



HEALTH EFFECTS OF PLANT FOOD AND POSSIBILITY OF REDUCTION OF HEALTH RISK

Martina Valachovicova¹, Jana Pribojova¹, Hana Padysakova¹, Dana Farkasova¹ and Shubhada Bopegamage²

¹Faculty of Nursing and Health Professional Studies Slovak Medical University in Bratislava, Slovakia

²Enterovirus Laboratory, Institute of Microbiology, Faculty of Medical Slovak Medical University in Bratislava, Slovakia

ARTICLE INFO

Article History:

Received 12th May, 2019

Received in revised form 23rd June, 2019

Accepted 7th July, 2019

Published online 28th August, 2019

Key words:

Nutrition, plant food, prevention in disease

ABSTRACT

Plants are fundamental components of the food chain in that they provide all essential nutrients to humans by animal food consumption. The vegetarian diets are rich in antioxidants, folic acid, vitamin D, iodine, carbohydrates, n-6 fatty acids, dietary fiber, magnesium, zinc, and relatively low in some essential amino acids, saturated fat, long-chain n-3 fatty acids. These facts are connected with certain health benefits or risks in population consuming exclusively or predominantly plant food. The study focused on monitoring the intake of selected nutrients and the determination of selected parameters to point out the possibilities of prevention in cardiovascular disease and diabetes.

Copyright©2019 *Martina Valachovicova et al.* This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Vitamins B12, D and n-3 fatty acids are not contained in plant food. Contents of methionine, iodine and carnitine in plant food sources are significantly reduced in comparison with animal sources. Iron, calcium and zinc absorption is inhibited by components of plant foods. Iron deficiency causes an inhibited synthesis of n-3 fatty acids. These facts relate to certain health risks in population consuming exclusively or predominantly plant food. From view of prevention of risks the application of food additives or pharmacological preparations to compensate the absence of nutrients in vegans – “strict vegetarians” or sufficient consumption of low-fat animal food in lacto-vegetarians and lacto-ovo-vegetarians is inevitable (Grusak and DellaPenna, 1999).

On the other hand, long-term nutritional surveys have shown an inverse relationship between diet with a predominance mostly a plant composition and the incidence of cancer, cardiovascular disease and the total mortality (Key *et al.*, 1999). A high and importantly, regular consumption of fruit and vegetables, dark or whole grain products, grain sprouts, pulses, plant oils and oil seeds rich in minerals, antioxidant vitamins, trace elements, flavonoids, complex carbohydrates, fiber, mono- and polyunsaturated fatty acids and nutrients together with an otherwise healthy lifestyle protect against degenerative disease.

The nutrient and non-nutrient components of plant foods, through a variety of mechanisms, are known to alter the risk of chronic diseases (Rajaram and Sabaté, 2000).

SUBJECTS AND METHODS

Randomly selected 1927 women and men apparently healthy non-obese (BMI<30 kg/m²) non-smoking subjects aged 20-65 years were divided into three groups. The group of vegetarians consumed plant food, dairy products and eggs. Semi-vegetarians with addition of white meat consumption (poultry) and fish consumption were the other group of alternative nutrition. The control group consisted of persons of general population on traditional mixed diet. The group characteristics are introduced in Table 1. The calculation of daily intake of nutrients was based on the data from standardized and validated dietary questionnaires. The questionnaire contained 159 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-

*Corresponding author: **Martina Valachovicova**

Faculty of Nursing and Health Professional Studies Slovak Medical University in Bratislava, Slovakia

cholesterol). The atherogenic index was expressed as a ratio of total cholesterol and HDL-cholesterol. Serum concentrations of insulin were detected by electro-chemiluminescence 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, E and beta-carotene were measured by HPLC (Lee *et al.*, 1992 and 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 the spring. The Student t-test was used for final evaluation.

RESULTS AND DISCUSSION

Our aim was to evaluate the health status of a randomly selected group, which was divided into three groups according to eating habits. It is known that nutrition affects human health. Therefore, the study focused on monitoring the intake of selected nutrients and the determination of selected parameters to point out the possibilities of prevention in cardiovascular disease and diabetes.

