DETERMINATION OF VITAMIN D, IRON AND N-3 FATTY ACIDS IN ADOLESCENTS WITH DIFFERENT EATING HABITS

Martina Valachovičová, Zuzana Slezáková, Jarmila Kristová, Vladimír Urbánek
Faculty of Nursing and Medical Professional Studies, Slovak Medical University in Bratislava, Bratislava, Slovakia

SUMMARY

Objectives: Proper eating habits ensure human health, so it is important to eat a balanced diet and learn the basics of proper eating from an early age. Adolescence is considered very important period for maintaining good health in terms of nutrition.

Methods: In the study, we monitored the eating habits of 182 adolescents aged 14–19 years with different eating habits (73 vegetarians and 109 nonvegetarians) using a questionnaire on nutrition and determination of selected blood parameters – vitamin D, iron and n-3fatty acids.

Results: Insufficient levels of vitamin D were determined in the whole group of adolescents, regardless of eating habits. Low iron concentrations negatively affect the biosynthesis of long-chain n-3 fatty acids, which was also reflected in our adolescents with alternative diets.

Conclusions: Based on the obtained results, it is necessary to place emphasis on the composition of the diet in adolescent age.

Key words: nutrition, vitamin D, iron, n-3 fatty acids, adolescent

Address for correspondence: M. Valachovičová, Faculty of Nursing and Medical Professional Studies, Slovak Medical University in Bratislava, Limbová 14, 833 01 Bratislava, Slovakia. E-mail: martina.valachovicova@szu.sk

https://doi.org/10.21101/cejph.a7048

INTRODUCTION

Adolescence is considered a critical period in a person's life. During this period big changes occur in a very short time, both physically and mentally. Eating habits in adolescence are based on increased energy intake, especially from refined starch food, sweet and sweetened drinks and insufficient intake of vegetables and fruits. Improper eating habits cause various eating disorders and are prevalent to adulthood (1, 2). During adolescence, it is not only obesity that is the most important nutritional problem today, but from a physiological point of view it is mainly the insufficient intake of some micronutrients (3).

Self-regulatory skills, social and physical environment are the most important determinants of a healthy diet, which can be changed by intervention strategies with different levels of effectiveness. Interventions aimed at normal behaviour and physical environment are considered more effective, as educational interventions generally have a limited impact on healthy eating (4). A varied diet ensures nutritional quality (intake of all nutrients). The nutritional quantity is ensured by the concentration of nutrients in the consumed food mixture corresponding to the recommended nutritional doses. These criteria for optimal nutrition are essential for the development and maintenance of human health (5). Vitamin B12, vitamin D and n-3 fatty acids are lacking in plant food sources. In particular, the content of methionine and lysine (also other essential amino acids), iodine and carnitine is significantly reduced compared to animal food (5–7). The absorption of iron, calcium, zinc, copper, manganese, and selenium can be inhibited by plant food components (8, 9). These facts may be associated with many health risks in the population with exclusive or dominant consumption of plant foods (10–12). To prevent these risks, it is necessary to consume food or pharmaceutical supplements to compensate for deficient nutrients.

The right composition of the diet ensures human health. We must place even more emphasis on eating habits during adolescence. Promoting adequate vitamin D status is important for older children and adolescents because the nutritional curve may develop during childhood (13). Several studies suggest that vitamin D directly or indirectly regulates up to 1,250 genes that affect human infectious, allergic and autoimmune conditions (14). Association between low levels of serum 25-hydroxyvitamin D and increased risk of developing several immune-related diseases and disorders, including psoriasis, diabetes type 1, multiple sclerosis, rheumatoid arthritis, tuberculosis, sepsis, and respiratory infection has been observed. Accordingly, a number of clinical trials aiming to determine the efficacy of administration of vitamin D and its metabolites for treatment of these diseases have been conducted with variable outcomes. Interestingly, recent evidence suggests that some individuals might benefit from vitamin D more or less than others as high inter-individual difference in broad gene expression in human peripheral blood mononuclear cells in response to vitamin D supplementation has been observed. Although it is still debatable what level of serum 25-hydroxyvitamin D is optimal, it is advisable to increase vitamin D intake and have sensible sunlight exposure to maintain serum 25-hydroxyvitamin D at least 30 ng/ml (75 nmol/l), and preferably at 40–60 ng/ml (100–150 nmol/l) to achieve the optimal overall health benefits of vitamin D (15, 16). Vitamin D is found in foods of animal origin, so an intake of this vitamin is insufficient in alternative diets (17).

