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NUTRITIONAL STATUS OF SUBJECTS WITH DOMINANT PLANT FOOD CONSUMPTION

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ABSTRACT

In three groups of apparently healthy subjects – vegetarians (plant food, dairy products, eggs), semi-vegetarians (as vegetarians with addition of white meat consumption) and non-vegetarians (control group on traditional mixed diet) were analyzed the dietary questionnaires of consumption frequency and measured the values of lipid profile, insulin resistance, homocysteine with determinants (vitamins B6, B9, B12) and plasma antioxidative vitamins (C, E, beta-carotene). Vegetarians and semi-vegetarians consumed the significantly reduced amount of cholesterol, saturated fatty acids, methionine, lysine, vitamin B12 and on the other hand, they have the significantly higher daily intake of polyunsaturated fatty acids, linoleic acid, alpha-linolenic acid, fiber, plant proteins, arginine, glycine, serine, alanine, folic acid (vitamin B9), vitamin B6, vitamins C, E and beta-carotene. Alternative nutrition groups vs. non-vegetarians have the significantly reduced concentrations of total and LDL-cholesterol, triacylglycerols, insulin as well as values of atherogenic index and insulin resistance. The vegetarian (but not semi-vegetarian) value of homocysteine is significantly increased as a consequence of the significantly reduced and low concentration of vitamin B12. Other two determinants of homocysteine degradation were significantly increased in serum of alternative nutrition groups. The both vegetarian groups have the significantly higher plasma concentrations of antioxidative vitamins and these values are in range of effective free radical disease reduction. The results of favourable values of cardiovascular risk markers and antioxidants document a beneficial effect of vegetarian nutrition in prevention of degenerative age-related diseases.

Keywords: vegetarian nutrition, intake of key nutrient, lipid profile, insulin resistance, antioxidative vitamin

INTRODUCTION

Plants are basal components of the food chain in that they provide all essential nutrients to humans either directly, or indirectly by animal food consumption. The complex diets that provide a nutrition quality (intake of all nutrients) and the nutrient concentrations in the consuming food mixture according with the recommended dietary allowances /RDAs/ (a nutrition quantity) are necessary to support human growth and health **(Craig, Mangels, 2009)**. 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 **(Krajčovičová-Kudláčková et al., 2003, Key et al., 2006, Allen 2008, Bortoli, Cozzolino, 2009, Sanders, 2009, Crowe et al., 2010)**. These facts are connected with certain health benefits or risks in population consuming exclusively or predominantly plant food.

The American dietetic association introduced that appropriately planned vegetarian diets are healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases **(Craig, Mangels, 2009).** Mortality and incidence of coronary disease events are indeed clearly lower in vegetarians **(Fraser, 2009)**. A combined analysis of two cohorts of Adventists in California, older cohorts of British and German vegetarians confirmed this result with a 32% higher coronary heart mortality rate in the non-vegetarians. Prevalence of diabetes is lower in Adventist vegetarians than in non-vegetarians. There is general agreement that red meat consumption increases the risk of colon or colorectal cancer. The idea that higher consumption of fruit and vegetables is associated with reduced all-cause mortality and reduced risks of some cancers has a longer history **(Fraser, 2009, Key et al., 2009).** Although vegetarian diets are healthful and are associated with lower risk of several chronic diseases, different types of vegetarians may not experience the same effects on health.

Several studies demonstrated that the observed deficiencies of nutrients in vegetarians are usually due to poor meal planning **(Leitzmann, 2005).** From view of prevention of health risks of vegan diet (strict vegetarians) or incorrectly planned vegetarian diet is inevitable the consumption of fortified food or nutritional and pharmaceutical supplements to compensation of absent or low-present nutrients in key vegetarian food **(Krajčovičová-Kudláčková et al., 2003, Leitzmann, 2005, Elmadfa, Singer, 2009)**.

The main goal of this study was to assess the selected cardiovascular risk parameters and plasma antioxidative vitamins in correlation to nutritional status in subjects with dominant consumption of plant food in comparison to general population.