In comparison with non-vegetarians, vegetarians in the Oxford vegetarian study had a 24 % reduction in mortality from ischemic heart disease IHD (death rate ratio DRR 0.76) (Key *et al.*, 1999). Whereas the non-vegetarians were divided into regular meat eaters (meat consumption at least once per week) and semi-vegetarians (fish consumption or meat consumption less than once per week), the IHD DRRs, when compared with regular meat eaters, were 0.78 in semi-vegetarians and 0.66 in vegetarians. The reduction in IHD among vegetarians is partially due to a low intake of saturated fat and cholesterol (Krajčovičová-Kudláčková *et al.*, 2000). Consumption of saturated fat (animal sources) has been found to be associated with hypercholesterolemia, while polyunsaturated fats (plant sources) were reported to have a cholesterol lowering effect. The hypolipidemic effect of monoene fatty acids (oleic acid – olive oil, rapeseed oil, sesame oil, hazelnuts, almonds) was discovered only quite recently. Consumption of cheese, eggs, total animal fat and dietary cholesterol were each strongly associated with IHD mortality as compared to those who ate relatively little of these foods. The DRRs are these foods were 2.47 for cheese, 2.68 for eggs, 3.29 for total animal fat and 3.53 for dietary cholesterol. The pooled analysis of five prospective studies also presented mortality data from cerebrovascular disease (Lampe, 1999). Nitrates enter the diet mainly through vegetables and are considered contaminants. A recent study suggests that nitrates lower systolic blood pressure, demonstrating that their intake would reduce the incidence of hypertension and cardiovascular mortality. Vegetarians have low systolic blood pressure and high nitrate intake from vegetables. Therefore, the authors of the study are recommended to include nitrates as a nutrient that is necessary for health (Ashworth and Bescos, 2017).

Dyslipidemia, hypertension and smoking are the main risk factors of atherosclerosis. The presence of obesity can increase

this risk. Vegetarians have a body mass index about 1-2 kg/m² lower than non-vegetarians (Table 1).

Table 1 Characteristic of groups and daily intake of selected nutrients.

	Non-vegetarians Semi-vegetarians	Vegetarians
n (m+w)	641	632
Average age (y)	40.0±1.7	40.8±1.8
BMI (kg/m ²)	24.3±0.4	22.7±0.3 **
range	19.3-28.5	18.9-25.7
>25	12 %	4 %
Duration of vegetar. (y)	-	19,1±0.7
Consumption of (times weekly)		22.0±0.7
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
Total proteins (g)	92.3±2.6	91.1±4.2
Animal proteins (g)	49.4±2.0	29.6±2.1 ***
Plant proteins (g)	42.9±1.6	61.5±3.8 ***
Fiber (g)	26.6±1.2	43.4±2.4 ***
Total fat (g)	90.4±1.8	87.3±2.8
Cholesterol (mg)	438±25	123±11 ***
Vitamin B12 (µg)	15.1±0.9	14.2±2.3
Folic acid (µg)	297±15	563±35 ***
Vitamin B6 (mg)	1.78±0.09	2.93±0.24 **
Vitamin C (mg)	81.3±2.2	171±16 ***
Vitamin E (mg)	12.4±0.4	16.2±1.5 **
beta-carotene (mg)	3.95±0.20	6.45±0.44 ***

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

Vegetarians are non-smokers and they have a normal blood pressure, or the incidence of hypertension is low (Appleby *et al.* 2002). In our repeated studies were found the significantly lower risk factors for atherosclerosis (total cholesterol, LDL-cholesterol, atherogenic index, triacylglycerols, saturated fatty acids) and the significantly higher values of parameters with antisclerotic properties (HDL-cholesterol, mono- and polyunsaturated fatty acids, linoleic acid, α-linolenic acid, vitamin E/cholesterol, vitamin E/triacylglycerols) (Table 2,3).

Table 2 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 ***

The results are expressed as mean±SEM * 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

Vegetarians have significantly reduced values of high sensitivity C-reactive protein as a consequence of higher consumption of fruits and vegetables (Szeto *et al.*, 2004),

which are rich sources of dietary salicylates and perhaps other anti-inflammatory compounds (Lawrence *et al.*, 2003).

Table 3 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 **	1.02±0.08 ***
> 3,8	7%	2%	0%
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 ***
Vitamin E/cholesterol (µmol/l)	4.91 ± 10	6.57±10 **	6.71±5.71 **
Vitamin E/triacylglycerols (µmol/l)	16.58±18	23.09±16**	23.36±8.89**

