ORIGINAL ARTICLE

Folate and vitamin B12 related to homocysteine and DNA damage in female university students

Folato e vitamina B12 relacionados com homocisteína e danos no DNA em estudantes universitárias

Folato y vitamina B12 relacionados con homocisteína y daño en el ADN en estudiantes universitarias

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ABSTRACT

Background and Objectives: It is not clear whether the increase in nutrition students' knowledge is associated with healthier eating behavior and fewer micronutrient deficiencies that can cause DNA damage. Deficiency in some vitamins can be a risk factor for increased homocysteine (Hcy) levels, a marker of cardiovascular risk. Therefore, this study aimed to verify whether dietary and serum folate and vitamin B12 are associated with Hcy levels and DNA damage in female university students. **Methods:** A cross-sectional study was conducted with female university students from southern Brazil. Folate, vitamin B12, and Hcy levels were determined in their diet or serum. DNA damage levels were assessed by the alkaline comet assay (index and frequency) and the buccal micronucleus assay (micronuclei frequency and binucleated cells frequency). **Results:** Correlation analyses did not show an association between Hcy levels and dietary or serum folate and vitamin B12 consumption. Dietary folate and vitamin B12 were associated with the index and frequency of damages; however, only serum folate was negatively associated with the index and frequency of damages. Additionally, the frequency of binucleated cells was negatively associated with dietary vitamin B12 and positively associated with serum levels. Serum folate was negatively associated with the frequency of micronuclei. Hcy levels were associated with the index and frequency of damages. **Conclusion:** These findings strengthen the role of healthier dietary patterns with adequate

micronutrients as a preventive strategy to reduce the risk of cardiovascular diseases. This approach should play a pivotal role in shaping health policies and advocating for appropriate food choices.

Keywords: *Folic Acid. Vitamin B 12. Homocysteine. Genomic instability, Cardiovascular Diseases.*

RESUMO

Justificativa e Objetivos: Não está claro se o aumento do conhecimento dos estudantes de nutrição está associado a um comportamento alimentar mais saudável, com menores deficiências de micronutrientes que podem induzir danos no DNA. A deficiência de algumas vitaminas pode ser um fator de risco para o aumento dos níveis de homocisteína (Hcy), um marcador de risco cardiovascular. Portanto, este estudo verificou se folato e vitamina B12 dietético e sérico estão associados aos níveis de Hcy e danos no DNA em estudantes universitárias. **Métodos:** Estudo transversal com universitárias do sul do Brasil. Determinou-se folato, vitamina B12 e Hcy dietético e séricos. Os níveis de danos no DNA foram avaliados pelo ensaio do cometa alcalino (índice e frequência) e pelo ensaio de micronúcleos bucais (frequência de micronúcleos e células binucleadas). **Resultados:** Análises de correlação não mostraram associação entre os níveis de Hcy com o consumo de folato e vitamina B12 dietético ou sérico. Folato e vitamina B12 dietéticos associou-se ao índice e frequência de danos, entretanto, somente folato sérico associou-se negativamente ao índice e frequência de danos. Ainda, a frequência de células binucleadas estava negativamente associada à vitamina B12 da dieta e positivamente associada aos níveis séricos. Folato sérico associou-se negativamente à frequência de micronúcleos. Os níveis de Hcy associou-se ao índice e frequência de danos. **Conclusão:** Esses achados fortalecem o papel de padrões alimentares mais saudáveis com micronutrientes adequados como estratégia preventiva visando a redução do risco de doenças cardiovasculares. Esta abordagem deve desempenhar um papel fundamental na formulação de políticas de saúde e na defesa de escolhas alimentares apropriadas.

Descritores: *Ácido Fólico. Vitamina B 12. Homocisteína. Instabilidade Genômica, Doenças Cardiovasculares.*

RESUMEN

Justificación y Objetivos: No está claro si el aumento del conocimiento de estudiantes de nutrición está asociado con un comportamiento alimentario más saludable, con menores deficiencias de micronutrientes que puedan inducir daños en ADN. La deficiencia de algunas vitaminas puede ser un factor de riesgo para el aumento de los niveles de homocisteína (Hcy), marcador de riesgo cardiovascular. Consiguiente, este estudio verificó si folato y vitamina B12 dietéticos y séricos están asociados con niveles de Hcy y daños en el ADN en estudiantes universitarias. **Métodos:** Estudio transversal con universitarias del sur de Brasil. Se determinaron folato, vitamina B12 y Hcy dietéticos y séricos. Los niveles de daño en el ADN se evaluaron por ensayo del cometa alcalino (índice y frecuencia) y el ensayo de micronúcleos bucales (frecuencia de micronúcleos y células binucleadas). **Resultados:** Los análisis de correlación no mostraron asociación entre los niveles de Hcy con folato y vitamina B12 dietéticos y séricos. Folato y vitamina B12 dietéticos se asociaron con índice y frecuencia de daños, pero, solo folato sérico se asoció negativamente con índice y frecuencia de daños. Además, la frecuencia de células binucleadas estaba negativamente asociada con la vitamina B12 de la dieta y

positivamente asociada con los niveles séricos. Folato sérico se asoció negativamente con la frecuencia de micronúcleos. Los niveles de Hcy se asociaron con índice y frecuencia de daños. **Conclusión:** Estos hallazgos refuerzan el papel de patrones alimentarios más saludables con micronutrientes adecuados como estrategia preventiva para reducir el riesgo de enfermedades cardiovasculares. Este enfoque debería desempeñar un papel fundamental en la elaboración de políticas de salud y en la promoción de elecciones alimenticias apropiadas.

