JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2005, 56, Suppl 1, 69–78
www.jpp.krakow.pl

Review article

M. NARUSZEWICZ, M. KOZŁOWSKA-WOJCIECHOWSKA

POTENTIAL PARAPHARMACEUTICALS IN THE TRADITIONAL POLISH DIET

National Food and Nutrition Institute, Warsaw, Poland

The basic parapharmaceuticals in the Polish diet include natural anti-oxidants - bioflavonoids found in berry fruit. They were proven to have the ability to regulate genetic transcription and increase the synthesis of nitric oxide which counteracts dysfunction of the vascular endothelium. They also display anti-oxidant action through the inhibiting effect on cyclooxygenase - COX-2, and increase the level of adiponectin. We have also more and more proof of the important biological role of short-chained fatty acids formed as a result of fermentation of fibre by probiotic bacteria. Through their effects on peroxisome proliferators activated receptors (PPAR), butyric and propionic acids may reduce the expression of adhesion molecules and exert anti-inflammatory action both in the gastrointestinal tract as well as systemically.

Key words: *bioflavonoids, fibre, probiotic bacteria, coronary artery disease, cancer*

INTRODUCTION

The achievements of contemporary medicine allowed significant prolongation of life of people practically all around the world. However, the increasing growth of metabolic civilisation diseases associated with the obesity epidemic forces us to search for new forms of prevention and treatment of arterial hypertension, hyperlipidaemia, and diabetes. These diseases are currently the most important risk factors for cardiovascular disorders which still remain the main cause of premature death also in Poland. Although with the use of new generations of drugs, i.e. statins or angiotensin converting enzyme inhibitors, ischaemic heart disease mortality may be reduced on average by 30 to 40%, such reduction is

associated with huge costs and side effects related to drug toxicity. This is particularly noticeable in the case of widely applied polypharmacotherapy. On the other hand, we now possess the spectacular results of the Lyon Diet Heart Study where lowering of the mortality risk by 70% was noted already after 27 months of use of the Mediterranean diet (1). This study allows one to draw a simple conclusion that a low-fat diet based on vegetables, leguminous fruit and seeds, nuts, and vegetable oil with the addition of alpha-linolic acid is the most beneficial from the point of view of inhibition of progress of arterial atherosclerosis.

The traditional Polish diet, particularly of the poorer social layers, is not as well known in the world as the French, Italian, or Chinese one, but with some of its health values it may play a significant role in the prophylaxis of both cardiovascular disorders as well as cancer. Berry fruit with a high content of bioflavonoids, groats and grits which are a source of soluble fractions of fibre, or fermentation products containing probiotic bacteria should be especially noted here. These products can be a valuable source of functional food of special health importance.

Berry fruit in the Polish diet as a source of anti-oxidative and anti-inflammatory compounds

A characteristic feature of the diet of North European countries is eating - in various forms - berry fruit rich in polyphenols including anthocyanins, flavonols, phenolic acids, and procyanidins. The majority of these compounds have potent anti-oxidative properties, but lately it has been underlined that anthocyanins might play a special biological role. Their activity regulating the expression of certain genes and/or transcription factors is pointed out. It should be noted that the most frequently occurring anthocyanins are cyanidine derivatives - conjugates with sugars, i.e. galactose, glucose, arabinose, and xylose.

An important element of evaluation of the biological role of anthocyanins as well as of (-) - epicatechines and (+) - catechines which accompany anthocyanins was the observation that these compounds are absorbed from the gastrointestinal tract into the general circulation. As a consequence, they display a significant effect on the formation of the anti-oxidative barrier at the level of cells and tissues, being at the same time more effective than vitamins E and C. In fact, the major clinical studies conducted so far in large populations of patients with ischaemic heart disease have not confirmed the effectiveness of vitamin E in inhibition of progression of atherosclerosis and lowering mortality due to ischaemic heart disease. On the other hand, the benefits of the Mediterranean diet rich in natural anti-oxidants in vascular disease prevention are unquestionable (2). Many experts point here to the significant role of flavonoids contained in red wine in inhibition of oxidative modifications of the LDL fraction (3,4). Indeed, so-called OX-LDL not only accelerates the formation of foam cells in the

atherosclerotic vascular bed, but also contributes to the dysfunction of the vascular endothelium (5).

