Serum homocysteine, folate, vitamin B_{12} and total antioxidant status in vegetarian children

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Abstract

Purpose: The results of several studies point to the positive role of vegetarian diets in reducing the risk of diabetes, some cancers and cardiovascular diseases. However, exclusion of animal products in vegetarian diets may affect the cobalamin status and cause an elevation of the plasma homocysteine level.

The aim of this study was to assess the effect of vegetarian diets on serum concentrations of homocysteine, folate, vitamin B_{12} and total antioxidant status (TAS) in children.

Material and methods: The study included 32 vegetarians (including 5 vegans), age 2-10 years. Dietary constituents were analyzed using a local nutritional programme. Serum homocysteine, folate and vitamin B_{12} were determined with fluorescence and chemiluminescence immunoassays. The concentration of TAS was measured by a colorimetric method.

Results: Average daily energy intake and the percentage of energy from protein, fat and carbohydrates in the diets of the studied children were just above or similar to the recommended amounts. It could be shown that vegetarian diets contain high concentrations of folate. In vegan diets it even exceeds the recommended dietary allowance. Mean daily intake of vitamin B_{12} in the studied diets was adequate but in vegans was below the recommended range. The serum concentrations of homocysteine, folate, vitamin B_{12} and TAS in vegetarian children remained within the physiological range.

Conclusions: The presented data indicate that vegetarian children, contrary to adults, have enough vitamin B_{12} in their diet (excluding vegans) and normal serum concentrations of homocysteine, folate and vitamin B_{12}. Therefore, in order to prevent deficiencies in the future, close monitoring of vegetarian children (especially on a vegan diet) is important to make sure that they receive adequate quantities of nutrients needed for healthy growth.

Introduction

The results of several studies show important benefits of vegetarian diets and a relation with reduced risk for such diseases as diabetes, obesity, heart diseases and several types of cancer [1-3]. However, exclusion of animal products in vegetarian diets may affect the cobalamin (vitamin B_{12}) status and cause an elevation of the plasma homocysteine (Hcy) level [4,5]. The predominant consumption of protein of plant origin in this kind of diets shifts homocysteine to the remethylation pathway, which requires vitamin B_{12} as a cofactor and methyltetrahydrofolate as a substrate. In vegetarian diets the intake of folic acid exceeds the recommended dietary allowance, whereas the intake of vitamin B_{12} is inadequate or even absent [6].

Adult vegetarians are at risk of developing hyperhomocysteinemia, which has been recognized as an independent cardiovascular risk factor. It is hypothesized that Hcy alters endothelial and smooth muscle cell functions by generating reactive oxygen species. The resulting increase in oxidative stress diminishes antioxidative capacity, which increases the risk for atherosclerotic vessel diseases in these subjects. Dietary folate deficiency causes insufficient formation of 5-methyltetrahydrofolate, which is needed as a donor of methyl-group in the remethylation of Hcy to methionine. Vitamin deficiencies (B_{12} and folate), enzyme mutations with partial loss of enzymatic activities (cystathionine-β-synthase, methionine synthase, methylentetrahydrofolate reductase polymorphisms), and renal insufficiency may produce moderate hyperhomocysteinemia (>15 μmol/L) [4,7].

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Table 1. Average daily energy and nutrient intakes of vegetarian children compared to recommended daily intake

	Daily intake	Recommended daily intake
Energy values (kcal)	1426.5±440.4	1300.0-2500.0
Energy from protein (%)	11.9±2.4	12.0-14.0
Energy from fat (%)	32.3±4.9	32.0
Energy from carbohydrates (%)	55.8±5.6	56.0-58.0
Folate (µg)	195.7±78.0	50.0-150.0
Vitamin B_{12} (µg)	1.6±1.3	1.0-2.0

Data are presented as mean values ±SD

Table 2. Serum concentrations of lipids, homocysteine, folate, vitamin B_{12} and TAS in vegetarian children

	Vegetarian children	Reference values
TC (mg/dL)	155.1±25.8	<170
HDL-C (mg/dL)	53.7±8.3	52-72
LDL-C (mg/dL)	91.0±24.5	<110
TG (mg/dL)	80.4±58.4	50-100
Homocysteine (µmol/L)	6.1±1.2	<8.0
Folate (ng/mL)	12.8±3.4	4.2-19.9
Vitamin B_{12} (pg/mL)	548.6±144.4	240-900
TAS (mmol/L)	1.20±0.1	1.16-1.40[25]

Data are presented as mean values ±SD and ranges

Hyperhomocysteinemia and decreased total antioxidant status (TAS) may partly counteract the beneficial lifestyle of vegetarians. No systemic studies have been carried out on homocysteine and TAS status in children on vegetarian diet.

