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# Folate intake in a Portuguese female student population and its relation to body mass index, physical activity level and intake of other nutrients

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**Abstract**.
**BACKGROUND:** Poor compliance to current recommendations of folic acid intake has been observed. Insufficient intakes of folate have been linked to neural tube defects in birth, anemia, neuropsychiatric disorders, some cancers and cardiovascular diseases.
**OBJECTIVE:** The present study aimed to examine folate intake and its relationship with body mass index, physical activity level and intake of other nutrients among female Portuguese university students.
**METHODS:** 67 female students with ages between 19 and 25 years old participated in the study. Dietary intake was assessed by prospective three days food records; physical activity level was evaluated based on reported time spent in several activities.
**RESULTS:** Mean body mass index (BMI) was $22.1 \pm 0.4\,kg/m^2$ (normal weight); mean physical activity level was $1.57 \pm 0.02$ (low active physical activity level). Mean folate intake was $193.3 \pm 9.4\,\mu g$/day, below the recommended dietary allowance of 400μg/day. A high prevalence of inadequate folate intake was found (49.2%).
**CONCLUSIONS:** Folate intake was not associated with physical activity categories or BMI values. A positive correlation was found between folate intake, vitamin C and vitamin A intakes. No correlation was found between folate intake and macronutrient intake.
Keywords: University students, Portugal, food records, nutrient intake, folate

## 1. Introduction

Folate is a B vitamin which is a key component in the synthesis and repair of DNA, providing for the synthesis of pyrimidine and purine nucleotides. Folate cycle is also linked to the methionine cycle, through regeneration of methionine from homocysteine [1]. Together, folate and methionine cycles are essential in one-carbon metabolism (transference of methyl groups), involving methylation of several biomolecules including DNA and proteins.

The association of folate deficiency and neural tube defects (NTD), mainly spina bifida and anencephaly is well established for a long time [2] and supplementation with the synthetic form of the vitamin, folic acid, around time of conception can reduce the risk of having a neural-tube defect pregnancy by more than 50% [3, 4]. More recently folate deficiency has been linked to other several health problems. A higher risk of emotional problems has been reported in children from mothers with prenatal folate deficiency [5]. Low folate intakes may also be associated with anxiety and depression illness [6, 7]. Moreover, folate intakes below the recommended levels have also been associated to

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a higher risk of major chronic non communicable diseases, such as cardiovascular diseases [8, 9], several common cancers [10] and neurological disorders in later life [11, 12].

University students are a population at risk of some micronutrient deficiency due to frequently adopted unhealthy nutritional habits, normally with low consumption of fruits and vegetables [13–15]. Particularly, folate intake in young adulthood seems to be associated with risk of hypertension development later in life [16]. Low physical activity levels of some university students are also a risk factor for cardiovascular disease [17]. This study aimed to assess folate intake and its relationship with body mass index, physical activity level and intake of other nutrients among 67 female Portuguese university students.

## 2. Materials and methods

### *2.1. Subjects, dietary and physical activity assessment*

The study was undertaken in Polytechnic Institute of Santarem, in Portugal. Participants were 67 female students at the second year of one of the Institution courses, with ages between 19 and 25 years old, who agreed to complete a three day consecutive report. After oral consent of subjects, anthropometric measures were obtained by a trained professional with subjects in light clothing and barefoot. Body weight was measured to the nearest 0.1 kg using a digital scale (TANITA, model SC-330) and height was measured to the nearest centimeter using a stadiometer (TANITA, model Leicester HR001); body mass index (BMI, kg/m$^2$) was calculated by dividing the weight (in kg) by the square of height (in m). BMI categories were: low weight, < 18.5 kg/m$^2$; normal weight, $\geq$18.5, <25.0 kg/m$^2$; excess weight 25.0 to 29.9 kg/m$^2$; obesity $\geq$30 kg/m$^2$ (WHO, 2005).

Dietary intake was assessed by prospective food records of three non-consecutive days (two week days and one weekend day), in accordance with recommendations to study dietary inadequacy in a population subgroup [18]. Participants were instructed to 1) write down everything they ate along the day, including all snacks, beverages and supplements, with the corresponding serving sizes according to a Portuguese food photographic manual [19]; 2) describe the type of food eaten and cooking process; 3) not to change any of their usual lifestyle and eating habits during the study. Food intakes were converted to nutrient intakes using a Portuguese food composition database [20].