Palabras Clave: *Ácido Fólico. Vitamina B 12. Homocisteína. Inestabilidad Genómica, Enfermedades Cardiovasculares.*

INTRODUCTION

Nutritional behaviors are multifaceted, influenced by various factors, including individual choices, cultural influences, and education.[1] Nutrition education plays a crucial role in promoting healthier dietary choices, equipping individuals with the knowledge and awareness necessary to make informed decisions about their eating habits.[2] In the context of higher education, university students pursuing degrees in nutrition undergo comprehensive and detailed training in the subject, which ideally results in a higher level of nutrition knowledge compared to individuals not pursuing such degrees.[3] Additionally, it is expected that, as students advance in their academic journey, those in the final semesters would display a more profound understanding of nutrition compared to their peers in the initial semesters. Furthermore, this unique population of university students of nutrition is often presumed to have better eating habits compared to other individuals due to their educational background and training in this field.

However, despite their potential advantage in nutrition knowledge and eating behaviors, unhealthy dietary practices among university students, including those in nutrition programs, can still be prevalent and may contribute to increased health risks.[4] Unhealthy eating behaviors have been identified as risk factors for various diseases, particularly cardiovascular diseases (CVD),[5] which continue to be a significant public health concern. In the context of CVD risk assessment, Homocysteine (Hcy), a non-essential amino acid containing sulfur, has emerged as a potential marker. Elevated levels of Hcy, resulting from the demethylation of methionine, have been associated with an increased risk of CVD.[6]

Notably, Hcy levels are modulated by the availability of folate (vitamin B9) and vitamin B12 in the body. Deficiencies in these essential vitamins can lead to the accumulation of Hcy, further exacerbating the risk of cardiovascular complications.[7] Folate and vitamin B12 play crucial roles in numerous physiological processes, including

their involvement in the synthesis of methionine and S-adenosylmethionine, acting as essential donors of methyl groups.[8] As a consequence, insufficient levels of these vitamins can induce DNA damage and disrupt DNA methylation processes, both of which are significant risk factors associated with elevated Hcy levels.[9]

While reference values for serum folate and vitamin B12 have been established based on disease prevention, their adequacy in minimizing chromosome damage and optimizing DNA methylation state remains a subject of inquiry.[8,10] Understanding the potential relationships between dietary intake, serum vitamin levels, Hcy concentrations, and DNA damage is essential in devising effective preventive strategies for CVD, particularly among university students of nutrition, who are vital in disseminating nutritional knowledge to the broader population.

Given the importance of nutrition in overall health and disease prevention, it is crucial to investigate factors influencing cardiovascular risk among university students of nutrition. This study aims to fill existing gaps in knowledge, focusing on the unique population of university nutrition students. Although these students are often assumed to have better eating habits due to their educational background, this study hypothesizes that poor eating practices may still be prevalent, contributing to health risks. Despite the existence of reference values for folate and B12 based on disease prevention, the adequacy of these levels in minimizing DNA damage remains an area of investigation. Therefore, this study proposes to explore the potential associations between dietary intake, serum folate and vitamin B12 levels, Hcy concentrations, and DNA damage in this specific population group. Due to the lack of studies in this area, this research will provide valuable insights into the consumption of folate and vitamin B12 and their potential relationship with cardiovascular risk and DNA damage among university students of nutrition, filling an important gap in the research. Therefore, this cross-sectional study aimed to verify whether dietary and serum folate and vitamin B12 are associated with Hcy levels and DNA damage in female university students.

METHODS

This is a descriptive cross-sectional study conducted from March to April 2016. The included subjects were female undergraduate students of nutrition, aged 19 to 50 years, enrolled in any semester at a community-university from Santa Cruz do Sul, Brazil. Exclusion criteria comprised subjects reporting atrophic gastritis, bariatric surgery, pregnancy or lactation, students taking drugs affecting folate and vitamin B12

metabolism, exposure to genotoxicants (e.g., cytotoxic drugs), and those with incomplete data or who did not sign the informed consent form were excluded. Participants were recruited by convenience sampling, with all nutrition course students invited via emails and/or other social media channels (including Instagram™ and Facebook™), and all efforts were made to avoid potential sources of bias in the study, such as selection bias or information bias.