Iron deficiency is a health risk factor. The bioavailability of iron in the diet is low in populations consuming monotonic plant foods. Recent studies have shown how the body regulates iron absorption and metabolism in response to changes in iron status by regulating up or down the regulation of important intestinal and hepatic proteins. Iron supplementation can be in the population using either food or nutritional supplements. Selective plant breeding and genetic engineering promise new approaches to improve the nutritional quality of dietary iron (18).

Current concepts and programmes in the Slovak Republic concerning the health and lifestyle of young people are a reflection of the implementation of the WHO strategic documents ("Health 21" – health for all in the 21st century, European Charter on Counteracting Obesity, European Action Plan for the Environment and Children's Health, International Working Group on Obesity). In connection with this issue, nursing has created a sufficient portfolio to support the symbiotic nature between health and proper nutrition. Through primary prevention, we want to create and acquire knowledge and habits that will lead to the promotion and strengthening of health.

The aim of the study was to determine the current state of selected nutritional markers in adolescents with different eating habits.

MATERIALS AND METHODS

Sample and Procedure

A dietary questionnaire and selected biochemical methods were used to determine selected nutritional markers in adolescents with different eating habits. The study included 182 adolescents aged 14–19 years, who were divided according to different eating habits into two groups: 73 vegetarians (the vegetarians who do not eat meat but eat milk and eggs) and 109 nonvegetarians (Table 1).

The observed group of adolescents came from Bratislava and its surroundings. Examinations were performed in spring. Including criteria: adolescents had to be subjectively healthy, that is, without cardiovascular disease, cancer, diabetes, thyroid disease, kidney and digestive tract disease. The probands indicated an approximately similar physical activity (no sports).

The calculation of daily intake of nutrients was based on the data from standardized and validated dietary questionnaires. The questionnaire contained 149 food items. The frequency of consumption was measured using four categories: almost never, times per day, per week or per month depending on food item. Trained workers checked the completeness of questionnaires. The obtained values from the questionnaire were distributed by a nutritional therapist. The conversion to nutrients was done by using self-developed software Nutrition based on the Slovak food composition database compiled by the Food Research Institute (Slovak Food Data Bank, 2015; ALIMENTA 4.2) (19). The results are further compared with the current recommended nutritional doses for the population of the Slovak Republic (9th revision) since 2015 (20).

Table 1. *Basic characteristics of the adolescent group (N = 182)*

Characteristics	Vegetarians n = 73	Nonvegetarians n = 109
Boys	32	50
Girls	41	59
Age min–max (years)	14–19	14–19
Average age (years)	16.1 ± 0.96	16.3 ± 1.13
Time of vegetarianism (years)	5.23 ± 0.63	–
Smokers	0	0

Results are expressed as means ± SEM

Laboratory Analysis

A blood sample was collected in a standard manner into 2 sampling syringes. One commercial syringe was with ethylenediaminetetraacetic acid (EDTA) with anticoagulant properties to obtain plasma and the other commercial syringe was without the addition of any anticoagulants to obtain serum. Adolescents at the time of collection fasted after standard food intake in the previous day.

Iron was determined by standard laboratory methods using a Vitros 250 automated analyser (Johnson & Johnson, USA).

Determination of vitamin D metabolites was performed by the classical equilibrium RIA method, which uses a goat polyclonal antibody against 25-hydroxyvitamin D with separation by a second antibody (donkey anti- goat). 25-Hydroxyvitamin D125I RIA Kit, Instruction Manual REF 68100E, DiaSorin, Stillwater, Minnesota 55082-0285, USA.

The fatty acid content was measured by gas chromatography after one-step transesterification as methyl esters and identified by comparing the retention times of standard fatty acids (21).