To evaluate physical activity level (PAL), participants reported the daily average time spent in the following activities: sleeping, self-care activities, transport (either by car, bus or train), work (sitting, standing or heavy non-mechanized work), walking (slow or rapid) household activities and leisure time activities (light and sport activities). PAL was calculated according to the equation: ($\Sigma$ daily hours spent in each activity $\times$ energy cost of the activity)/24 h [21]. In order to compare nutrient intake according to BMI and physical activity levels, two groups of BMI and two groups of physical activity were defined. The two groups defined for BMI were normal weight (group 1) *versus* excess weight plus obesity (group 2, BMI $\geq$25.0). The two groups defined for physical activity were sedentary and low active (group A, PAL <1.6) *versus* active and very active (group B, PAL $\geq$1.6), as defined by the Institute of Medicine (IOM, 2005b).

Estimated energy requirement (EER) was calculated as basal metabolism requirement (BMR) multiplied by PAL [21]; confidence interval (CI) for EER was calculated as mean $\pm$ 2 standard deviation (SD).

The prevalence of inadequate micronutrient intakes was determined using the Estimated Average Requirement (EAR) cut-point method, estimating prevalence of inadequacy as the percentage of individuals with usual intakes below the EAR [22].

### *2.2. Statistical analysis*

Nutrient distribution was analyzed after adjustment for total energy intake, using percentages of total energy intake for macronutrients and the nutrient residual model for vitamins and minerals [23]. Statistical analysis was performed with IBM SPSS software, version 20.0 for Windows. Normality was assessed by Kolmogorov-Smirnov test. The *t*-test and Mann-Whitney-U test were used to compare two independent samples of normal and non-normal distribution, respectively. Statistical significant differences were considered for $P < 0.05$. Values are presented as mean $\pm$ standard error of the mean (SEM).

## 3. Results

After energy adjustment, all tested variables were normal except energy intake, carbohydrates, total fat and calcium. Mean age of study participants was 20.3 ± 0.3 years and mean BMI was 22.1 ± 0.4 kg/m$^2$, ranging from 17.0 to 30.6. Most of the participants had normal weight (74%), 18% had excess weight, 1.5% were obese and 6% had low weight. Mean physical activity level was 1.57 ± 0.02, which is in the low active physical activity category (IOM, 2005b), ranging from 1.12 – 2.29 (Table 1), with 59.1% of the participants in the sedentary and low active group (group A), and 40.9% in active and very active group (group B). No significant differences in BMI values were observed between the two physical activity groups ($p = 0.340$).

The most consumed food group was dairy, followed by carbohydrate and protein sources (Fig. 1). Dairy, including milk (half fat), yogurt and cheese accounted for 31% of the daily food intake. As carbohydrate main sources, potato, rice and pasta were consumed at meals (17% of daily food intake), while bread, breakfast cereals and cookies were consumed at breakfast and between meals (16% of daily food intake). Protein sources such as meat, fish and eggs accounted for 16.5% of total food intake. Vegetables and fruits were the less consumed foods (Fig. 1), providing for 8% and 12% of total food intake, respectively.

Participants' mean nutrient intakes are described in Table 2. The mean estimated energy requirement for the studied population was 2105 kcal/day (or in Mega Joules, 8.8 MJ/day); SD 297 kcal/day (1.2 MJ/day), with a confidence interval of 1511 to 2699 kcal/day (6.3 to 11.3 MJ/day). Twenty-six participants reported energy intake below the estimated confidence interval (41%) and only one reported an energy intake superior to the estimated confidence interval. From the twenty-six lower intakes, nineteen (73%) reported an intake between 25 and 50% less than the estimated energy requirement.

Mean intake of carbohydrates, total fat and protein was within the recommended intervals (Table 3). However, a prevalence of inadequacy was observed for carbohydrate and fat, with 37% of participants reporting a lower intake of carbohydrates and 20% of participants reporting a higher intake of total fat (Table 3).

Table 1
Distribution of participants in physical activity categories (total $n = 67$)

| Group | Lifestyle | PAL[1] | *n* | % |
|---|---|---|---|---|
| A | Sedentary | ≥1.0 <1.4 | 6 | 9.1 |
| | Low active | ≥1.4 <1.6 | 33 | 50 |
| B | Active | ≥1.6 <1.9 | 25 | 37.9 |
| | Very active | ≥1.9<2.5 | 2 | 3.0 |

[1]Categories of lifestyle were defined according to intervals of Physical Activity Level [24].

