

## POSSIBLE HEALTH RISKS IN SUBJECTS WITH DOMINANT PLANT FOOD CONSUMPTION

### MOŽNÉ ZDRAVOTNÉ RIZIKÁ U ĽUDÍ S PREVAŽNOU KONZUMÁCIOU RASTLINNEJ POTRAVY

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#### ABSTRACT

In two groups of apparently healthy non-obese non-smoking women aged 20-30 years – 79 vegetarians (39 lacto-ovo-vegetarians /plant food, dairy products, eggs/, 40 semi-vegetarians /as lacto-ovo-vegetarians with addition of white meat and fish consumption/) and 81 non-vegetarians (control group on traditional mixed diet) were analyzed the dietary questionnaires of food-frequency and measured the blood concentrations of vitamins B9, C,  $\beta$ -carotene, B12, D and concentrations of iron. Young women in both groups had similar values of body mass index, concentrations of vitamin C, vitamin B9 and  $\beta$ -carotene. In vegetarian vs. non-vegetarian group was found the significantly increased daily intake of fiber, whole grain products, pulses, seeds and nuts. These findings suggest that both nutritional groups had the similar nutritional regimen from view of fruit and vegetables and different from view of other key vegetarian food commodities. Vitamin B12, vitamin D and long-chain n-3 fatty acids are not contained in plant food. Bioavailability of iron from food can be lower in presence of phytic acid (from whole grain products and pulses) and fiber (pulses, seeds, nuts, whole grains). In group of lacto-ovo-vegetarians (narrow range of animal food consumption) vs. non-vegetarian or semi-vegetarian groups were found the significantly reduced concentrations of vitamin B12, vitamin D and iron with a greater incidence of deficient values (49 % vs. 13 and 15 % for vitamin B12, 67 % vs. 46 and 50 % for vitamin D, 44 % vs. 20 and 30 % for iron). Long-chain n-3 fatty acid intake (eicosapentaenoic and docosahexaenoic) in lacto-ovo-vegetarian group was significantly reduced and very low (no fish consumption) in comparison to non-vegetarians and semi-vegetarians. Intake of these acids in semi-vegetarians vs. non-vegetarians was non-significantly increased. The substrate for long-chain n-3 fatty acid

animal food and dominant consumption of plant food can be connected with possible health risks (higher incidence of deficient values of vitamin B12, vitamin D, iron and long-chain n-3 fatty acids).

**Keywords:** vegetarian nutrition, vitamin B12, vitamin D, iron, n-3 fatty acids

## ABSTRAKT

V dvoch skupinách subjektívne zdravých neobéznych žien veku 20-30 rokov – 79 vegetariánov (39 lakto-ovo-vegetariánov /rastlinná potrava, mliečne výrobky, vajíčka/, 40 semi-vegetariánov /ako lakto-ovo-vegetariáni s prídavkom konzumácie bieleho mäsa a rýb/) a 81 nevegetariánov (kontrolná skupina na tradičnej zmiešanej strave) boli analyzované dietetické dotazníky frekvencie konzumácie a merané v krvi koncentrácie vitamínov B9, C, -karoténu, B12, D a koncentrácie železa. Mladé ženy oboch skupín mali rovnaké hodnoty body mass indexu a koncentrácií vitamínu C, vitamínu B9 a -karoténu. V skupine vegetariánov vz nevegetariánska skupina bol zistený signifikantne zvýšený denný príjem vlákniny, celozrnných produktov, strukovín, semien a orieškov. Tieto nálezy napovedajú, že obe nutričné skupiny mali rovnaký nutričný režim z hľadiska ovocia a zeleniny a rozdielny z pohľadu ďalších kľúčových vegetariánskych potravinových komodít. Vitamín B12, vitamín D a dlhoreťazové n-3 mastné kyseliny chýbajú v rastlinnej potrave. Dostupnosť železa z potravy môže byť znížená v prítomnosti kyseliny fytovej (z celozrnných produktov a strukovín) a vlákniny (strukoviny, semená, oriešky, celozrnné výrobky). V skupine lakto-ovo-vegetariánov (úzke rozpätie konzumácie živočíšnej potravy) vz nevegetariánska a semi-vegetariánska skupina boli zistené významne redukované koncentrácie vitamínu B12, vitamínu D a železa so širším výskytom deficitných hodnôt (49 % vz 13 a 15 % pre vitamín B12, 67 % vz 46 a 50 % pre vitamín D, 44 % vz 20 a 30 % pre železo). Príjem dlhoreťazových n-3 mastných kyselín (ikozapentaénová a dokozahexaénová) v skupine lakto-ovo-vegetariánov bol v porovnaní so skupinou nevegetariánov a semi-vegetariánov signifikantne redukovaný a veľmi nízky (žiadna konzumácia rýb). Príjem kyselín u semivegetariánov vz nevegetariáni bol nevýznamne zvýšený. Substrát pre biosyntézu dlhoreťazových mastných kyselín – kyselina -linolénová bol vegetariánmi v oboch skupinách vz. nevegetariáni významne viac konzumovaný (hlavne ľanové semená). Výsledky napovedajú, že limitovaná konzumácia živočíšnej potravy a dominantná konzumácia rastlinnej potravy môže byť spojená s možnými zdravotnými rizikami (vyšší výskyt deficitných hodnôt vitamínu B12, vitamínu D, železa a n-3 mastných kyselín).