Folate and vitamin B12 intake determination were obtained using three 24-hour food recalls. To estimate regular intake, three food recalls were performed on two weekdays and one weekend day, during the period of one week. To minimize potential memory errors and ensure consistency in the responses, the questionnaires were administered by a properly trained individual. Students completed food recalls, in which they reported all food intakes (type and amount of food and liquids) in the last 24 hours. The quantification of folate and vitamin B12 consumption was determined using the DietWin® software. For evaluating the adequacy of folate and vitamin B12 intake, reference values from the Dietary Reference Intake (DRI) were used, following the Estimated Average Requirements (EAR).[11]

A qualified professional performed blood collection on the day scheduled by a specialized professional. Students were requested to fast for at least four hours. The blood collected (5 mL) was used in the biochemical dosage of Hcy, folate, and vitamin B12, in addition to comet assay. At the same opportunity, oral mucosa cells were also collected to perform the Buccal Micronucleus Cytome (BMCyt) assay.

Blood quantifications of serum folate, vitamin B12, and Hcy were performed in a clinical analysis laboratory using the methodology of competitive chemiluminescent immunoassay on a Immulite 2000 Immunoassay System (Siemens Healthineers). Measurement of these blood parameters was conducted using commercial kits from Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA. All procedure was performed following the manufacturer's recommendations. The equipment was subjected to controls and limit of quantification as defined by the manufacturer's guidelines. Reference values used were: 4.44 to 13.56 µmol/L for Hcy, above 3.50 ng/mL for folate, and 174 to 878 pg/mL for vitamin B12, following laboratory standards. Moreover, another reference value for vitamin B12 (490 pg/mL) was considered as the safe lower threshold.[12]

The alkaline comet assay of blood cells was performed as described by Molz et al.[13] In total, 5 µL of whole blood (mixed with heparin) was embedded in 95 µL of low

melting point agarose (LMP) (0.75%) over a slide precoated with agarose, and subsequently, a coverslip was gently placed over that slide. After the mixture solidified, the coverslips were removed and the slides were put in a freshly prepared lysis solution containing high salt and detergent concentrations (2.5 M NaCl, 100 mM EDTA, 10 Mm Tris, pH 10–10.5, with freshly added 1% Triton X-100 (v/v) and 10% DMSO) (v/v) for a minimum of 1 h under refrigeration. Subsequently, the slides were exposed for 20 min to an alkaline solution (300 mM NaOH and 1 mM EDTA, pH>13) for DNA unwinding and to express the alkali-labile sites as single-strand breaks. The slides were then immediately subjected to an electrical current (electrophoresis in the same solutions at 300 mA and 25 V (0.90 V/cm) for 15 min at 4 °C to induce the migration of DNA fragments in the direction of the current. After that, the slides were washed with neutralization buffer (Tris 0.40M, pH 7.5) and fixed. Silver nitrate was used in the staining process of DNA. All procedures were conducted under dim yellow light to prevent DNA damage induced by ultraviolet radiation.

For each individual, two slides were prepared and 100 cells were randomly selected and analyzed (50 per slide, two slides per individual) using an optical microscope. Slides were coded to allow a blinded analysis, making it impossible to identify the individual, and the coding was performed by two properly trained individuals. Damage was determined visually by cells classification (comet morphology) in five classes of DNA migration, ranging from 0 damage (no damage, circular morphology only head and no tail) to damage 4 (maximum damage, tail expressively larger than head). Thus, the damage index for 100 cells ranged from 0 (no damage) to 400 (all cells with maximum damage). Damage frequency (%) was calculated using the relationship between the number of cells with damage (classified from 1 to 4) and the total of 100 cells from the sample.

BMCyt assay was performed following the protocol by Thomas et al.[14] Firstly, oral mucosa cells were collected using a cervical brush, which was then shaken into a microtube containing 1 mL of methanol. The brush was discarded from the microtubes and 20 μL of DMSO were added for subsequent centrifugation at 3,500 g for 3 min. Then, 200 μL of supernatant were aspirated off and more 200 μL of methanol were added. A pipette tip was used to dissociate the cells (this procedure was repeated three times). Next, 400 μL of supernatant were discarded, and 100 μL of the remaining cell suspension were distributed onto clean microscope slides (two slides per individual). After that, the slides were treated with HCl and Schiff's reagent, let dry overnight, and then stained following

the Feulgen method.[14] The slides were later analyzed using an optical microscope. A total of 2,000 differentiated cells were evaluated for the presence of DNA damage and the score from 1,000 cells was evaluated to determine the frequency of abnormal cells. They were classified according to the cytological and nuclear features in indicative of DNA damage (micronuclei and/or nuclear buds), cytokinetic defects (binucleated cells) and/or cell death (karyorrhexis, pyknotic and karyolytic cells), based on nuclear/cytoplasmic ratio, nuclear morphology, and texture.[14] Results are expressed as counts per 1,000 cells. Microscope slides were coded to allow a blinded analysis, making it impossible for the evaluator to know the subject identification. The analysis of slides was conducted by two examiners (two slides per subject).