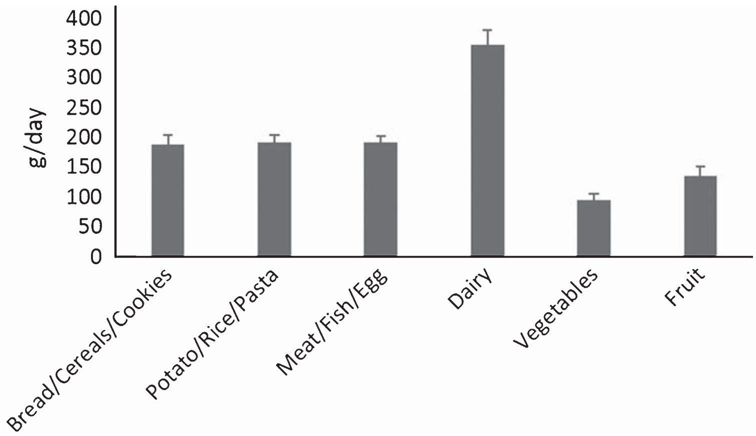


Fig. 1. Daily intake of different food groups.

Table 2
Mean daily intake of studied nutrients (energy adjusted; $n = 67$)

| | Mean ± SEM | Range |
|---|---|---|
| *Total energy intake* | | |
| kcal/day | 1640.8 ± 55.5 | 926.3 – 2704.3 |
| MJ/day | 6.9 ± 0.2 | 3.9 – 11.3 |
| *Macronutrient intake (% of total energy/day)* | | |
| Carbohydrate | 47.3 ± 0.7 | 31.1 – 58.1 |
| Fat | 31.1 ± 0.7 | 17.9 – 53.8 |
| Saturated Fat | 10.7 ± 0.4 | 4.5 – 19.5 |
| Protein | 21.3 ± 0.7 | 13.8 – 41.1 |
| *Micronutrient intake* | | |
| Vitamin A (mg of retinol equivalents/day) | 506.8 ± 39.7 | 36.6 – 2064.0 |
| Vitamin C (mg/day) | 98.9 ± 7.3 | 16.3 – 243.8 |
| Folate (μg of folate equivalents/day) | 193.3 ± 9.4 | 51.4 – 371.6 |
| Calcium (g/day) | 723.9 ± 33.2 | 179.2 – 1473.3 |
| Iron (μg/day) | 9.7 ± 0.5 | 3.3 – 25.4 |

Table 3
Prevalence of inadequacy of macronutrient intake

| | % of participants with values | | | DRI (adults) |
|---|---|---|---|---|
| | Below DRI | Within DRI | Above DRI | |
| Carbohydrate | 32 | 68 | 0 | 45 – 60[1] 45 – 65[2] |
| Fat | 2 | 79 | 20 | 20 – 35[2,3] |
| Protein | 0 | 97 | 3 | 10 – 35[2] |

DRI – Dietary Reference Intakes, distribution range for macronutrients as percentage of total energy intake per day; [1][25], [2][24], [3][26].

Table 4
Prevalence of inadequacy of vitamin A, vitamin C, folate, calcium and iron (% of participants with values below EAR; total $n = 67$)

| | % below EAR ($n$) | EAR (females 19-30 years) |
|---|---|---|
| Vitamin A | 31.1 (19) | 500 μg/day |
| Vitamin C | 15.8 (9) | 60 mg/day |
| Folate | 49.2 (30) | 320 μg/day |
| Calcium | 41.0 (25) | 800 mg/day |
| Iron | 20.0 (12) | 8.1 mg/day |

EAR –Estimated Average Requirements [24].

Mean intakes of vitamin A, folate, calcium and iron (Table 2) were below the recommended dietary allowances (RDA), respectively 700 μg/day for vitamin A, 400 μg/day for folate,1000 mg/day for calcium and 18 mg/day for iron [24]; only mean vitamin C intake was superior to the RDA value of 75 mg/day [24]. Prevalence of inadequate micronutrient intake is presented in table 4 and refers to the percentage of study participants with intakes below the EAR. The highest prevalence of inadequacy was observed for folate (49.2%) and calcium (41%) and the lowest was observed for vitamin C (15.8%).