**Kľúčové slová:** vegetariánske stravovanie, vitamín B12, vitamín D, železo, n-3 mastné kyseliny

## PODROBNÝ ABSTRAKT

spojených ochorení, ako to uvádza Americká dietetická asociácia. Protektívny účinok rastlinnej potravy na zdravie však nemusí byť vždy rovnaký; je rozhodujúci pomer konzumácie rastlinnej a živočíšnej potravy. Všeobecne v rastlinnej potrave chýba vitamín B12, vitamín D a dlhoreťazové n-3 mastné kyseliny. Dostupnosť niektorých minerálnych a stopových látok môže byť znížená vplyvom kyseliny fytovej a vlákniny, ktoré sú prítomné v rastlinnej potrave. Rastlinná potrava je ďalej relatívne chudobná na niektoré esenciálne aminokyseliny, na saturované tuky, retinol, jód a zinok a relatívne bohatá na sacharidy, n-6 mastné kyseliny, vlákninu, karotenoidy, kyselinu listovú, vitamín C, vitamín E, magnézium a mnohé bioaktívne komponenty ako sú polyfenoly, hlavne flavonoidy, katechíny a fytoestrogény. Kľúčové komponenty vegetariánskej potravy sú cereálie, strukoviny, orešky, semená, ovocie a zelenina. Uvedené skutočnosti o zložení vegetariánskej potravy prinášajú mnohé zdravotné benefity, ale môžu prinášať aj zdravotné riziká pre populáciu konzumujúcu výlučne alebo prevažne rastlinnú potravu.