The Statistical Package for the Social Sciences (SPSS) version 23.0 (IBM, Armonk, USA) was used for data tabulation. Descriptive statistics was presented as means and standard deviations or frequencies and percentages. Numerical data were tested for normal distribution and equality of variances by the Shapiro-Wilk test. The evaluation of adequacy for folate, vitamin B12 intake, serum levels of folate, vitamin B12, and Hcy were classified into distribution percentiles. Pearson correlation analyses were performed to test the association between normal parameters, and Spearman correlation was applied for non-normal parameters. This aimed to assess the relationship between folate and vitamin B12 intake, serum levels of folate, vitamin B12, and Hcy. Additionally, it also investigated the association between the DNA damage index and frequency with folate and vitamin B12 intake, serum levels of folate, vitamin B12, and Hcy. The significance level used was p<0.05.

The study was approved by the research ethics committee from the University of Santa Cruz do Sul/UNISC (protocol no. 1.432.400/2016; CAAE: 52822115.8.0000.5343) and followed the guidelines established by 466/2012 - 510/2016 - 580/2018 Brazilian resolution. All participants singed an informed consent form, indicating their voluntary agreement to participate. Additionally, a unique identification code was assigned to each participant to guarantee unbiased examination of test outcomes and respect privacy.

RESULTS

In this study, 47 women with a mean age of 24.8±6.7 years were evaluated. Table 1 presents the folate and vitamin B12 inadequacy, evaluated via diet and blood. Our results showed that 100% of the women presented inadequate folate intake and 48.9% of

vitamin B12, according to the EAR.[11] Regarding serum vitamins levels, 6.4% of women were deficient in serum folate. In addition, 10.6% of the women had vitamin B12 levels below 174 pg/mL and 93.6% of the women had levels below 490 pg/mL. None of the women had deficient Hcy levels (Table 1) nor had Hcy levels above 13.56 µmol/L.

Table 1. Evaluation of folate, vitamin B12 intake, and serum levels of folate, vitamin B12, and homocysteine (n=47).

Dietary	EAR	Mean±SD	Regular intake distribution percentiles									Evaluation comments
			10	20	30	40	50	60	70	80	90	
Folate (µg/day)	320.0	103.7±46.3	54.0	60.0	70.8	82.7	93.9	109.7	122.4	143.0	171.2	Inadequacy prevalence of 100.0%
Vitamin B12 (µg/day)	2.0	2.0±1.3	0.8	1.0	1.4	1.6	1.8	2.1	2.2	2.5	3.6	Inadequacy prevalence of 48.9%

Serum levels	Deficiency	Mean±SD	Serum levels distribution percentiles									Evaluation comments
			10	20	30	40	50	60	70	80	90	
Folate (ng/mL)	Lower than 3.5	7.5±3.2	4.0	5.0	5.5	6.2	7.4	8.0	8.9	9.4	12.4	Inadequacy prevalence of 6.4%
Vitamin B12 (pg/mL)	Lower than 174.0[a]	249.7±100.5	111.8	176.6	197.2	213.4	224.0	255.0	292.2	312.0	362.2	Inadequacy prevalence of 10.6%
	Lower than 490.0[b]											Inadequacy prevalence of 93.6%
Homocysteine (µmol/L)	Lower than 4.4	8.0±1.8	5.7	6.5	6.7	7.4	8.1	8.5	8.8	9.5	9.9	Inadequacy prevalence of 0.0%

[a]according to laboratory standards.
[b]considering 490 pg/mL as the safe lower threshold.

Additional analyses showed that Hcy levels were not correlated with folate and vitamin B12 intake or serum levels (p>0.05; Supplementary Figure 1).