Vast evidence is available from experimental studies indicating that OX-LDL in endothelial cells inhibits the activity of nitric oxide synthase, which lowers in turn the relaxing activity of the vascular wall. Recently, several studies appeared which documented that cyanidine glucosides contained in blackberries had protective action on endothelial cells through the inhibition of formation of peroxynitrite which contributed to the destructive generation of nitric oxide (6). It is particularly important when the cell becomes deficient in L-arginine, because then nitric oxide synthase commences the generation of cytotoxic quantities of peroxynitrate. The above mechanism may be especially important for the pathogenesis of endothelial dysfunction in inhabitants of the North European countries whose diet has a low content of L-arginine.

Apart from the action on the formation of cytotoxic radicals, cyanidine glucosides may also selectively bind to MAP kinases, through which they exert an effect on phosphorylation processes and gene expression. Therefore it may be hypothesised that they have potential properties of signalling molecules. Japanese researchers proved that cyanidin-3-glucoside, contained mainly in blackberries, enhances the expression of nitric oxide synthase through the Src-ERK1/2 signalling pathway (7). Moreover, it is possible that the activation of this signalling pathway may counteract the processes of apoptosis of endothelial cells, potentially preventing atherosclerotic plaque rupture and reducing the risk of coronary events. The possibility of influencing the apoptotic processes by supplementing the diet in compounds contained in berry fruit is extremely attractive from the point of view of the spreading epidemics of cardiovascular insufficiency.

The compounds found in berries - (-) epigallocatechin together with quertin - have specific anti-apoptotic properties through inhibition of expression of bax protein and the cleavage and activation of caspase-3 (8).

In view of the increasing concern about the adverse effects of non-steroidal anti-inflammatory drugs - COX-2 inhibitors, and in particular about their effects increasing the risk of coronary events, natural compounds affecting the activity of cyclooxygenase enjoy more and more interest. For a long time it has been indicated that anthocyanins present also in cherries inhibit COX-2 activity in the manner similar to ibuprofen or naproxen (9).

The mechanism of anti-inflammatory action of anthocyanins has not been fully elucidated but they are thought to inhibit prostaglandin synthesis.

It should be underlined that anthocyans seem to be safer in their action than other C0X-2 inhibitors, because they inhibit oxidation of the LDL fraction through their direct antioxidant activity. In contrast, non-steroidal anti-inflammatory drugs do not have such properties.

A new and attractive issue in the potential use of cyanidine glucosides in the prophylaxis of metabolic disorders is their effect on increased expression of the

adiponectin gene (10). This gene is located on chromosome 3, and thus near the genes of predisposition to diabetes and ischaemic heart disease. Adiponectin is an animal protein with the structure similar to collagen produced mainly by the adipose tissue. In physiological concentrations, it increases beta-oxidation of fatty acids and insulin sensitivity of tissues (11). It displays also cytoprotective action through the mechanism of inhibiting the migration of smooth muscle cells and lowering the expression of adhesion molecules. The decrease in the adiponectin level was found to be characteristic for obesity and to contribute to the endothelial function impairment.

A significant number of clinical studies confirm that hypoadiponectinemia is associated with advanced vascular atherosclerosis and ischaemic heart disease (12). A reverse correlation between the concentration of the C-reactive protein (CRP) and the level of adiponectin in the subcutaneous adipose tissue in persons with angiographically confirmed atherosclerosis was also observed. This may lead to the conclusion that adiponectin displays also an anti-inflammatory action.

The fact that cyanidine and its glucoside increase adiponectin secretion in the white adipose tissue through stimulation of AMP-activated protein kinase in rats on a diet supplemented with anthocyanins suggests the possibility of the potential use of these compounds in the treatment of the metabolic syndrome and endothelial dysfunction. This is all the more possible as anthocyanins seem to have a metabolic action similar to the widely used drug, metformin. At the same time, a question arises to what extent the observations made at the cellular level and/or on laboratory animals apply to the affected patient.