V dvoch skupinách subjektívne zdravých neobéznych žien veku 20-30 rokov – 79 vegetariánov (39 lakto-ovo-vegetariánov /V-LO, rastlinná potrava, mliečne výrobky, vajčka/, 40 semi-vegetariánov /V-S, ako lakto-ovo-vegetariáni s prídavkom konzumácie bieleho mäsa a rýb/) a 81 nevegetariánov (NV, kontrolná skupina na tradičnej zmiešanej strave) boli analyzované dietetické dotazníky frekvencie konzumácie a merané v krvi koncentrácie vitamínov B9, C, -karoténu, B12, D a koncentrácie železa. Mladé ženy oboch skupín mali rovnaké hodnoty body mass indexu a koncentrácií vitamínu C, vitamínu B9 a -karoténu. V skupine vegetariánov vz nevegetariánska skupina bol zistený signifikantne zvýšený denný príjem vlákniny, celozrnných produktov, strukovín, semien a oreškov. Tieto nálezy napovedajú, že obe nutričné skupiny mali rovnaký nutričný režim z hľadiska ovocia a zeleniny a rozdielny z pohľadu ďalších kľúčových vegetariánskych potravinových komodít. Vitamín B12, vitamín D a dlhoreťazové n-3 mastné kyseliny chýbajú v rastlinnej potrave. Dostupnosť železa z potravy môže byť znížená v prítomnosti kyseliny fytovej (z celozrnných produktov a strukovín) a vlákniny (strukoviny, semená, orešky, celozrnné výrobky). V skupine lakto-ovo-vegetariánov (úzke rozpätie konzumácie živočíšnej potravy) vz nevegetariánska a semi-vegetariánska skupina boli zistené významne redukované koncentrácie vitamínu B12 (205 vz 285 a 274 pmol/l /P<0,001/), vitamínu D (17,4 vz 22,1 a 20,2 ng/ml /P<0,01/) a železa (12,4 vz 15,1 a 14,8 mol/l /P<0,05/) so širším výskytom deficitných hodnôt (49 % vz 13 a 15 % pre vitamín B12, 67 % vz 46 a 50 % pre vitamín D, 44 % vz 20 a 30 % pre železo). Príjem dlhoreťazových n-3 mastných kyselín (ikozapentaénová /EPA/ a dokozahexaénová /DHA/) v skupine lakto-ovo-vegetariánov bol v porovnaní so skupinou nevegetariánov a semi-vegetariánov signifikantne redukovaný a veľmi nízky v dôsledku žiadnej konzumácie rýb (EPA 0,057 vz 31,30 a 37,06 mg /P<0,001/, DHA 0,033 vz 25,75 a 34,12 mg /P<0,001/). Príjem kyselín u semivegetariánov vz nevegetariáni bol nevýznamne zvýšený. Substrát pre biosyntézu dlhoreťazových mastných kyselín – kyselina -linolénová - bol vegetariánmi v oboch skupinách vz nevegetariáni významne viac konzumovaný (hlavne ľanové semená) (2,48 g V-LO /P<0,001/ a 1,60 g V-S /P<0,05/ vz 1,35 g NV).

Výsledky napovedajú, že limitovaná konzumácia živočíšnej potravy a dominantná konzumácia rastlinnej potravy môže byť spojená s možnými zdravotnými rizikami (vyšší výskyt deficitných hodnôt vitamínu B12, vitamínu D, železa a n-3 mastných kyselín). Tieto zdravotné riziká však neznižujú kľúčovú

ochorenia, diabetu, metabolického syndrómu, mnohých typov rakoviny a ďalších ochorení, ako je demonštrované vo vedeckej literatúre. Zdravotným rizikám možno ľahko predchádzať, je nutné monitorovanie rizikových parametrov, konzumácia fortifikovanej potravy alebo užívanie nutričných a farmaceutických suplementov.

## INTRODUCTION

Mortality and incidence of coronary disease events are indeed clearly lower in subjects with dominant plant food consumption (Fraser, 2009). A combined analysis of two cohorts of Adventists in California, older cohorts of British and German vegetarians confirmed this result with relative risk for rate coronary heart mortality 0.68. Occurrence of diabetes is lower in Adventist vegetarians than in non-vegetarians. Subjects with week consumption of all meats were 29 % more likely (relative to vegetarians) to develop diabetes during the 17 year follow-up. Long-term adherence (over a 17 year interval) to a diet that included at least weekly meat intake was associated with a 74 % increase in odds of diabetes relative to long-term adherence to a vegetarian diet (Vang et al, 2008). The results of a greater sample of Adventist population (n=60,903) shown that a prevalence of type 2 diabetes (2002-2006) increased from 2.9 % in vegans, 3.2 % in lacto-ovo-vegetarians, 6.1 % in semi-vegetarians to 7.6 % in non-vegetarians (Tonstad et al, 2009). There is generally known that red meat consumption increases the risk of colon or colorectal cancer. The higher consumption of fruit and vegetables is associated with reduced all-cause mortality and reduced risks of some cancers (Fraser, 2009; Key et al, 2009). In group of 29,163 British vegetarian men and women (fish eaters and lacto-ovo-vegetarians) was incidence of cancers 3.93 % vs. 6.8 % in group of 32,403 non-vegetarians (meat eaters) after an average follow-up of 12.2 years. Relative risk for stomach cancer was 0.29 in fish eaters and 0.36 in vegetarians (compared with meat eaters), for cancers of the lymphatic and haematopoietic tissues 0.85 in fish eaters and 0.55 in vegetarians.