Correlation analyses identified important relationships between the index and the frequency of DNA damage with vitamins in diet and serum. Folate intake (r=0.177; p=0.025 and r=0.302; p=0.026, respectively; Figure 1a, c) and vitamin B12 intake (r=0.364, p=0.015 and r=0.340, p=0.024, respectively; Figure 1b, d) were significantly associated with the damage index and frequency in DNA.
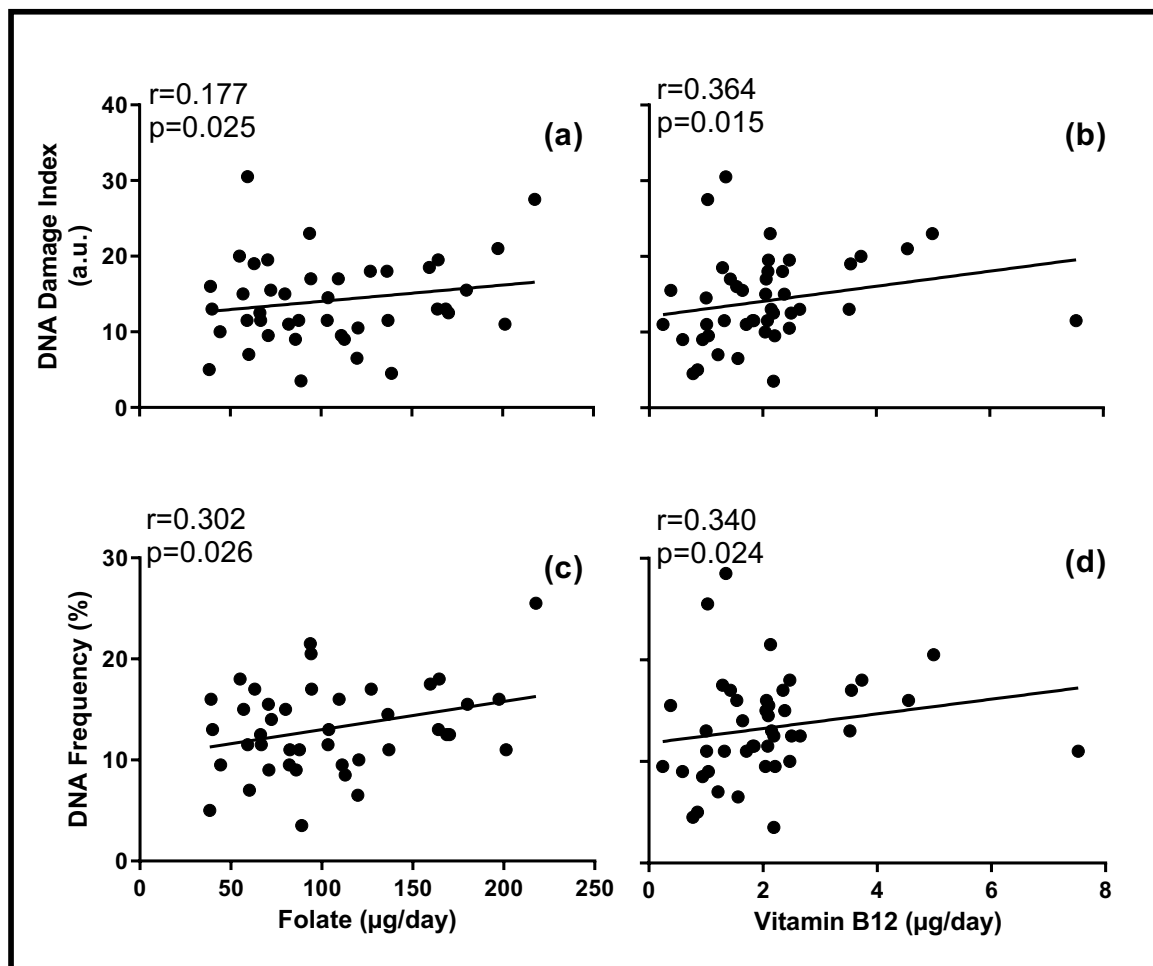
Figure 1. Association between DNA damage index and frequency with folate (a, c) and vitamin B12 (b, d) intake. r: correlation coefficient and p: significance level according to Pearson's or Spearman's tests.

In addition, only folate serum level was negatively associated with damage index and frequency (r=−0.406, p=0.008 and r=−0.430, p=0.05, respectively; Figure 2a, d). Hcy levels were significantly positively associated with damage index and frequency (r=−0.273, p=0.038 and r=−0.342, p=0.027, respectively; Figure 2c, f).
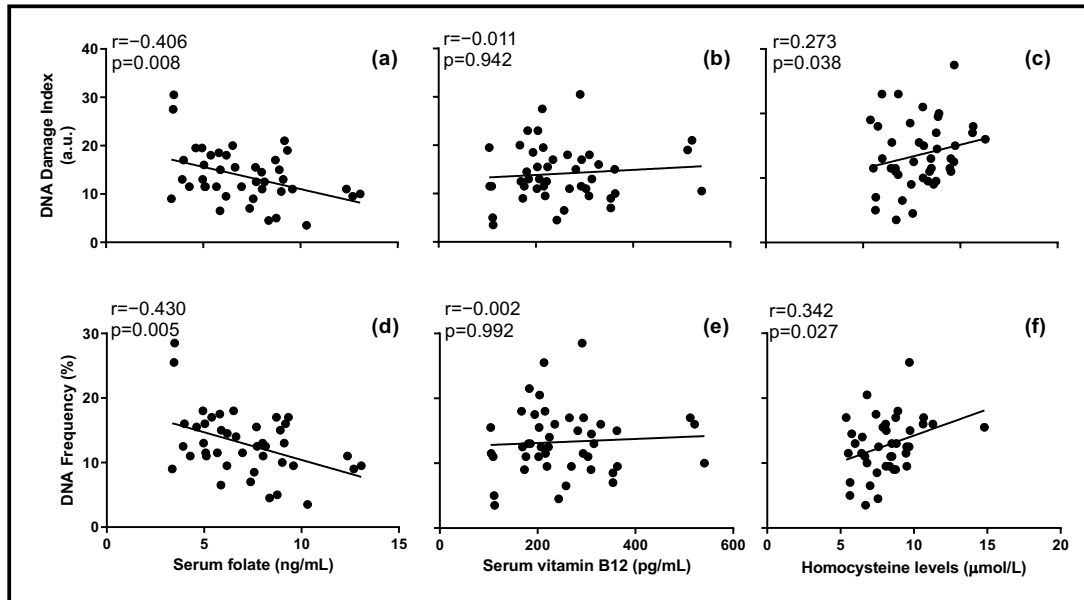
Figure 2. Association between DNA damage index and frequency with folate (a, d), vitamin B12 (b, e), and homocysteine (c, f) serum levels. r: correlation coefficient and p: significance level according to Pearson's or Spearman's tests.