Unfortunately, a very limited number of the respective clinical studies exist which consistently with the medicines assessment standards would deal with the precise role of bioflavonoids in the prophylaxis and treatment of ischaemic heart disease. Nevertheless, a research group from Israel has recently published several studies indicating the positive clinical effects of bioflavonoids contained in pomegranate juice, also rich in antocyans (13). Particular attention should be paid also to the most recent publication of this group which found that already after 1 year of supplementation of the diet with pomegranate juice, the progression of atherosclerotic lesions in cervical vessels decreased by 30% in comparison with placebo in patients aged 65 to 75. This was accompanied by a decrease in the systolic pressure and LDL oxidation (14).

Also our studies with the use of the preparation obtained from chokeberry fruit containing over 20% of anthocyanins in patients with a history of myocardial infarction have confirmed the potent therapeutic action of these bioflavonoids (15). Apart from the significant reduction in systolic and diastolic pressure, we have also found lowered concentrations of C-reactive protein and increased plasma adiponectin. Such effect has not been observed in the placebo group.

It should be emphasised that all patients receiving the chokeberry extract have been routinely treated with statins, ACE inhibitors, and aspirin, and thus the

positive effects of supplementation observed were independent of the treatment used for at least 6 months.

Kasha (grits and groats) and linen as a source of anti-atherosclerotic and anticancer compounds

Kasha (grits and groats, e.g. roasted buckwheat, barley, pearl barley, and hulled millet) has always played an important role in the Polish culinary tradition, and its health values cannot be overestimated. The nutritional value of kasha in comparison with the worldwide-popular rice is much higher. And thus, kasha contains much more fibre, thiamine, niacin as well as potassium and magnesium than rice. Nevertheless, due to the fact that the Polish population is characterised by high prevalence of environmental hypercholesterolaemia (above 70% of adults), the possibility of using the fibre from kasha to lower the level of the atherogenic LDL fraction becomes an issue of an utmost importance (16,17).

Due to the physiological action of fibre on the human body, this substance has been classified into water-soluble and -insoluble fractions. Water-soluble fibre includes pectins and hemicelluloses with various ramification level, extracted from neutral gum solutions (beta-glucans and mucilages), undergoing bacterial fermentation in the gastrointestinal tract, and displaying an effect on metabolic transformations of carbohydrates and fats.

On the other hand, the insoluble fraction consists of cellulose, hemicelluloses extracted from acid solutions, and lignins, which affect the function of the intestines - both the small bowel as well as the large bowel, particularly the colon. The role of this fraction comes down to acceleration of the intestinal transit and to the absorption and binding of some substances, also toxic ones, from the gastrointestinal tract. As shown by a meta-analysis of numerous epidemiological studies, strict correlation exists between the dietary content of fibre and the incidence of colorectal carcinoma (18).

The role of the fibre is not only to improve the function of the large bowel through transit time shortening, increase in the faecal material volume, and regulate the defecation process. It also plays an important role in reduction of the cholesterol level in the blood serum. Susceptibility of fibre to fermentation processes under the influence of the intestinal microflora is determinant for the inhibition of the hepatic cholesterol synthesis. This results from the formation of short-chain fatty acids such as acetic, propionic, butyric, and capronic acids. This ability is displayed above all by pectin and guar gum. Cellulose which does not have the ability to bind biliary acids is totally resistant to fermentation processes. This ability to bind biliary acids, which are a vehicle for cholesterol, leads to an inhibition of the reverse transport of such acids to the liver, which results in lowered cholesterol level in the blood serum. Biliary acid binding also contributes to the decreased lithogenicity of the bile, which facilitates the reduction in the risk of choledocholithiasis (19).

Epidemiological observations confirm that the populations whose nutrition is rich in dietary fibre have usually lower total cholesterol levels, which applies especially to the LDL-cholesterol. It was found that vegetables, leguminous plants, oat and barley bran and kasha, where the water-soluble fractions predominate, are most effective in lowering the serum cholesterol level. It is assumed that their intake can lower the cholesterol level by 5% to 10% on average. It has been reported that, at doses below 35g daily of dietary fibre with predominating water-soluble fractions, the baseline cholesterol level may be lowered even by 25%. On the other hand, no significant changes in the HDL fractions under the influence of fibre have been found (17,20).