Statistical Analysis

Commercial programs Excel 2000 and Statgraphics for Windows, version 1.4 and PASW Statistics 18 were used for statistical processing of the obtained data. The following methods were used: the standard deviation (SD) was compared by F-test and the difference or similarity of the means of the observed groups ($x1 = x2$) was tested by t-test, as well as the hypothesis $x1 < x2$ resp. $x1 > x2$. The importance of the statistics was set at 95% ($p = 0.05$). The comparison was supplemented by determining the percentage occurrence of risk values of the monitored parameters.

Ethical Statement

All investigations were carried out in accordance with the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of the Slovak Medical University in Bratislava. All subjects enrolled in the study or their legal representatives signed informed consent. The study was approved by the governing schools in the Bratislava region. Parents were informed about the study through the school administration and can drop out if their child disagrees. Participation in the study was completely voluntary and anonymous without explicit incentives to participate.

RESULTS

Basic descriptive parameters of adolescents are shown in Table 1. Adolescents on a traditional diet had a mean plasma concentration of 25 (OH) D of 20.1 ng/ml and vegetarian adolescents had a plasma concentration of 16.9 ng/ml. Our results in Table 2 suggest that vegetarian adolescents, despite the same conditions as nonvegetarian adolescents with reduced sunlight, had significantly reduced plasma vitamin D levels. Vegetarian adolescents were found to have a higher incidence of deficits than nonvegetarian adolescents (60% vs. 42%). However, vitamin D concentrations in both groups are at lower than recommended values, i.e., below 30 ng/ml, so it is essential that adolescents be given nutritional supplements in the winter and early spring months.

Iron concentrations are significantly reduced in the group of adolescents with alternative diet compared to nonvegetarian adolescents. Hyposideremia occurred in 19% of nonvegetarian adolescents and 38% of vegetarian adolescents (Table 2).

The study sample was divided according to iron concentration into three groups, considering that low iron concentrations in vegetarians may cause a deficiency of long-chain n-3 fatty acids, as these are absent in a plant-based diet and are only found in cold water fish. The first group consisted only of nonvegetarian adolescents with normal iron, in the second group we included only adolescent vegetarians who had iron concentrations in the norm, and the third group included only vegetarian adolescents who had iron deficiency. In the group of vegetarian adolescents who had iron deficiency, we measured significantly reduced plasma levels of eicosapentaenoic acid and docosahexaenoic acid compared to nonvegetarian adolescents and vegetarians with normal serum iron levels (Table 3).

In addition to known disorders and diseases related to iron deficiency, it is important to determine the concentrations of n-3 fatty acids, because iron deficiency negatively affects their biosynthesis (22–25). Alternatively eating adolescents versus traditional eating adolescents consumed significantly reduced and very small amounts of eicosapentaenoic acid and docosahexaenoic acid as a consequence of not consuming fish (trace amounts come from different fat spreads with the addition of fish or fish oil) (Table 4). The intake of eicosapentaenoic and docosahexaenoic acid was not significantly increased in either group (Table 4), but the intake of α-linolenic acid was significantly increased although it did not reach the recommended 2 g/day.

Table 2. *Concentrations of vitamin D and iron in serum of the study group*

Variables	Vegetarians n = 73	Nonvegetarians n = 109	p-value
Vitamin D (ng/ml)	16.9 ± 1.03	20.1 ± 1.03	< 0.01
< 20 (ng/ml)	60%	42%	
Iron (μmol/l)	12.3 ± 0.9	14.8 ± 0.9	< 0.05
< 10 (μmol/l)	38%	19%	

Results are expressed as means ± SEM
The prevalence of iron and vitamin D deficiency was determined in our laboratory in a sample of 14–19 years old girls.