Table 5
Comparison of nutrient intake between Body Mass Index groups

| | Normal weight | Excess weight + obese | *P* |
|---|---|---|---|
| Energy | 1634.5 ± 58.3 | 1584.8 ± 142.6 | *0.545* |
| CHD | 47.9 ± 0.8 | 46.4 ± 1.1 | *0.355* |
| Fat | 30.8 ± 0.8 | 32.5 ± 1.3 | *0.177* |
| Protein | 21.6 ± 0.8 | 19.7 ± 1.1 | *0.166* |
| Vit A | 516.2 ± 47.3 | 448.4 ± 61.9 | *0.393* |
| Vit C | 104.2 ± 7.9 | 66.1 ± 15.7 | *0.053* |
| Folate | 192.9 ± 10.1 | 200.4 ± 27.2 | *0.801* |
| Calcium | 766.9 ± 34.7 | 503.4 ± 66.3 | *0.004* |
| Iron | 10.3 ± 0.6 | 7.4 ± 0.7 | *0.004* |

Comparisons for energy, Carbohydrates (CHD), fat, protein and calcium were performed by Mann-Whitney U test (non-normal variables). All other comparisons were performed by 2-tailed *T*-test. Significant differences marked at bold ($p < 0.05$).

Table 6
Comparison of nutrient intake between Physical Activity groups

| | Sedentary + low active | Active + very active | *P* |
|---|---|---|---|
| Energy | 1588.7 ± 72.1 | 1715.3 ± 89.4 | *0.376* |
| CHD | 46.1 ± 1.0 | 48.6 ± 1.1 | *0.165* |
| Fat | 30.9 ± 0.9 | 31.4 ± 1.2 | *0.668* |
| Protein | 21.6 ± 0.9 | 20.9 ± 1.0 | *0.614* |
| Vit A | 500.3 ± 59.2 | 522.9 ± 55.5 | *0.782* |
| Vit C | 106.5 ± 8.7 | 93.4 ± 12.9 | *0.408* |
| Folate | 181.6 ± 12.6 | 210.5 ± 13.7 | *0.121* |
| Calcium | 663.2 ± 35.5 | 819.2 ± 56.1 | *0.019* |
| Iron | 9.5 ± 0.7 | 10.1 ± 0.9 | *0.615* |

Comparisons for energy, Carbohydrates (CHD), fat, protein and calcium were performed by Mann-Whitney U test (non-normal variables). All other comparisons were performed by 2-tailed *T*-test. Significant differences marked at bold ($p < 0.05$).

Tables 5 and 6 present the comparison of nutrient intake according to BMI and physical activity groups. Folate intake was not different between normal weight and excess weight/obese students, or between sedentary/low active and active/very active students. No significant differences were observed in other nutrient intakes according to BMI and physical activity groups, with the exception of calcium and iron. Intakes of these minerals were higher in normal weight students than in excess weight/obese students. Calcium was also higher in active/very active students than in sedentary/low active students (Table 6). Folate intake was positively correlated to intakes of both vitamin C (Pearson correlation of 0.315) and vitamin A (Pearson correlation of 0.282).

## 4. Discussion

Reported percentages for prevalence of overweight and obesity in Portuguese females from 18 to 64 years old are 42.6% and 19.3%, respectively [27]. In the present study a much lower prevalence was observed for university females between 18 and 26 years (18% and 1.5 % respectively). A study undertaken in Portugal has shown that prevalence of both overweight and obesity increases with age until approximately 60 years old [28], suggesting that the prevalence of the studied population will tend to rise. The observed overweight prevalence is higher than the values of 10 to 13% found for female university students in other countries [15, 29, 30] When analyzing physical activity, only 40.9% of the female students met the current recommendations of an active lifestyle (PAL $\geq$1.6)[31]. Other studies have also reported more than 50% of sedentary lifestyle among university students [17, 32].

Some university students may develop unhealthy eating habits while adapting to a new reality. Away from home and consuming more commercially prepared meals than cooking for themselves, they may have a poorer diet quality and be at risk of nutrient imbalance and/or deficiency [33].

This study has shown that most students have an adequate intake of macronutrients, with 97%, 79% and 68% of the students meeting the current recommendations for protein, fat and carbohydrate respectively (Table 3). Other studies with university students have reported mean intakes of carbohydrate and protein within the recommended intervals for female students, but values in total fat intake higher than recommended [34–36]. Although most students of the present study meet the recommendations for macronutrient intake, we could still find some students with over consumption of total fat (20%) and under consumption of carbohydrates (37%). In another study carried in the North of Portugal overconsumption of fat (20.6%) and under consumption of carbohydrates (13.7%) was also observed in females ranging from 19 to 40 years old [37]. Regarding saturated fat, European recommendations do not set an upper limit, just suggest limiting the intake of this nutrient [26]. However, in 2004 FAO recommended an intake of saturated fat lower than 10% of total energy intake [38]. Comparing the results with this limit, 45% of the participants reported higher intakes in saturated fat.