The mechanism of the hypolipaemic action of fibre has not been fully elucidated. The most probable is the mechanism of binding biliary acids in the intestine; other mechanisms are also suggested, such as modification (inhibition) of cholesterol synthesis in the liver through the action of short-chain fatty acids. It is also hypothesised that the diet rich in complex carbohydrates and dietary fibre inhibits lipogenesis. Therefore, the hypoglycaemic action of fibre may be multifactor, which still requires many studies (21).

Many studies presented for years indicate a beneficial role of dietary fibre in the reduction of postprandial glycaemia and insulin response. Such action of fibre was observed in healthy persons and in patients with various types of diabetes. Fibre was found to delay gastric and small intestinal emptying, resulting in deceleration of glycaemic response and prolonging the time of food presence in the intestines, causing decreased absorption of glucose and triglycerides, which ultimately resulted in a decreased demand for insulin and oral hypoglycaemic agents such as sulphonylureas. One of the mechanisms of such action is the increase in viscosity of the food material through the formation of gels which slow down the transit in the small intestine. Water-insoluble fibre fractions were shown to accelerate and the water-soluble fibre fractions were shown to decelerate the passage of food material through the small intestine. It should be therefore concluded that for patients with increased glucose and cholesterol levels, above all kasha, leafy, tuberous, and root vegetables, oats, and fruit should be recommended, as they are a rich source of soluble fractions of dietary fibre. For diabetics it is assumed that the daily intake of dietary fibre, and in particular of pectins, should be at least 15g per 1000 kcal (22).

When considering the nutritional value of Polish kasha we cannot disregard the fact that it is a source of vitamin B1 or B2, i.e. a water-soluble and unstable vitamin which - as not accumulated in the body - has to be delivered every day with food. In spite near 75% of soluble vitamins (thiamin and riboflavin) from kasha are destroyed during boiling processes, still the rest are important source for the body. Its role in the body comes down to participation in energy transformation processes, in regulation of the pentose cycle reactions leading to the formation of ribose necessary for the synthesis of nucleotides and NADPH, and in neurophysiological processes. It is a component of many enzymes, in particular of

decarboxylase of ά-ketoacids and transketolase participating in the indirect metabolism of carbohydrates. Moreover, thiamine plays an important role in the nervous tissue where its active form is the thiamine triphosphate acting on the physiological activity of neurotransmitters - acetylcholine and serotonin (19).

Many studies indicate that it participates also in the transmission of nervous impulses. Coverage of the daily requirement for thiamine, which for adults is ca. 1 mg on average, is at the level of ca. 80%. Therefore, the above-discussed types of kasha may be considered one of the best dietary sources of thiamine. As mentioned above, kasha is also an important dietary source of riboflavin (vitamin B2), the deficiency of which is a health concern all over the world, especially in the developing countries. Insufficient intake of riboflavin may cause many enzymatic and metabolic disorders such as changes in gluconeogenesis or increased accumulation of glycogen in the liver. Prolonged deficiency of this vitamin results in lowered DNA and RNA levels in some tissues and reduced beta-oxygenation and dehydrogenation of fatty acids. Moreover, its insufficient intake contributes to the decrease in the linolic, linolenic, and arachidonic acid levels in the blood serum and in the liver. It prevents lipid peroxidation and maintains glutathion, which prevents the harmful effects of free radicals in the body, in the reduced form. Its importance for human physiology is wider; it is responsible for incorporation of iron into erythrocytes, counteracts cataract, and is indispensable for normal functioning of the immune system. Therefore, each dietary source of this vitamin is very valuable in everyday nutrition.

Another Polish product which may be used for functional food is linseed oil. In unrefined cold pressed linseed oil, as much as one half of its composition is alpha-linolenic acid of the omega-3 family, and the remaining half is ca. 20% of oleic acid and a similar quantity of linolic acid of the omega 6 family. Owing to its composition, linseed oil fortifies the immune system, lowers cholesterol and hypertension, and is beneficial in the anticancer diet. Linseed oil in the form of unrefined cold pressed oil is not available in food stores due to the instability of the omega-3 acid. In fact, all polyunsaturated fatty acids undergo rapid oxidation under the influence of light, air, and high temperature. Therefore, they have to be stored in refrigeration conditions, without the access of light or air, which is not always possible. However, as shown by the Lyon Diet Heart Study, this type of fat has an extraordinarily potent beneficial effect on the reduction of the cardiovascular risk resulting from atherosclerosis, and therefore a real effort should be undertaken to make it available (1).