Appropriately planned vegetarian diets are healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases as introduced the American dietetic association (Craig and Mangels, 2009). Different types of vegetarians may not experience the same effects on health. In general, vegetarian diets provide relatively large amounts of cereals, pulses, nuts, fruits and vegetables (Key et al, 2006). In terms of nutrients, vegetarian diets are usually rich in carbohydrates, n-6 fatty acids, dietary fiber, carotenoids, folic acid, vitamin C, vitamin E and magnesium, and relatively low in some essential amino acids, saturated fat, long-chain n-3 fatty acids, retinol, vitamin B12, vitamin D, iodine and zinc (Hunt, 2003; Krajčovičová-Kudláčková et al, 2003; Key et al, 2006; Sanders, 2007; Allen, 2008; Crowe et al, 2011). These facts are connected with certain health benefits (as is introduced above) or risks in population consuming exclusively or predominantly plant food.

Eliminating all animal products from the diet increases the risk of certain nutritional deficiencies (Craig, 2009) because vitamins B12 and D and long-chain n-3 fatty acids are not contained in plant foods. Iron status of subjects with preference of plant food may be decreased because of the limited

bioavailability of this mineral in presence of some plant components (Hunt, 2003). The main goal of this study was to assess the possible and assumed health risks in subjects with dominant consumption of plant food (with or without white meat and fish consumption) in comparison to general population on traditional mixed diet.

## SUBJECTS AND METHODS

Randomly selected 160 apparently healthy non-obese ( $BMI < 30 \text{ kg/m}^2$ ) non-smoking women aged 20-30 years were divided into two groups. The non-vegetarian (control) group consisted of 81 persons of general population on traditional mixed diet (Slovak medical university workers and students of Bratislava universities). The group of vegetarians ( $n=79$ ) consumed plant food, dairy products and eggs (sub-group of lacto-ovo-vegetarians,  $n=39$ ) or consumed as addition still white meat (poultry) and fish (sub-group of semi-vegetarians,  $n=40$ ). Alternative nutrition subjects were selected from data base of previous research university projects. The group characteristics are introduced in Table 1. 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 146 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 conversion to nutrients was done by using self-developed software Nutrition based on the Slovak food composition database compiled by the Food Research Institute (SFDB, 1999).

Blood was sampled after an overnight fasting by a standard procedure. EDTA was used as an anticoagulant. Plasma concentrations of vitamins C and  $\beta$ -carotene were measured by HPLC (Čerhata et al, 1994; Lee et al, 1992). Serum vitamin B12 and folic acid (vitamin B9) concentrations were determined using Elecsys immunoassay (Roche tests). Serum 25-hydroxyvitamin D (25/OH/D) concentration was determined by RIA method (IDS Ltd, UK). Serum iron concentration was measured by standard laboratory method using Vitros 250 autoanalyzer (Johnson & Johnson, UK). The intake of vitamins, mineral and trace elements in natural form only was allowed (no supplementation). The Student t-test was used for final evaluation.

## RESULTS AND DISCUSSION

Vitamin B12 is absent in plant food and bacteria in the distal part of small intestine are its only source in subjects with exclusive plant consumption, therefore vitamin B12 deficiency is one of the risk factors of alternative nutrition (Krajčovičová-Kudláčková et al, 2000a). Vitamin deficiency can lead to several adverse health consequences: folate trapping in the methylation cycle and