The aim of this study was to assess the effect of vegetarian diets on serum concentrations of homocysteine, folate, vitamin B_{12} and total antioxidant status in children.

Material and methods

The study included 32 vegetarian children (14 girls, 18 boys) aged 6.5±4.2 years who had been referred to the Department of Nutrition of the Institute of Mother and Child (Warsaw) for dietary consultation. The principal difference among various vegetarian diets was the extent to which certain products were avoided. In this tested group there were:

– lacto-ovovegetarians (n=21), who did not consume meat, poultry or fish, but ate eggs and dairy products,

– lacto vegetarians (n=1), who excluded eggs,

– ovovegetarians (n=5), who ate eggs, but excluded milk products,

– vegans (n=5), who excluded all foods of animal origin.

This research was approved by the institutional review board and informed consent was obtained from parents of the examined children. Dietary constituents were analyzed using the nutritional programme Dietetyk2® (National Food and Nutrition Institute, Warsaw) and completed with supplementation data.

Venous blood samples were obtained after overnight fast. Serum was prepared by centrifugation at 1000 x g at 4°C and total cholesterol (TC), high-density lipoprotein HDL (HDL-C), low-density lipoprotein LDL (LDL-C) and triglycerides (TG) concentrations were determined enzymatically with kits from Bio-Merieux (France) using Cobas Mira analyzer (Roche, Switzerland). Remaining serum samples were frozen and collected for other analyses. Total Hcy was measured with a fluorescence polarization immunoassay on IMX analyzer (Abbott, USA). Folate and vitamin B_{12} were determined with a chemiluminescence immunoassay (Elecsys, Roche, Switzerland). TAS was measured by a colorimetric method using kits from Randox Laboratories Ltd (GB).

Results

Tab. 1 shows the average daily energy and nutrients intakes of vegetarian children including the intake of folate and vitamin B_{12}, compared to nutrition recommendations. Mean daily energy intake was 1426.5±440.4 kcal and the percentage of energy from protein was 11.9±2.4, from fat 32.3±4.9 and from carbohydrates 55.8±5.6. These amounts were just above or similar to the lower limit of the recommended values. Moreover, vegetarians have a high intake of folate (195.7±78.0 µg/day). Mean daily intake of vitamin B_{12} was in the reference range (1.6±1.3 µg/day), but in 9 individuals (including all the vegans) was below the recommended values (<1 µg/day).

In vegetarian children serum concentrations of total cholesterol and LDL-cholesterol levels (*Tab. 2*) were low but in the physiological range. Triglycerides concentrations were in the middle, whereas HDL-cholesterol were close to the lower limit of the reference range. The mean serum concentrations of homocysteine (6.1±1.2 µmol/L), folate (12.8±3.4 ng/L) and vitamin B_{12} (548.6±144.4 pg/mL) were in the physiological range. Total antioxidant status in vegetarians was in the range of 1.16-1.40 mmol/L. Concentrations of TAS below the lowest value of omnivorous children were found only in 2 vegetarians.

Discussion

According to present knowledge there are several positive as well as negative consequences of vegetarianism on health status [1-3,5]. The risk of dietary inadequacy increases with the number and degree of restrictions on the food groups that are consumed, willingness to use fortified foods or nutrient supplements, and to accept medical advice. The principal difference among various vegetarian diets is the extent to which animal products are avoided. In this respect the lacto-ovovegetarian diet being the most permissive and the vegan diet the most restrictive.