Probiotic flora in the Polish diet as a source of immunutrition

In the traditional diet of Poles, probiotic bacteria have constituted an important element of health maintenance. Such bacteria as *Lactobacillus plantarum* and *L. rhamnosus*, which are a constant element of fermented food, not only ensured the preservation of its nutritional value, but also stimulated immunity mechanisms.

We currently know that these probiotic bacteria have a unique system of degradation of two amino acids, i.e. tyrosine and arginine which is a substrate for nitric oxide synthesis (23). Owing to this mechanism, our gastrointestinal tract is protected against infections caused by *Candida albicans*, *Escherichia coli*, *Shigella, Salmonella*, and also *Helicobacter pylori*. We cannot forget that probiotic bacteria have the ability to eliminate nitrates from the diet, which they reduce to nitrites, which in turn contributes to the formation of nitric oxide (24). This additional mechanism is initiated in the mouth and continued in the stomach at pH below 2. Thus, if we have the appropriate number of *L. plantarum* bacteria in the gastrointestinal tract, they are able to eliminate up to 90 to 100% nitrates from vegetables.

Another important feature of probiotic bacteria is their participation in fibre fermentation, which leads to the formation of short-chain fatty acids. Butyric and propionic acids may play a special biological role. We know that butyric acid may change the morphology and ultrastructure of human colonic epithelial cell and endothelial cells (25). In fact, it is a potent anti-proliferative agent stimulating apoptosis of cancer cells (26). Butyrate was also shown to improve healing and reduce inflammation of intestinal mucosa through a reduction in activation of the transcription factor NFκB (27). This has an effect on bacterially stimulated synthesis of proinflammatory cytokines (28). In our studies we have also proven that butyric acid at concentrations observed in humans reduced the expression of adhesion molecules VCAM-I and ICAM-I in vascular epithelial cells (29). This may indicate anti-atherosclerotic action of short-chain fatty acids and indirectly explain the role of fibre in prevention of cardiovascular disorders.

As it has been suggested recently, also propionic acid is a strong anti-inflammatory agent, since through its effect on transcription factor PPARα it may lower the expression of COX 2, and thus act similarly as non-steroidal anti-inflammatory drugs. Therefore, it is possible that through concomitant increase in the intake of fibre and probiotic bacteria we will contribute to the increase of propionic acid in the circulation. The consequence of that may be a reduction in inflammation and the related oxidative stress in the body. Indirect evidence for such mechanism is provided by our clinical studies in which we administered to tobacco smokers *Lactobacillus plantarum 299V* in the form of functional food product (30). As a result, we have obtained a marked reduction in the level of oxidative stress evaluated with the use of concentration of F_2-isoprostane in the serum. In addition, we have found significant lowering in concentration of interleukine-6 and fibrinogen, and thus parameters related to associated chronic inflammation.

In connection with the commonly used Western-type diet devoid of fibre - we observe a dramatic drop in the presence of *L. plantarum* in the gastrointestinal tract. This is also associated with the wide use of antibiotic treatment and stress which contribute to elimination of these bacteria from the human body. An example may be the fact that astronauts are practically devoid of *L. plantarum* after their return to the Earth, which is accompanied by an increase in the

Enterobacteriaceae-type flora (31). Therefore, it is necessary to supplement the Western diet with the food which carries fermentation bacteria.

Summary

Production of functional food dynamically developing on the worldwide scale was based on knowledge on biologically active substances naturally occurring in food. Nevertheless, we still lack clinical studies with so-called hard endpoints, in which the direct effect of functional food on health would be proven.

So far it has only been evidenced that soy protein and plant sterols can actively lower the cholesterol level, which indirectly contributes to the lowering of the ischaemic heart disease risk.