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

The term folate is used as a generic name for group of compounds based on the folic acid structure. Folate or vitamin B9 and vitamin B12 are two of the 13 essential vitamins. Vitamin B9 and B12 enter the body through diet or supplementation. 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. Vitamin B12 deficiency is one of the risk factors of alternative nutrition. Vitamin deficiency can lead to several adverse health consequences: folate trapping in the methylation cycle and subsequent impaired DNA biosynthesis, pernicious anemia hematologically like that caused by folate deficiency, elevated atherogenic blood homocysteine and neural tube defects (Varela-Moreiras *et al.*, 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. Mean serum vitamin B12 was highest among general population - 281 pmol/l, intermediate among vegetarians - 182 pmol/l and lowest among vegans - 122 pmol/l (Gilsing *et al.*, 2010). Despite 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. Hcy was shown to be an independent risk factor cardiovascular disease, which includes peripheral vascular disease, coronary artery disease, cerebrovascular disease and venous thrombosis. It has long been reported that vitamin B9 and B12 have a positive effect on vascular diseases, related to the reduction in homocysteine. Thus, it is possible that hyperhomocysteinemia is not a common primary cause of atherothrombotic disorders in the general population, but rather a marker of systemic or endothelial oxidative stress that is a major mediator of these disorders (Hoffman, 2011). It has been suggested that hyperhomocysteinemia may promote the production of hydroxyl radicals which are the initiators of lipid peroxidation process. Despite high incidence of hyperhomocysteinemia in vegetarians the risk increased products of lipid peroxidation

were found in 8 % of subjects only because of a better antioxidative status in comparison to subjects of general population. Research has shown the beneficial effects of folates not related to homocysteine decline, suggesting independent properties. One possible mechanism may be free radical scavenging and antioxidant activity. Free radicals play an important role in the oxidative stress leading to many disorders and diseases. In particular, reduced folic acid derivatives have been found to act directly and indirectly to induce antioxidant effects. Folates interact with the endothelial enzyme NO synthase and, exert effects on the cofactor bioavailability of NO and thus, on peroxynitrite formation (Stanger and Wonisch, 2012). Vitamin B12 is not present in plant sources. This fact may partly weaken the positive effects of vegetarian nutrition in prevention of cardiovascular disease. This can be easily reparable by increase of consumption of dairy products with low fat content or by consumption of food additives and by use of pharmacological preparations with vitamin B12 content.

Subjects consuming predominantly plant food may be at lower risk of dying from diabetes than omnivores (Snowdon and Philips, 1985). 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. The important role in regulation of carbohydrate and lipid metabolism plays a type of fiber. Diets from soluble fiber sources (oat, barley, legumes) had much more marked effect compared with diet containing insoluble fiber. High fiber foods such as beans deliver more bulk with less energy, take longer to eat and increase satiety. This indirectly helps reduce the incidence of obesity, a major risk factor for type 2 diabetes. Hyperinsulinemia and insulin resistance are critical components of the metabolic syndrome which is associated with abdominal obesity and are the early manifestations of type 2 diabetes. The insulin resistance syndrome is composed of risk factors for cardiovascular disease including insulin resistance with hyperinsulinemia, atherogenic dyslipidemia, hypertension, and abdominal obesity. Diets rich in carbohydrates with a low glycemic index (whole grains, beans, legumes) and with a high fiber content produce slow carbohydrate absorption and thus flat postprandial rises in blood glucose, minimal postprandial insulin secretion and maintenance of insulin sensitivity. There was a documented decrease of insulin resistance (IR/HOMA) from 3.44 to 2.16 in patients with coronary artery disease consuming whole grain and legume food for 16 weeks (Yang *et al.*, 2001). 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. In our study on healthy vegetarians with normal weight aged 19-64 years was recorded the average IR (HOMA) value 1,02 (vs. 1.87 in non-vegetarian group, P<0.001) and the values were independent of age (vs. significant age dependence in non-vegetarians) (Table 3).

The composition of dietary proteins has the potential to influence the balance of glucagon and insulin activity. Soy protein, as well as many other plant proteins are higher in non-essential amino acids in comparison to animal protein sources. Glucagon promotes (and insulin inhibits) cAMP-dependent mechanisms that down-regulate lipogenic enzymes and cholesterol synthesis, while up-regulating hepatic LDL receptors (McCarty, 1999). Vegetarians had a significantly

higher intake of arginine, glycine and serine. When dietary protein is relatively high in non-essential amino acids, the down regulation of insulin and up-regulation of glucagon is a logical consequence. The effect of a chronic increase in glucagon activity means a reduction in de novo lipogenesis, decreasing fat storage, a reduction in cholesterol synthesis and in circulating LDL cholesterol, a reduction in triacylglycerol synthesis.

CONCLUSION

Alternative eating population 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 cardiovascular disease and diabetes.

Acknowledgment

This publication was created by realization of research project “Health effects of plant food and the possibility of reduction of health risks”, ITMS code: 26240220022.