Vegetarian diets in children can be healthy only, if they are well balanced and a variety of foods is consumed. It is also important to be sure that all nutrients are consumed at appropriate levels [3,8,9]. Adequate energy intake is very important for children and adolescents, because growth and development

are the most intensive at these age spans. Vegetarians usually consume less total protein than omnivores but their intakes are usually satisfactory if energy intakes are adequate. Protein quality is also of potential concern, because plant proteins are limited in some amino acids (lysine, cysteine, tryptophan, methionine) [10]. Most vegetarian diets are lower in fat, saturated fat but higher in polyunsaturated and monounsaturated fats than non-vegetarian ones. Intakes of carbohydrates, especially complex carbohydrates and dietary fiber, tend to be higher among vegetarians than among omnivores, and more in line with dietary recommendations [3,9].

Our results indicate that in the tested vegetarian children the mean daily energy intake and the percentage of energy from protein, fat and carbohydrates were similar or just above the lower limit of the recommended values.

In general vegetarians have relatively low serum cholesterol, lipoproteins and triglycerides concentrations [11-13]. However, recent studies have shown higher serum concentrations of homocysteine in vegetarians than in omnivores. The Hcy concentration increases as the vegetarian diet becomes more restrictive and peaks in vegans [4,7,14-17]. Hermann et al. [7] and Krajcovicowa-Kudlackova et al. [18] observed that about 20-30% young vegetarian had moderate hyperhomocysteinemia ($>15 \mu$mol/L).

High Hcy occurrence in children is not yet fully recognized. Our previous results, similar to the studies of other, indicated that Hcy concentration in healthy omnivorous children were half of that reported in adults [19,20]. In the presented study concerning children on vegeterian diets, we have confirmed these conclusions and have observed mean values of homocysteine in children that are lower by 40% compared to adult vegetarians.

Elevated serum homocysteine, caused by its prooxidative activity might be associated with non-efficient antioxidant protection and results in lipid peroxidation [21,22]. We have previously shown that mean serum concentrations of antioxidant vitamins A and E in prepubertal vegetarian children were statistically lower as compared to those in non-vegetarians but comparable to the reference range [23]. Total antioxidant status (TAS) values represent a mixed antioxidant capacity contributed among other compounds mainly to vitamin C, vitamin E, vitamin A and β-carotene [24]. In our group of prepubertal vegetarian children mean values of TAS were similar and in the same range as reported by others in omnivorous children and in adult vegetarians [7,25,26].

Ullegaddi et al. [22] observed that therapy with antioxidants and B-group vitamins inhibited an effect of elevated total plasma homocysteine. Several studies reported that mild hyperhomocysteinemia in vegetarians could be a consequence of vitamin B₁₂ deficiency [18,27]. Vegetarian diets are typically high in folic acid because of high intakes of fruits and vegetables but are low in vitamin B₁₂, which is found in its most bioavailable form only in meat [17,28]. Vitamin B₁₂ deficiency may take years to develop because the body is able to store sufficient quantities of this vitamin (in vegetarians mainly from vitamin supplementation). This is a problem not only because megaloblastic anemia may develop, but also nerve cells tend to be depleted in B₁₂. Another problem is that high folate level can mask this kind

of anemia and B₁₂ deficiency may not be detected until after the onset of neurological symptoms.

In the group of studied children high intake of folate and normal mean daily intake of vitamin B₁₂ was observed. Only in vegan children the intake of folate was above 200 μg/day and vitamin B₁₂ below the recommended values. In the serum of all tested children concentrations of folate and vitamin B₁₂ were in the middle of the reference range.

Therefore, it seems to be important to closely monitor vegetarian children to make sure that they receive adequate quantities of nutrients needed for healthy growth. It is also necessary to look for negative consequences of these diets, such as hyperhomocysteinemia and deficient total antioxidant status (TAS), which may partly counteract the beneficial lifestyle of vegetarians.

Conclusions

The presented results indicate that vegetarian prepubertal children, contrary to adults, have enough vitamin B₁₂ (excluding vegans) in their diet and normal serum concentrations of homocysteine, folate and cobalamin. Therefore, in order to prevent future deficiencies, close monitoring of vegetarian children (especially on a vegan diet) is important to make sure that they receive adequate quantities of nutrients needed for healthy growth.

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