Table 3. *Plasma profile of polyunsaturated fatty acids (%) of total and iron concentration in the blood of adolescents*

PUFA profile and iron value	Nonvegetarians n = 109	Vegetarians n = 73				
	Iron in the norm	Iron in the norm	p-value[a]	Iron deficiency	p-value[a]	p-value[b]
Iron (μmol/l)	15.80 ± 0.35	15.00 ± 0.51	n.s.	8.97 ± 0.47	< 0.001	< 0.001
18:2 n-6	28.70 ± 0.32	34.60 ± 0.60	< 0.001	35.20 ± 1.16	< 0.001	n.s.
20:4 n-6	6.95 ± 0.09	6.51 ± 0.12	n.s.	6.19 ± 0.16	< 0.01	n.s.
18:3 n-3	0.61 ± 0.05	0.81 ± 0.04	< 0.01	0.83 ± 0.08	< 0.01	n.s.
20:5 n-3	0.55 ± 0.3	0.53 ± 0.20	n.s.	0.35 ± 0.04	< 0.01	< 0.01
22:6 n-3	1.6 ± 0.04	1.5 ± 0.08	n.s.	1.07 ± 0.14	< 0.01	< 0.01
PUFA	40.31 ± 0.71	45.74 ± 0.61	< 0.001	45.70 ± 1.12	< 0.001	n.s.
PUFA n-6	39.1 ± 0.55	43.32 ± 0.62	< 0.001	44.10 ± 1.55	< 0.001	n.s.
PUFA n-3	3.11 ± 0.09	3.15 ± 0.07	n.s.	2.38 ± 0.06	< 0.05	< 0.05

Adolescents were divided into three groups – nonvegetarians with iron in the norm, vegetarians with iron in the norm and vegetarians with iron deficiency.
Results are expressed as means ± SEM; [a]statistically significant values of vegetarians versus nonvegetarians; [b]statistically significant values of vegetarians with iron deficiency versus values of vegetarians with normal iron concentration; n.s. – not significant

Table 4. *Intake of n-3 fatty acids*

Fatty acids	Vegetarians n = 73	Nonvegetarians n = 109	p-value
Eicosapentaenoic acid (mg/day)	31.7 ± 5.03	29.16 ± 4.02	n.s.
Docosahexaenoic acid (mg/day)	28.57 ± 4.01	26.74 ± 3.93	n.s.
α-linolenic acid (g/day)	1.91 ± 0.09	1.10 ± 0.05	< 0.05

Results are expressed as means ± SEM; n.s. – not significant

Table 5. Daily intake in g/day

Food commodities	Vegetarians n = 73	Nonvegetarians n = 109	p-value
Dietary fibre	33.1 ± 1.3	22.7 ± 1.1	< 0.001
Whole grain products	144.1 ± 11.9	94.7 ± 10.1	< 0.05
Legumes	8.1 ± 1.9	10.1 ± 1.6	< 0.05
Soybeans and products thereof	53.1 ± 7.3	13.7 ± 2.3	< 0.001
Oilseeds	4.9 ± 0.9	11.6 ± 2.9	< 0.05
Nuts	8.3 ± 1.9	14.5 ± 1.9	< 0.05

Results are expressed as means ± SEM

The fibre reduces the absorption of various substances from the intestine, and thus in many cases reduces the risk of introducing unwanted substances into the body. Food commodities containing fibre and folic acid are consumed more in adolescents with alternative diets than in adolescents with traditional diets (Table 5).

Dietary questionnaires analyses showed that vegetarian adolescents consume significantly more soy, whole grain products, seeds, and nuts (Table 5).