Opposed to macronutrient intakes, mean intakes of all the studied micronutrients were below the recommended dietary allowances (vitamin A, folate, calcium and iron), with the exception of vitamin C. A high prevalence of inadequacy was found for folate (49%) and calcium (41%). Female university students were also reported to have low intakes of vitamin A, folate and calcium in Spain [35], high inadequacy of calcium intakes (46%) in Austria [39] and high inadequacy of folate (64 %) and iron (81%) intakes in Turkey [34]. In Jordan, among women of 15 to 49 years of age, the prevalence of folate insufficiency was 82.9% [40].

The inadequacies found for these micronutrients reflect the low consumption of fruits, vegetables, grain cereals and dairy products reported among university students [13–15, 17, 32]. In this study, although dairy products were the most consumed food products, they still did not meet the national recommendations of 2 to 3 portions of dairy per day, which corresponds to about 500 to 700 g of dairy products, thus explaining the high calcium inadequacy. The positive correlation observed in the present study between folate intake and vitamin C and vitamin A intakes is probably related to the low observed intake of fruits and vegetables, which was 229 g, almost half of the recommended value of 400 g per day.

In the present study, calcium intake was significantly higher in the normal weight BMI group than in the overweight/obese group, which is in agreement with other studies relating lower BMI or body fat to higher calcium intake and milk consumption [41, 42]. Iron intake was also significantly higher in the normal weight BMI group (Table 5). Higher intakes of these nutrients may reflect a higher conscience of normal weight individuals towards healthy food habits.

Calcium was the only nutrient with a higher intake in the active physical activity group than in sedentary/low active group. In another study undertaken in Portugal, means of energy and nutrient intake, including calcium, did not differ significantly among active and sedentary females of more than 40 years old [43]. In an European study, folate levels were also not different between inactive, moderately inactive, moderately active and active females [44].

In the North of Portugal a 58.2% inadequacy prevalence of folate was reported in women prior to pregnancy (median intake of 293.5 μg/ day), increasing to 90.8% during pregnancy (median intake of 314.6 μg/ day) [37]. Interestingly, in spite of such high prevalence of inadequacy, Portugal is the European country with the lowest prevalence of NTDs [45]. This may be due to intake of vitamin supplements before pregnancy as well as folic acid supplementation during pregnancy, which has not been assessed in previous studies.

Mean intake of folate in this study was within the range reported in Europe (200 to 300 μg per day), which is below the RDA [44]. On the contrary, in United States of America the mean intake of folate is above the RDA, with only 26% prevalence of inadequacy [46]. This reflects the implementation of the mandatory fortification program of cereal-based foods with folic acid in the USA since 1996, which caused a decrease of 19 to 32% in NTDs [47]. Other countries like Canada, Chile, Brazil and South Africa have also implemented mandatory fortification of foods with folic acid, with a concomitant decrease in NTDs [47]. In Europe no such recommendation has been advised, since a possible association between high folic acid intakes and cancer risk has not been yet completely ruled out [48]. However, in EU there is a recommendation for women who might become pregnant to take folic acid supplements in order to reduce the risk of NTD occurrence. A recent Canadian study found a correlation between supplemental folic acid intake and optimal red blood cell folate concentration for NTDs risk reduction, in women of child bearing age, stressing the importance of folic acid supplementation prior to pregnancy [49].

Although there are several studies describing the frequency of different food groups consumption in university students, there is not much information on macronutrient and micronutrient intakes. To our knowledge this is the first study assessing folate intake in female university students in Portugal. The relation of folate intake with non-communicable disease risk factors such as physical activity and BMI as well as correlation with other nutrient intakes, is an important information to help identifying students at folate deficiency risk and defining food policies for university population. Further studies in Portugal should aim to assess folate status biomarkers such as serum folate or red blood cell folate (RCB) in a larger population, encompassing several universities, and its association with several lifestyle and dietary factors including use of vitamin supplements.

## 5. Conclusions

A high inadequacy prevalence of folate intake was observed in young female university students. However, folate intake does not seem to be associated with physical activity or BMI since no significant differences in folate intake were found between different physical activity groups and BMI groups. A positive correlation was found between folate intake, vitamin C and vitamin A intakes. No correlation was found between folate intake and macronutrient intake.

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