SUBJECTS AND METHODOLOGY

Randomly selected 143 apparently healthy non-obese (BMI<30 kg/m^2) non-smoking subjects aged 20-60 years (64 men, 79 women) were divided into three groups. The group of vegetarians (n=41) consumed plant food, dairy products and eggs. Semi-vegetarians (n=44) with addition of white meat consumption (poultry) and fish consumption were the other group of alternative nutrition. The non-vegetarian (control) group consisted of 58 persons of general population on traditional mixed diet (Slovak medical university workers). 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 **(Slovak Food Data Bank, 1999)**.

Blood was sampled after an overnight fasting by a standard procedure. Serum concentrations of total cholesterol, HDL-cholesterol, triacylglycerols and glucose were measured using standard laboratory methods. Values of LDL-cholesterol were calculated in according with the Friedewald formula (LDL-cholesterol = total cholesterol – triacylglycerols/2.2 – HDL-cholesterol). The atherogenic index was expressed as a ratio of total cholesterol and HDL-cholesterol. Serum concentrations of insulin were detected by electro-chemiluminiscence immunoassay (Roche Elecsys Insulin Test). Insulin resistance values IR/HOMA/ (HOMA – homeostasis model assessment) were calculated from fasting concentrations of insulin and glucose: IR /HOMA/ = insulin x glucose/22.5. Plasma concentrations of total homocysteine were measured by HPLC **(Houze et al., 2001).** EDTA was used as an anticoagulant. Serum vitamin B12 and folic acid (vitamin B9) concentrations were determined using Elecsys immunoassay (Roche tests). Serum vitamin B6 values were detected by HPLC method (Chromsystems test). Plasma concentrations of vitamins C and E, and beta-carotene were measured by HPLC **(Lee et al., 1992, Čerhata et al., 1994).**

The intake of vitamins, mineral and trace elements in natural form only was allowed (no supplementation). The study was realized in half of May in duration of six days. The Student t-test was used for final evaluation.

RESULTS AND DISCUSSION

Consumption of animal fat (saturated) raises total and LDL-cholesterol, while polyunsaturated fats (plant sources) have a cholesterol lowering effect. Sufficient intake of food rich in dietary fiber is associated with a lower risk of cardiovascular disease; the soluble and insoluble fibers reduce plasma total and LDL-cholesterol. The hypocholesterolemic effect of fiber is due to an increase in bile-acid binding and fecal sterol excretion. Fermentation of soluble fiber is produced short-chain fatty acids that inhibit hepatic cholesterol synthesis. In addition to unsaturated fat and fiber, there are components of plant food that have the ability to reduce cardiovascular risk (saponins in legumes, plant proteins, antioxidant nutrients, selenium, polyphenols and flavonoids **(Carroll, Kurowska, 1995, Krajcovicova-Kudlackova et al., 2005, Key et al,. 2006, Erkkila et al., 2008).** Previously, the dietary recommendation to reducing cardiovascular risk was aimed at decreasing total and saturated fat intake from meat consumption. Actually, this alone may not be sufficient. The consumption of a variety of plant foods is necessary to favourable modify lipid and lipoprotein profile **(Rajaram, Sabate, 2000)**.

Plant protein consumption decreases cardiovascular risk. For a longer time, experimental studies described, that animal proteins with higher content of essential amino acids in comparison to plant proteins induce an elevation of plasma total and LDL-cholesterol concentrations. Conversely, plant protein consumption can prevent from hypercholesterolemia **(Carroll, Kurowska, 1995).** The higher intake of methionine and lysine from animal proteins has an unfavourable effect on phospholipid metabolism **(Krajcovicova-Kudlackova et al., 2005).**