DISCUSSION

Vitamin D is known to affect bone health (26, 27) because it plays an important role in regulating calcium and phosphorus metabolism. Age-related factors may play a role in increasing the risk of vitamin D deficiency. During childhood and adolescence, hypovitaminosis D is mostly a consequence of a poor and unbalanced diet, even in developed countries. During the first years of life, exclusive breastfeeding without adequate sun exposure or vitamin D supplementation is an important risk factor for vitamin D deficiency, whereas, in adolescence, the frequent consumption of fast and junk food is a relevant risk factor. Other risk factors include obesity, diseases interfering with vitamin D activation or fat absorption, lifestyle and drugs (28). In our study, we found a reduced level of vitamin D in randomly selected adolescents with different eating habits. However, this finding does not apply only to adolescents with a dominant consumption of plant-based food, but to the entire studied group. Vitamin D concentrations were below the limit in both groups, which indicates not only a low intake of nutrients and therefore a poor composition of the diet, but also movement in the sun. Adolescents with a dominant consumption of plant foods containing mainly whole grains, legumes, seeds and nuts have been found to reduce the use of minerals and trace elements in foods (23). Plant foods contain potent iron-absorption inhibitors, including phytates, oxalates, and phenolic compounds. Although iron intake among vegetarians is often higher than among nonvegetarians, iron requirements for vegetarians is about 1.8 times higher compared to nonvegetarians (28). Thus, iron intake among vegetarians falls short of their needs. The vegetarians who eat a varied and well-balanced diet are not at any greater risk of iron deficiency anaemia, compared to nonvegetarians. Compared to nonvegetarians, vegetarians may often have lower serum ferritin but their ferritin is still within the normal range. Findings from several studied showed low, often below normal, ferritin levels among vegetarians. Thus, if their conclusion is accurate, it can be assumed that vegetarian children and adolescents included in these studies did not have varied and well-balanced diet. A conclusion that iron status among vegetarians varies and depends on findings from specific studies seems more accurate (29). In our study, we did not find a difference in iron deficiency between girls and boys, i.e., by gender, but iron concentrations were significantly reduced in vegetarian adolescents. Iron deficiency negatively affects the normal course of biosynthesis of long-chain polyunsaturated n-3 fatty acids from α-linolenic acid by reducing δ-6 desaturase activity. This enzyme provides the conversion of α-linolenic acid to stearidonic fatty acid, which is a substrate for the production of eicosapentaenoic acid catalysed by δ-5 desaturase. Based on these data, we divided our group according to iron concentrations. Low iron concentrations in adolescents with alternative diets negatively affect the biosynthesis of long-chain n-3 fatty acids.

People with a dominant consumption of plant foods may be deficient in long-chain n-3 fatty acids because these are absent in plant foods, they are found only in cold water fish. Eicosapentaenoic acid and docosahexaenoic acid and fish intake are important for reducing the risk of coronary heart disease and for reducing triacylglycerols values in blood (30). Although there is no officially recommended nutritional dose for eicosapentaenoic and docosahexaenoic acid, various scientific institutions recommend that approximately 10% of α-linolenic acid intake (the starting substrate for n-3 fatty acid biosynthesis) should be consumed as eicosapentaenoic acid and docosahexaenoic acid, i.e., 200 mg, as the recommended nutritional doses for α-linolenic acid are 2.0 g/day (20, 24).

CONCLUSIONS

Based on the results, a higher intervention in the correct composition of the diet of adolescents is needed in nursing practice as part of primary prevention, which will be enriched with nutrients and exercise in the sun.

Acknowledgements

The paper was prepared with the support of an internal project SMU in Bratislava, “Vitamin D as a marker of human health.”, 06/2021-SVG1.

Conflict of Interests

None declared

Authors' Contributions

MV drafted the manuscript, ZS and MV coordinated the publication process, MV and VU analysed samples. VU and JK provided the primary data. All authors read and approved the final manuscript.