similar to that caused by folate deficiency, elevated atherogenic blood homocysteine and neural tube defects (Varela-Moreiras, 2009). Consumption of dairy products and eggs in lacto-ovo-vegetarians, moreover the intake of white meat in semi-vegetarians, provides a better possibility to meet the needs for vitamin B12 (Krajčovičová-Kudláčková et al, 2000a, 2007). In our samples of volunteers we found the significantly reduced serum vitamin B12 concentration in group of lacto-ovo-vegetarians vs. non-vegetarian but also semi-vegetarian group (Table 1) with deficient values in 49 % of subjects vs. 13 % and 15 % in non-vegetarian and semi-vegetarian subjects. From view of global perspectives for vitamin B12 deficiency prevention, vegetarians, particularly vegans (only plant food), must be advised to monitor their blood vitamin B12 status and to use vitamin B12 fortified foods or take vitamin B12 supplements if necessary (Elmadfa and Singer, 2009).

Table 1. Iron and vitamins B9, B12, D, C,  $\beta$ -carotene blood concentrations  
Tabuľka 1. Koncentrácie železa a vitamínov B9, B12, D, C,  $\beta$  karoténu v krvi

	NV	V	V-S	V-LO
n	81	79	40	39
average age (y)	24.3±0.3	25.6±0.3		
BMI (kg/m <sup>2</sup> )	21.7±0.4	21.5±0.3		
>25	10 %	6 %		
>30	0	0		
duration of vegetar. (y)	-	9.58±0.48		
vitamin B9 (nmol/l)	23.5±1.0	23.1±1.2		
vitamin C ( $\mu$ mol/l)	57.9±1.8	55.7±1.5		
$\beta$ -carotene ( $\mu$ mol/l)	0.350±0.041	0.382±0.030		
vitamin B12 (pmol/l)	285±13	240±11 ^	274±15	205±16 *
<179	13 %	32 %	15 %	49 %
vitamin D (ng/ml)	22.1±1.2	18.8±1.0 °	20.2±1.0	17.4±1.2 ^
<20	46 %	58 %	50 %	67 %
iron ( $\mu$ mol/l)	15.1±0.7	13.6±0.8	14.8±1.0	12.4±1.1°
<10	20 %	37 %	30 %	44 %

The results are expressed as mean±SEM; NV – non-vegetarians, V – vegetarians, V-S – semi-vegetarians, V-LO – lacto-ovo-vegetarians, °P<0.05, ^P<0.01, \*P<0.001

Subjects who limit their intake of animal products may be a greater risk of vitamin D deficiency than non-vegetarians, because foods providing the highest amount of vitamin D are all from animal sources (Chan et al, 2009). The authors Crowe et al. (2011) described that plasma 25(OH) vitamin D concentrations reflected the degree of animal product exclusion. Meat eaters had the highest mean intake of vitamin D (3.1  $\mu$ g/day) and mean plasma 25(OH)D concentration (77.0 nmol/l) and vegans (exclusive intake of plant

food) the lowest (0.7 g/day and 55.8 nmol/l, respectively). In our presented study at the same sun exposure (blood samples collected in spring April-May) was found the significantly reduced plasma vitamin D concentration in lacto-ovo-vegetarians while in semi-vegetarians (white meat eaters) was this concentration similar in comparison to non-vegetarians (red and white meat eaters) (Table 1). In vegetarian group vs. meat eater groups was found the higher incidence of deficient vitamin D values (67% vs. 46 % /non-vegetarians/ and 50 % /semi-vegetarians/) (Table 1).

Table 2. Daily intake of selected food and nutrients  
Tabuľka 2. Denný príjem vybranej potravy a živín

	NV	V	V-S	V-LO
Eicosapentaenoic acid (mg)	31.30±4.22	18.81±3.41°	37.06±5.18	0.057±0.036 *
docosahexaenoic acid (mg)	25.75±4.11	17.07±3.63	34.12±3.88	0.033±0.018 *
-linolenic acid (g)	1.35±0.06	2.03±0.13 *	1.60±0.09 °	2.48±0.23 *
fiber (g)	26.2±1.2	34.4±1.3 *		
whole grain products (g)	97.3±10.2	140.1±14.2 °		
pulses (g)	10.9±1.6	18.4±2.3 ^		
soya and its products (g)	7.7±2.2	53.2±7.7 *		
seeds (g)	5.0±1.0	13.7±2.6 ^		
nuts (g)	9.3±1.7	14.9±1.9 °		