Vegetarians and semi-vegetarians in present study consumed the significantly reduced amount of cholesterol and saturated fatty acids (Tables 2,3) and on the other hand, they have the higher daily intake of polyunsaturated fatty acids, linoleic acid and alpha-linolenic acid. Tables 2, 3 introduce also that both alternative nutrition groups have significantly higher intake of fiber, plant proteins and significantly reduced consumption of methionine and lysine. Favourable values of cardiovascular risk markers as a consequence of vegetarian nutrition are introduced in Table 4. Concentrations of total cholesterol, LDL-cholesterol and triacylglycerols and values of atherogenic index are in vegetarians and semi-vegetarians vs. non-vegetarians significantly reduced. Vegetarians consume significantly reduced and very low amounts of n-3 fatty acids (eicosapentaenoic and docosahexaenoic acids) (Table 3) as a consequence of no fish consumption. On the other hand, intake of substrate for biosynthesis of n-3 fatty acids – alpha-linolenic acid is significantly higher. There is no evidence of adverse effects on health with lower n-3 fatty acid intake **(Sanders, 2009).**

Subjects with dominantly plant food consumption may be at lower risk of type 2 diabetes occurrence than subjects on traditional mixed diet **(Tonstad et al., 2009).** Complex carbohydrates with low glycemic index are slowly absorbed and thus they have a beneficial effect on glucose control, hyperinsulinemia, insulin resistance and blood lipids **(Reaven, 2000).** Hyperinsulinemia and insulin resistance are critical components of the metabolic syndrome and are the early manifestations of type 2 diabetes. The significantly reduced values of insulin resistance (IR/HOMA) as well as insulin serum concentrations were found in vegetarians and semi-vegetarians vs. non-vegetarians (Table 4). Composition of dietary proteins has the potential to influence the balance of glucagon and insulin activity **(McCarty, 1999).** Plant proteins are higher in non-essential amino acids in comparison to animal protein sources **(Krajcovicova-Kudlackova et al., 2005)**. Glucagon promotes (and insulin inhibits) cAMP-dependent mechanisms that down-regulate lipogenic enzymes and cholesterol synthesis, while up-regulating hepatic LDL receptors. Essential amino acids are relatively more effective for releasing insulin, whereas non-essential amino acids (arginine and pyruvigenic amino acids) are effective in glucagon secretion. The effect of a chronic increase in glucagon activity by regular and sufficient intake of plant proteins means a reduction in lipogenesis, cholesterol and triacylglycerol synthesis. In presented groups of alternative nutrition was found the significantly higher intake of arginine, glycine, serine and alanine (Table 3).

Vegetarians and semi-vegetarians consume significantly more antioxidative vitamins C,E and beta-carotene (Table 2) and plasma concentrations of these vitamins are significantly higher (Table 4) in comparison to non-vegetarians and they are in value range of effective reduction of free radical disease – more than 50 μmol/l for vitamin C, more than 0.4 μmol/l for beta-carotene, more than 30 μmol/l for vitamin E **(Krajčovičová-Kudláčková et al., 2004).** Alternative nutrition subjects have significantly reduced values of lipid peroxidation though they consume higher amounts of polyunsaturated fatty acids with greater oxidation ability. Products of DNA damage are also significantly reduced in vegetarians vs. non-vegetarians **(Krajčovičová-Kudláčková et al., 2008)**. This fact is a consequence of the better antioxidative status of vegetarian nutrition. Damage of molecules during the oxidative stress has been implicated in the pathogenesis of chronic age-related diseases such as cancer and cardiovascular disease. Improved antioxidant status helps to inhibit oxidative damage, and thus can prevent pathological changes. The endogenous antioxidants are inadequate prevent damage completely, so that diet-derived antioxidants have an important role in lowering free radical disease risk.

The favourable values of lipid and non-lipid cardiovascular risk markers in vegetarians and semi-vegetarians as well as the high plasma antioxidative vitamin concentrations document a beneficial effect of vegetarian nutrition in prevention and treatment of degenerative age-related diseases. The exception from markers is atherogenic homocysteine (HCy). During the last decade, several observational studies about HCy as a predictor for atherosclerosis risk showed that the overall risk for vascular disease is small **(Kaul et al., 2006).** In prospective longitudinal studies, a weak association between HCy and atherothrombotic vascular disease was reported, compared to retrospective case-control and cross-sectional studies with stronger association.