Regarding the BMCyt assay, only vitamin B12 intake was negatively associated with binucleated cells frequency (r=−0.302, p=0.021; Figure 3d). In serum, there was a significant negative association between folate and the micronuclei frequency (r=−0.275; p=0.034; Figure 4a) and a significant positive association between vitamin B12 and binucleated cells frequency (r=0.276, p=0.032; Figure 4e). Hcy levels were not significantly associated with micronuclei frequency nor binucleated cells frequency (p>0.05).
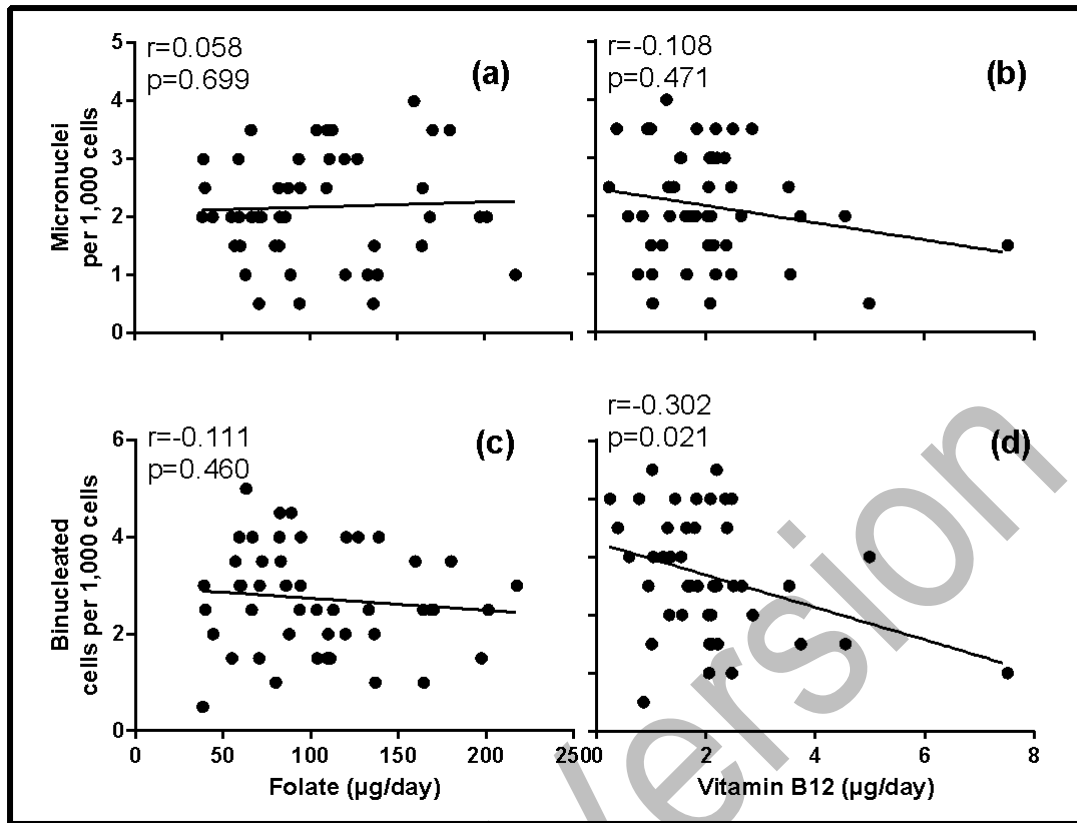
Figure 3. Association between micronuclei and binucleated cells frequency with folate (a, c) and vitamin B12 (b, d) intake. r: correlation coefficient and p: significance level according to Spearman's test.
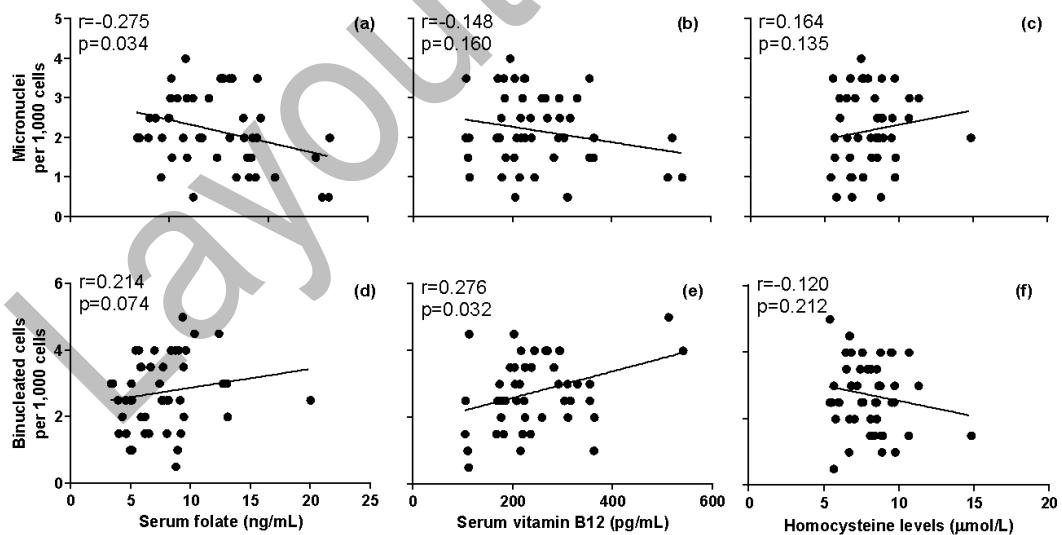