References

1. Grusak, M.A., DellaPenna, D. 1999. Improving the nutrient composition of plants to enhance human nutrition and health. *Annu.Rev.Plant Physiol.Plant Mol.Biol.*, 50:133-161.
2. Key,T.J., Davey,G.K., Appleby,P.N. 1999. Health benefits of a vegetarian diet. *Proc. Nutr. Soc.*, 58:271-275.
3. Rajaram,S., Sabaté,J. 2000. Health benefits of a vegetarian diet. *Nutr.*, 16: 531-533.
4. Slovak Food Data Bank. 1999. Food Research Institute, Bratislava.
5. Houze, P., Gamra, S., Madelaine, I., Bousquet, B., Gourmel, B. 2001. Simultaneous determination of total plasma glutathione, homocysteine, cysteinylglycine, and methionine by high performance liquid chromatography with electrochemical detection. *J. Clin. Lab. Anal.*, 15: 144-153.
6. Lee, B.L., Chua, S.C., Ong, H.Y., Ong, C.N. 1992. High performance chromatographic method for routine determination of vitamins A and E, and beta-carotene in plasma. *J. Chromatogr.*, 581: 41-43.
7. Čerhata, D., Bauerová, A., Ginter, E. 1994. Ascorbic acid determination in serum by high performance liquid chromatography and its correlation with spectrophotometric determination. *Ces. Slov. Farm.*, 43: 166-168.
8. Key,T.J., Fraser,G.E., Thorogood,M. 1999. Mortality in vegetarians and nonvegetarians, detailed findings from a collaborative analysis of 5 prospective studies. *Am. J. Clin. Nutr.*, 70: 516-520.
9. Krajčovičová-Kudláčková,M., Blažiček,P., Babinská,K. *et al.* 2000. Traditional and alternative nutrition – levels of homocysteine and lipid parameters in adults. *Scand. J. Clin. Lab. Invest.*, 60: 657-664.
10. Lampe, J.W: Health effects of vegetables and fruits. *Am. J. Clin. Nutr.*, 70,1999,475.
11. Ashworth, A., Bescos, R. 2017. Dietary nitrate and blood pressure: Evolution of a new nutrient? *Nutr Res Rev*, 30: 208-219.
12. Appleby, P.N., Davey,G.K., Key,T.J. 2002. Hypertension and blood pressure among meat eaters, fish eaters, vegetarians and vegans in EPIC-Oxford. *Publ.Health Nutr.*, 5: 645-654.
13. Richter, V., Rassoul, F., Hentschel, B. *et al.* 2004. Age dependence of lipid parameters in the general population and vegetarians. *Z. Gerontol. Geriat.*, 37: 207-213.
14. Szeto, Y.T., Kwok, T.C., Benzie, I.F. 2004. Effect of a long-term vegetarian diet on biomarkers of antioxidant status and cardiovascular disease risk. *Nutrition*, 20:863-866.
15. Lawrence, J.R., Peter, R., Baxter, G.J., Robson, J., Graham, A.B., Paterson, J.R. 2003. Urinary excretion of salicylic and salicylic acids by non-vegetarians, vegetarians and patients taking low dose aspirin. *J.Clin.Pathol.*, 56: 649-650.
16. Varela-Moreiras, G., Murphy, M.M., Scott J.M. 2009. Cobalamin, folic acid, and acid and homocysteine. *Nutr Rev.*,67: 69-72.
17. Gilsing, A.M.J., Crowe, F.L., Lloyd-Wright, Z., Sanders, T.A., Appleby, P.N., Allen, N.E., Key, T.E. 2010. Serum concentrations of vitamin B12 and folate in British male omnivores, vegetarians and vegans, results from a cross-sectional analysis of the EPIC-Oxford cohort study. *Eur J Clin Nutr.*, 64: 933-939.
18. Hoffman, M. 2011. Hypothesis – hyperhomocysteinemia is an indicator of oxidant stress. *Med Hypotheses.*, 77: 1088-1093.
19. Stanger, O., Wonisch, W. 2012. Enzymatic and non-enzymatic antioxidative effects of folic acid and its reduced derivatives. *Subcell Biochem.*, 56:131-151.
20. Snowdon, D.A., Phillips, R.L. 1985. Does a vegetarian diet reduce the occurrence of diabetes? *Am. J. Publ. Health*, 75: 507-511.
21. Jang,Y., Lee, J.H., Kim, O.Y., Park, H.Y., Lee, S.Y. 2001. Consumption of whole grain and legume powder reduces insulin demand, lipid peroxidation, and plasma homocysteine concentration in patients with coronary artery disease. *Arterioscl. Thromb. Vasc. Biol.*, 21: 2065-2071.
22. Zavaroni,I., Bonini,L., Gasparini,P. *et al.* 1999. Hyperinsulinemia in a normal population as a predictor of non-insulin dependent diabetes mellitus, hypertension, and coronary heart disease, the Barilla factory revisited. *Metabolism*, 48: 984-994.