The results are expressed as mean±SEM; NV – non-vegetarians, V – vegetarians, V-S – semi-vegetarians, V-LO – lacto-ovo-vegetarians  
°P<0.05, ^P<0.01, \*P<0.001

Subjects with exclusive and predominant consumption of plant food can suffer from long-chain n-3 fatty acid deficiencies. These acids are only contained in fish. EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid) and fish intake are primarily important for coronary disease risk reduction and triacylglycerol lowering (Kris-Etherton, 2009). Although there is no recommended dietary allowance (RDA) for EPA and DHA, various organization recommended that approximately 10 % of -linolenic (ALA) acid RDA (2.0 g/day) can be consumed as EPA and/or DHA (=200 mg/day). Lacto-ovo-vegetarians vs. non-vegetarians and semi-vegetarians in presented study consumed significantly reduced and very low amounts of n-3 fatty acids (Table 2) as a consequence of no fish consumption (trace amounts are from various oil spreads with fish addition). On the other hand, intake of substrate for biosynthesis of n-3 fatty acids ALA is significantly higher (as a consequence of higher consumption of linseeds, olive, rapessed and soybean oils, walnuts) and it is higher as recommended 2 g. Analyses of food-frequency questionnaires shown, that lacto-ovo-vegetarians consume significantly more soya, seeds and nuts (Table 2). There is no evidence of adverse effects on health with lower n-3 fatty acid intake (Sanders, 2007). In our previous studies we shown that plasma

concentrations of EPA and DHA are similar in lacto-ovo-vegetarians and non-vegetarians (Krajčovičová-Kudláčková et al, 2000b). Intake of EPA and DHA in semi-vegetarians vs. non-vegetarians was non-significantly increased (Table 2) and intake of ALA was significantly increased but it was lower than 2 g.

In subjects with exclusive or dominant plant food consumption was observed the decrease of bioavailability of trace elements because of the high content of phytic acid (whole grains, legumes) and/or dietary fiber (whole grains, pulses, seeds, nuts) (Hubt, 2003). These introduced food commodities and fiber are significantly more consumed in vegetarians vs. non-vegetarians (Table 2). We found that serum iron concentration is significantly reduced in group of lacto-ovo-vegetarians (Table 1) with higher incidence of deficient values (44 % vs. 20 % in non-vegetarian group and 30 % in semi-vegetarian group). Iron deficiency causes the lower biosynthesis of long-chain polyunsaturated fatty acids by  $\Delta$ -6 desaturase activity reduction. This enzyme provides the conversion of ALA to stearidonic acid (substrate for EPA production by  $\Delta$ -5 desaturase activity). In group of iron deficient lacto-ovo-vegetarians vs. non-vegetarians and iron normal lacto-ovo-vegetarians we found the significantly reduced plasma concentrations of EPA and DHA (0.36 % vs. 0.57 % and 0.53 % EPA, 1.27 % vs. 1.80 % and 1.71 % DHA; fatty acids expressed as % from total fatty acids) (Krajčovičová-Kudláčková et al, 2000b).

## CONCLUSION

The findings of higher incidence of deficient values of vitamin B12, vitamin D, n-3 fatty acids and iron suggest that limited consumption of animal food and dominant consumption of plant food can be connected with possible health risks. Vitamin B12, vitamin D and long-chain n-3 fatty acids are lacking in plant food. Iron bioavailability from plant food can be lower in presence of phytic acid and fiber. These health risks may be prevented by monitoring of parameters, consumption of fortified food and nutrition or pharmaceutical supplements. The health benefits of sufficient intake of various plant food in prevention of cardiovascular disease, diabetes, metabolic syndrome, many cancers and other diseases are demonstrated in scientific literature (Krajčovičová-Kudláčková et al, 2000, 2008; Key et al, 2006, 2009; Vang et al, 2008; Craig and Mangels, 2009; Fraser, 2009; Tonstad et al, 2009).

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