Vegetarians and mainly vegans (strict vegetarians) suffer from mild hyperhomocysteinemia as a consequence of lower vitamin B12 intake and lower serum concentrations **(Krajcovicova-Kudlackova et al., 2007, Allen, 2008, Elmadfa, Singer, 2009).** Vitamin B12 is absented in plant food. In evaluated group of vegetarians was recorded significantly higher concentration of total HCy and significantly reduced intake and serum concentration of vitamin B12 (Tables 2,4). Hyperhomocysteinemia (>15 μmol/l) was recorded in 17 % of subjects. Semi-vegetarian HCy values are similar with those in non-vegetarians and occurrence of mild hyperhomocysteinemia is low (5 % vs. 3 % in non-vegetarians). Semi-vegetarians consume the animal food in wider range and thus they have the significantly higher intake of vitamin B12 than vegetarians (Table 2). The second and third from nutritional determinants of HCy metabolism (folic acid and vitamin B6) are significantly higher in vegetarians – their intake and serum concentrations (Tables 2,4). In spite of generally weak atherogenic activity of Hcy the findings of higher Hcy values in vegetarians indicate the recommendation of low fat dairy product consumption in greater variety.

Table 1. Characteristic of groups

	Non-vegetarians	Semi-vegetarians	Vegetarians
n (m+w)	58 (26+32)	44 (20+24)	41 (18+23)
Average age (y)	40.0±1.7	40.8±1.8	38.8±2.0
BMI (kg/m^2)	24.3±0.4	22.7±0.3 **	22.2±0.4 ***
range	19.3-28.5	18.9-25.7	18.2-25.8
>25	12 %	5 %	5 %
Duration of vegetar. (y)	-	9.8±0.7	11.0±0.7
Consumption of (times weekly)			
red meat	3.04±0.15	-	-
white meat (poultry)	2.15±0.13	3.25±0.17	-
fish	1.04±0.10	1.17±0.08	-

The results are expressed as mean±SEM ** P<0.01 *** P<0.001

Table 2. Daily intake of selected nutrients

	Non-vegetarians	Semi-vegetarians	Vegetarians
Total proteins (g)	92.3±2.6	91.1±4.2	76.6±3.3 ***
Animal proteins (g)	49.4±2.0	29.6±2.1 ***	14.8±1.8 ***
Plant proteins (g)	42.9±1.6	61.5±3.8 ***	61.8±4.0 ***
Fiber (g)	26.6±1.2	43.4±2.4 ***	44.3±2.9 ***
Total fat (g)	90.4±1.8	87.3±2.8	74.6±3.2 ***
Cholesterol (mg)	438±25	123±11 ***	60.5±7.7 ***
Vitamin B12 (μg)	15.1±0.9	14.2±2.3	7.2±1.9 ***
Folic acid (μg)	297±15	563±35 ***	616±51 ***
Vitamin B6 (mg)	1.78±0.09	2.93±0.24 **	2.92±0.19 ***
Vitamin C (mg)	81.3±2.2	171±16 ***	165±12 ***
Vitamin E (mg)	12.4±0.4	16.2±1.5 **	14.9±1.1 *
beta-carotene (mg)	3.95±0.20	6.45±0.44 ***	6.89±0.72 ***