Figure 4. Association between micronuclei and binucleated cells frequency with folate (a, c) and vitamin B12 (b, d) serum levels. r: correlation coefficient and p: significance level according to Spearman's test.

DISCUSSION

This study evaluated the consumption and blood levels of both folate and vitamin B12 in female university students to assess whether there was an association with

cardiovascular risk and/or genomic damage. It was expected that nutrition university students would present a low prevalence of folate inadequacy and vitamin B12 in diet and serum due to their knowledge on nutrition. However, our results showed that all female students had inadequate folate intake, with nearly half of them (48.9%) showing vitamin B12 inadequacy, following EAR values according to the DRI.[11] Pereira et al.[15] evaluated Brazilian undergraduate and graduate students aged 36.2±9.4 years and observed that 12% and 11.1% of the participants presented a possible insufficient intake of folate and vitamin B12, respectively, values smaller than the ones found in this study. Besides that, Manios et al.[16] also evaluated the coefficients of variation (CV) of food intake, finding 44.7% for folate and 65% for vitamin B12. The standard CV of DRI for healthy women is 62% for folate and 294% for vitamin B12 in the same age group of the population studied.[11] These values are much higher than ours (44.24% to folate intake and 61.8% to vitamin B12), showing that our subjects were more homogeneous than the subjects used by the DRI and similar to the study by Manios et al.[16]

In this study, the prevalence of folate serum levels inadequacy was lower than in the diet (6.4% vs. 100%). As for vitamin B12 serum levels, 10.6% of the individuals had levels below 174 pg/mL, and 93.6% of women had figures below 490 pg/mL. Those results were lower when compared to an Australian study that evaluated young adult women.[9]

Hcy is a metabolite of methionine (since humans do not acquire Hcy by food) and a key intermediate metabolite in the folate cycle-linked metabolic processes of remethylation and transsulfuration, producing L-methionine and L-cysteine, respectively.[17] Remethylation requires vitamin B12 and 5-methyltetrahydrofolate (active folate) as methyl donors. In the transsulfuration process, Hcy is irreversibly converted to cysteine and it can be utilized in protein synthesis and glutathione (GSH) production (beneficial antioxidant). In this context, lack of the various B-vitamins, such as B12 and folate could elicit an excessive level of Hcy (hyperhomocysteinemia), a modifiable risk factor for CVD.[8]

Hyperhomocysteinemia has been classified as moderate (16 to 30 µmol/L), intermediate (31 to 100 µmol/L), and severe (>100 µmol/L).[18] In this study, none of the women had Hcy above 16 µmol/L. Studies have also reported that Hcy levels above 10 µmol/L are associated with an increased risk of cardiovascular disease.[19] In our study, only 8.5% of women had Hcy levels above 10 µmol/L.

In addition, low folate intake or folate metabolism abnormalities, as well as vitamin B12 deficiency, can lead to Hcy elevation.[6] We did not observe an association between serum Hcy levels and folate or vitamin B12 intake. Nevertheless, only one subject (2.13%) presented an elevated level of Hcy (14.8 μmol/L) and none of them had it below 4.4 μmol/L. However, serum vitamin B12 above 490 pg/mL prevents functional deficiency more reliably.[12] Based on this criterion, 93.6% of the sample was at risk of vitamin B12 deficiency. Han et al.[20] also evaluated serum folate levels and found 10% inadequacy and, similarly to our study, found that 5% of the subjects had serum Hcy higher than 15 μmol/L. Besides that, they found that serum Hcy was negatively correlated with serum folate, which is contrary to our results. Folsom et al.[21] found a negative association between Hcy and vitamin B12, differing from our study, in which we did not find an association between these variables.

