by previous studies.

Regarding the TAG concentrations between vegetarian diets and non-vegetarian diets, the studies have shown some controversy during the previous decades. However, in a meta-analysis of cross-sectional and cohort studies, Zhang et al. (2013) suggested that vegetarian diets could help lower TAG levels. Results from the present study, however, did not evidence differences between the groups, reinforcing that the vegetarian and non-vegetarian students presented similar diet intake pattern, with no benefits for the vegetarians.

Despite the apparent higher intake of fiber among the vegetarians, a low percentage of volunteers met the EAR, totaling 36.7 and 24% of vegetarians and non-vegetarians, respectively. The low consumption of fiber could probably be associated with low intake of legumes and dark green vegetables, which also may have influenced the low intake of Fe and zinc.

In relation to Fe intake, the present study found significant differences only among women, lower in the non-vegetarian group. However, DRIs suggest Fe intake 1.8 times higher for vegetarians to compensate for the lower bioavailability of this mineral (Institute of Medicine [IOM], 2001). This recommendation was not achieved in this study. Among men, besides similar Fe intake, closer values to EAR were noted when compared to women. Two studies have found similar values (Hawk et al., 2012; Shridhar et al., 2014). Shridhar et al. (2014) found average intake of Fe between 25.5 mg and 22.4 mg in vegetarians and non-vegetarians, respectively, while in another study, this adjustment was found only in males, with an average intake of 18.5 mg (Hawk et al., 2012). Part of the discrepancy between the studies is due to food fortification, different evaluation methodologies and different consumption patterns.

In general, results from Experiment 1 evidenced that the inadequacy of micronutrients prevailed among students of both groups, which may be associated with inadequate food choices among university students, as pointed out by De Piero, Bassett, Rossi & Samman (2015). In vegetarians, these choices still may be associated with the reasons influencing adherence to this type of diet. Those individuals motivated by factors related to health, show greater concern about the adequacy of the diet when compared to those who were motivated by ethical issues.

Results of hematological parameters, on the other hand, did not indicate anemia, given that plasma hemoglobin levels were similar between groups. Hemoglobin is an intracellular protein with high sensitivity to the malnutrition process when compared to the other proteins for nutritional analysis. This indicator has been used in previous studies evaluating the nutritional status of specific populations (Moore, Pawloski, Rodriguez, Lumbi & Ailinger, 2009). In vegetarians, some findings suggested that there is no significant difference in hemoglobin concentrations between vegetarians and non-vegetarians (Haider, Schwingshackl, Hoffmann & Ekmekcioglu, 2018). Besides, the lower platelet count was verified in the vegetarian group, this level was within the normal range.

Regarding TXRF spectroscopy, it proved to be a powerful tool for determining multi-element concentrations in human blood samples. The simple dilution with ultrapure water was sufficient to identify the nutrition-relevant elements K, Fe, Cu and Zn, which is agreement with the literature (Stosnach & Mages, 2009).

From the nutritional point of view, results indicated no differences in blood mineral content between vegetarians and non-vegetarians. On the other hand, Haider et al. (2018) in a systematic review and meta-analysis, showed that vegetarians are more likely to have lower Fe stores compared with non-vegetarians. However, some considerations may be addressed to this topic.

Fe and Zn are the trace minerals of greatest concern when considering the nutritional value of vegetarian diets (Schupbach, Wegmuller, Berguerand, Bui & Herter-Aeberli, 2017). According to Hunt (2003), with elimination of meat and increased intake of phytate-containing legumes and whole grains, the absorption of both Fe and Zn is lower in vegetarian than with non-vegetarian diets. The health consequences of lower Fe and Zn bioavailability are not clear, especially in industrialized countries with abundant, varied food supplies, where nutrition and health research has generally supported recommendations to reduce meat and increase legume and whole-grain consumption. In addition, supplements or fortified products can potentially fulfill requirements for vitamin and mineral consumption (Schupbach, Wegmuller, Berguerand, Bui & Herter-Aeberli, 2017). This is supported by Craig (2010), who stated that, although a vegetarian diet can meet current recommendations for all of these nutrients, the use of supplements and fortified foods provides a useful shield against deficiency.

Notwithstanding it is clear that vegetarians have lower Fe stores, adverse health effects from lower Fe and Zn absorption have not been demonstrated with varied vegetarian diets in developed countries, and moderately lower Fe stores have even been hypothesized to reduce the risk of chronic diseases (Hunt, 2003). In fact, high Fe stores are recognized as a risk factor for certain non-communicable diseases, such as type II diabetes. Thus, it is recommended that not only vegetarians but also non-vegetarians should regularly control their Fe status and improve their diet regarding the content and bioavailability of Fe by consuming more plants and less meat (Haider et al., 2018).

Conclusion

Both dietary groups showed adequate intakes for some macronutrients, and deficiencies for some micronutrients. On average, the university students presented adequate biochemical and hematological profile, and the absent of meat did not improve their nutritional status. However, the high incidence of abnormal parameters among the students, regardless the dietary pattern, raises concerns about the high prevalence of poor eating habits and cardiovascular risk factors among young university students.

Thus, it is important for academics to be aware of its potential nutritional limitations, particularly the vegetarian ones. Food and nutrition education programs in the academic context could contribute to set up autonomous and healthy subjects, regardless the type of diet chosen.

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