The results are expressed as mean±SEM * P<0.05 ** P<0.01 *** P<0.001

Table 3. Daily intake of selected key amino and fatty acids

	Non-vegetarians	Semi-vegetarians	Vegetarians
Amino acids			
Methionine (g)	1.68±0.08	1.26±0.06 **	1.03±0.05 ***
Lysine (g)	5.21±0.25	4.11±0.21 **	3.12±0.20 ***
Arginine (g)	4.29±0.21	5.44±0.23 **	5.64±0.22 **
Glycine (g)	3.56±0.13	4.79±0.16 ***	5.00±0.14 ***
Serine (g)	4.01±0.18	4.46±0.16 *	4.76±0.16 **
Alanine (g)	3.28±0.11	4.73±0.17 ***	4.90±0.18 ***
Fatty acids			
Myristic (g)	2.66±0.17	1.82±0.11 **	1.56±0.10 ***
Palmitic (g)	15.53±0.69	12.46±0.47 **	8.94±0.59 ***
Stearic (g)	9.03±0.43	6.48±0.39 **	4.20±0.35 ***
Saturated (g)	33.82±2.12	24.59±1.84 **	18.04±1.644 ***
Oleic (g)	28.70±0.81	28.23±1.43	27.10±1.72
Monounsaturated (g)	30.94±0.82	29.62±1.67	27.98±1.60
Linoleic (g)	19.06±0.71	24.11±1.22 **	23.28±1.12 **
alpha-linolenic (g)	1.64±0.09	1.98±0.17 *	2.06±0.18 *
Eicosapentaenoic (mg)	37.20±4.11	31.91±2.23	0.42±0.19 ***
Docosahexaenoic (mg)	38.47±3.67	33.02±2.45	0.47±0.14 ***
Polyunsaturated (g)	21.38±0.56	26.35±1.26 **	26.98±0.92 ***

* P<0.05 ** P<0.01 *** P<0.001
Saturated: butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic acids
Monounsaturated: palmitoleic, oleic acids
Polyunsaturated: linoleic, alpha-linolenic, arachidonic, eicosapentaenoic, docosahexaenoic acids

Table 4. Lipid profile, insulin resistance, concentrations of homocysteine and its determinants and antioxidative vitamin concentrations

	Non-vegetarians	Semi-vegetarians	Vegetarians
Total cholesterol (mmol/l)	5.34±0.09	4.78±0.08 **	4.56±0.14 ***
HDL-cholesterol (mmol/l)	1.48±0.05	1.49±0.05	1.46±0.04
LDL-cholesterol (mmol/l)	3.17±0.11	2.66±0.05 **	2.52±0.12 **
Triacylglycerols (mmol/l)	1.58±0.05	1.36±0.05 **	1.31±0.09 **
Atherogenic index	3.87±0.12	3.36±0.12 **	3.26±0.12 ***
Glucose (mmol/l)	5.10±0.07	4.94±0.06	4.67±0.06 **
Insulin (mU/l)	8.22±0.39	6.31±0.30 **	6.01±0.37 ***
IR (HOMA)	1.87±0.10	1.42±0.08 **	1.22±0.08 ***
Homocysteine (μmol/l)	9.69±0.33	10.8±0.4	12.6±0.6 ***
Vitamin B12 (pmol/l)	270±10	242±13	187±13 ***
Folic acid (nmol/l)	17.6±0.9	23.3±0.7 ***	25.6±0.9 ***
Vitamin B6 (μg/l)	4.80±0.29	6.93±0.36 **	7.26±0.41 ***
Vitamin C (μmol/l)	39.8±1.7	55.7±1.4 ***	58.0±1.8 ***
Vitamin E (μmol/l)	26.2±0.9	31.4±0.8 **	30.6±0.8 **
beta-carotene (μmol/l)	0.295±0.012	0.456±0.015 ***	0.476±0.016 ***

** P<0.01 *** P<0.001

CONCLUSION

Subjects with dominant plant food consumption vs. non-vegetarians consume the significantly reduced amounts of cholesterol, saturated fatty acids, methionine, lysine and on the other hand, they have the significantly higher daily intake of protective food (polyunsaturated fatty acids, linoleic acid, alpha-linolenic acid, fiber, plant proteins – mainly amino acids arginine, glycine, serine, alanine, vitamins B6, B9 and antioxidative vitamins). This protective nutritional habit reflect in the favourable values of lipid and non-lipid cardiovascular risk markers in vegetarians and semi-vegetarians as well as the high plasma antioxidative vitamin concentrations with effective reduction of oxidative stress and thus with preventive effect against free radical diseases. Possible higher values of weakly atherogenic homocysteine as a consequence of vitamin B12 deficiency can be prevented by sufficient consumption of low fat animal food of greater variety (or vitamin supplements in strict vegetarians). The described findings document a protective effect of vegetarian nutrition against degenerative age-related diseases.

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