O'Keefe et al. evaluated only the folate intake, finding that among young women aged from 21 to 27 years, the intake of 200 μg/day of folate was negatively correlated with plasma Hcy,[22] being significantly higher than when compared with the ingestion of 300 to 400 μg/day of folate. These results show that elevated Hcy levels are associated with lower folate levels in the diet, possibly due to the need for this nutrient to prevent Hcy accumulation by converting Hcy into methionine. Another study, which evaluated university students in South Korea, found a negative correlation of folate with serum Hcy levels.[20] We did not evaluate folate supplementation; however, a meta-analysis assessing the effect of folic acid supplementation on the risk of cardiovascular disease found that it did not decrease the levels of Hcy in all the studies analyzed.[23] Thus, the authors did not recommend the supplementation of folic acid to decrease the risk of cardiovascular disease.

However, it has been reported that folate and vitamin B12 may present an important role in DNA metabolism.[24] These vitamins are required for the synthesis of methionine and S-adenosyl methionine, used for the maintenance of methylation patterns in DNA. Folate and Vitamin B12 deficiencies can lead to elevated DNA damage rate and altered DNA methylation, important risk factors for the increase of Hcy status.[3] Using comet assay, we found a significant association between folate and vitamin B12 in the diet with DNA damage (both index and frequency of damage). Regarding BMCyt assay, only vitamin B12 intake was negatively associated with binucleated cells. In addition, serum folate levels presented an inverse association with the index and frequency of DNA damage, as well as with the micronuclei frequency. No association was found between

serum vitamin B12 levels and DNA damage by comet assay, but serum vitamin B12 was associated with binucleated cells frequency. Milić et al. found a significant association between serum vitamin B12 and increased DNA damage using the comet assay.[25] These authors also reported that a higher serum vitamin B12 concentration was associated with a lower frequency of micronuclei, corroborating our study, in which we found this tendency but without statistical significance (Figure 4). According to Fenech, the increased micronuclei frequency is an important biomarker associated with defects in the metabolic pathways that requires folate and vitamin B12.[9]

Increased or slightly increased Hcy might contribute to DNA damage induction. We observed a significant association between serum Hcy and DNA damage (both index and frequency of damage). Regarding DNA damage evaluated by the lymphocyte micronucleus assay, the study of Fenech found that elevated plasma Hcy was associated with increased micronucleus formation.[9] We did not find this association, possibly due to the low age range of our subjects and since only one subject had increased level of Hcy. Besides that, Fenech[9] also showed that the micronuclei frequency is lower when serum Hcy is below 7.5 µmol/L and when the plasmatic vitamin B12 is higher than 300 pmol/L.

Our study holds some limitations that should be highlighted. Firstly, the 24-hour food recall method, though widely acknowledged as the gold standard for assessing food intake, is dependent on respondents' full cooperation, memory, and honesty. As a result, it may lead to potential underestimation or overestimation of their actual intake. To mitigate this, efforts were made to ensure participants understood the importance of accurate reporting. Secondly, the cross-sectional design of this study limits its capacity to establish a causal relationship between the analyzed variables. To address this limitation, future research should consider employing longitudinal studies or experimental designs to explore causal associations more effectively. Despite these limitations, the study gains strength in evaluating nutrition course students, as there are only a few existing studies on this specific topic. This uniqueness adds value to the findings and offers valuable insights into an underexplored area of research.

In conclusion, the results from this study showed that Hcy levels were not associated with folate and vitamin B12 intake or serum levels in this population of female university students. However, folate and vitamin B12 intake were positively associated with damage index and frequency, whereas only folate serum was negatively associated with damage index and frequency. Furthermore, binucleated cells frequency was negatively associated with vitamin B12 intake and positively associated with serum

levels. There was a significant negative correlation between micronuclei frequency and folate, but not serum levels. Additionally, Hcy levels were correlated with damage index and frequency, but not with micronuclei or binucleated cells frequency. Our findings strengthen the role of healthier eating patterns with adequate micronutrients, mainly folate and vitamin B12 intake.

Considering the results of this study, future research can deepen the understanding of the relationship between Hcy, folate, and vitamin B12 associated with genomic instability in the female university population. Consequently, further studies are necessary to investigate mechanisms related to the consumption of folate and vitamin B12, exploring direct influences on cellular processes or mediating factors and identifying protective or modulating mechanisms for these cellular events. Longitudinal and intervention studies are also recommended to assess dietary patterns over time, aiming to analyze the relationship with Hcy, folate, and vitamin B12, providing a more profound understanding of these biomarkers and whether adequately adjusting the intake of folate and vitamin B12 can positively impact cellular damage markers and Hcy levels.

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Luana Beatriz Limberger performed the experiment and wrote the manuscript. **Patrícia Molz** performed the experiment, analyzed the data, and wrote the manuscript. **Caio Fernando de Oliveira** and **Jane Dagmar Pollo Renner** performed the experiment and helped with constructive discussions. **Silvia Isabel Rech Franke** designed and guided the research.

All the authors read, critically evaluated, gave their feedback, and edited the manuscript.