REFERENCES

1. Biro FM, Wien M. Childhood obesity and adult morbidities. Am J Clin Nutr. 2010;91(5):1499S-1505S.
2. Harris KM, Gordon-Larsen P, Chantala K, Udry JR. Longitudinal trends in race/ethnic disparities in leading health indicators from adolescence to young adulthood. Arch Pediatr Adolesc Med. 2006;160(1):74-81.
3. Moreno LA, González-Gross M, Kersting M, Molnár D, de Henauw S, Beghin L, et al.; HELENA Study Group. Assessing, understanding and modifying nutritional status, eating habits and physical activity in European adolescents: the HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) Study. Public Health Nutr. 2008 Mar;11(3):288-99.
4. De Ridder D, Kroese F, Evers C, Adriaanse M, Gillebaart, M. Healthy diet: Health impact, prevalence, correlates and interventions. Psychol Health. 2017;32(8):907-41.
5. Gusak MA, DellaPenna D. Improving the nutrient composition of plants to enhance human nutrition and health. Annu Rev Plant Physiol Plant Mol Biol. 1999;50:133-61.
6. Krajčovičová-Kudláčková M. Health risk of alternative nutrition. Klin Biochem Metab. 2001;9(4):187-93. (In Slovak.)
7. Allen LH. Causes of vitamin B12 and folate deficiency. Food Nutr Bull. 2008;29(2 Suppl):S20-34.
8. Gibson RS. Content and bioavailability of trace elements in vegetarian diets. Am J Clin Nutr. 1994;59(5 Suppl):1223S-32S.
9. Leitzmann C. Vegetarian diets - what are the advantages? Forum Nutr. 2005;(57):147-56.
10. Elmadfa I, Singer I. Vitamin B12 and homocysteine status among vegetarians: a global perspective. Am J Clin Nutr. 2009;89(5):1693S-8S.
11. Sabaté J. The contribution of vegetarian diets to human health. Forum Nutr. 2003;56:218-20.
12. Craig WJ. Health effects of vegan diets. Am J Clin Nutr. 2009;89(5):1627S-33S.
13. Scientific Advisory Committee on Nutrition. Vitamin D and health. SACN; 2016.
14. Saggese G, Vierucci F, Prodam F, Cardinale F, Cetin I, Chiappini E, et al. Vitamin D in pediatric age: consensus of the Italian Pediatric Society and the Italian Society of Preventive and Social Pediatrics, jointly with the Italian Federation of Pediatricians. Ital J Pediatr. 2018;44(1):51. doi: 10.1186/s13052-018-0488-7.
15. Charoenngam N, Holick MF. Imunologic effects of vitamin D on human health and disease. Nutrients. 2020;12(7):2097. doi: 10.3390/nu12072097.
16. Chan J, Jaceldo-Siegl K, Fraser GE. Serum 25-hydroxyvitamin D status of vegetarians, partial vegetarians, and nonvegetarians: the Adventist Health Study-2. Am J Clin Nutr. 2009;89(5):1686S-92S.
17. Crowe FL, Steur M, Allen NE, Appleby PN, Travis RC, Key TJ. Plasma concentrations of 25-hydroxyvitamin D in meat eaters, fish eaters, vegetarians and vegans: results from the EPIC-Oxford study. Public Health Nutr. 2011;14(2):340-6.
18. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet. 2007 Aug 11;370(9586):511-20.
19. Slovak Food Composition Database Online [Internet]. Bratislava: National Agricultural and Food Centre; c2010 [updated 2022 Dec 19; cited 2022 Aug 25]. Available from: http://www.pbd-online.sk/.
20. Recommended nutrition doses for population of the Slovak republic (9th revision). Vestník MZ SR. 2015 Jun 19;63 Pt 4-5:19-28. (In Slovak.)
21. Lapage G, Roy CC. Direct transesterification of all classes of lipids in one step reaction. J Lipid Res. 1986;27(1):114-20.
22. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014;19(2):164-74.
23. Hunt JR. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. Am J Clin Nutr. 2003;78(3 Suppl):633S-9S.
24. Kris-Etherton PM, Grieger JA, Etherton TD. Dietary reference intakes for DHA and EPA. Prostaglandins Leukot Essent Fatty Acids. 2009;81(2-3):99-104.
25. Sanders TA. DHA status of vegetarians. Prostaglandins Leukot Essent Fatty Acids. 2009;81(2-3):137-41.
26. EFSA Panel on Dietetic Products, Nutrition and Allergies. Dietary reference values for vitamin D. EFSA J. 2016;14(10):4547. doi: 10.2903/j.efsa.2016.4547.
27. Aswall JE. Health for all in the 21st century a policy framework for the WHO European region. Int J Occup Med Environ Health. 2000;13(1):5-13.
28. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M; Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. Pediatrics. 2008;122(2):398-417.
29. Saunders AV, Craig WJ, Baines SK, Posen JS. Iron and vegetarian diets. Med J Aust. 2013;199(S4):S11-6.
30. Burns-Whitmore B, Froyen E, Heskey C, Parker T, San Pablo G. Alpha-linolenic and linoleic fatty acids in the vegan diet: do they require dietary reference intake/adequate intake special consideration? Nutrients. 2019;11(10):2365. doi: 10.3390/nu11102365.

Received August 10, 2021
Accepted in revised form August 25, 2022