REFERENCES

1. De Lorgeril M, Salen P, Martin JL., et al. Mediterranean diet, traditional risk factors and the rate of cardiovascular complications after myocardial infraction. Final report of the Lyon Diet Heart Study. *Circulation* 1999;99: 779-785
2. Meagher EA. Treatment of atherosclerosis in the new millennium: is there a role for vitamin E? *Prev Cardiol*. 2003;6 :85-90
3. De Lorgeril M, Salen P, Martin J-L, et al. Wine drinking and risk of cardiovascular complications after recent acute myocardial infraction. *Circulation* 2002; 106:1465-1469
4. Stocker R, O,Halloran RA. Dealcoholized red wine decreases atherosclerosis in apolipoprotein E gene-deficient mice independently of inhibition of lipid peroxidation in the artery wall. *Am J Clin. Nutr* 2004; 79: 123-30.
5. Bonetti PP, Lerman LO, Lerman A.. Endothelial Dysfunction a Marker of Atherosclerotic Risk. *Arterioscler Thromb Vasc Biol* 2003; 23: 168-175.
6. Serraino I, Dugo L, Dugo P, et al. Protective effects of cyanidin-3-O-glucoside from blackberry extract against peroxynitrite-induced endothelial dysfunction and vascular failure. *Life Science* 2003; 73: 1097-1114.
7. Xu JW, Ikeda K, Yamon Y. Cyanidin-3-glucoside, a typical anthocyanin pigment, induces expression of endothelial nitric oxide synthase through Src-ERK1/2 signaling pathway. *Atherosclerosis* 2003; suppl. 4, 2, 281(abstract).
8. Choi YJ, Kang JS, Park JHY, et al. Polyphenolic Flavonoids Differ in Their Antiapoptotic Efficacy in Hydrogen Peroxide-Treated Human Vascular Endothelial Cells. *Am .Soc. for Nutr. Sciences* 2003; 985-991.
9. Seeram N.P, Momin RA., Nair MG, Bourquin LD. Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries. *Phytomedicine* 2001; 8: 362-369.
10. Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules adipocyte-derived plasma protein adiponectin. *Circulation* 1999; 100: 2473-2476.
11. Pischon T, Girman CJ, Hotamisligil GS, et al. Plasma adiponectin levels and risk of myocardial infraction in men. *JAMA* 2004; 291: 1730-1737.
12. Tsuda T, Ueno Y, Aoki H, et al. Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. *Biochem. Biophys. Res. Commun.* 2004; 316: 149-157.
13. Aviram M, Dornfeld L. Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. *Atherosclerosis* 2001:158:195-198.

14. Aviram, M, Rosenblat D, Gaitini, S, et al. Pomegranate juice consumption for 3 years by patients with carotid artery stenosis reduces common carotid intima-media thickness, blood pressure and LDL oxidation. *Clin. Nutr.* 2004; 23: 423-433.
15. Naruszewicz M, Daniewski M, Łaniewska I, et al. Effect of anthocyanins from chokeberry (Aronia melanocarpa) on blood pressure, inflammatory mediators and cell adhesion molecules in patients with a history of myocardial infarction (MI). *Atherosclerosis* 2003; 4 suppl.2: 143 (abstract).
16. Jenkins DJ, Jenkins A.L, Wolever TM, et al. Fiber and starchy foods: gut function and implications in disease. *Am J Gastroenterol* 1986; 81: 920-930.
17. Kendall CW, Emam A, Augustin LS, Jenkins DJ. Resistant starches and health. *J AOAC Int* 2004; 87: 769-774.
18. Bingham SA, Day NE, Luben R., et al. Dietary fibre in food and protection against colorectal cancer in The European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *Lancet* 2003; 361: 1496-1501.
19. Slavin J. Why whole grains are protective: biological mechanisms. *Proc Nutr Soc* 2003; 62: 129-134.
20. Jensen M.K, Koh-Banerjee P, Hu FB, et al. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. *Am J Clin Nutr* 2004; 80: 1492-1499.
21. Truswell AS. Cereal grains and coronary heart disease. *Eur J Clin Nutr* 2002; 56: 1-14.
22. Toeller M. Fibre cpnsumption, metabolic effects and prevention of complications in diabetic patients: epidemiological evidence. *Dig Liver Dis* 2002; 34 suppl 2: S145-149.
23. Jonsson C, Clausen E, Raa J. Arginine degradation by a Lactobacilus plantarum strain from fish. *System Appl Microbiol* 1983; 4:148- 151.
24. Duncan C, Dougall H, Johnston P, et al. Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate. *Nature Med* 1995; 1: 546-551.
25. Gaschott T, Maassen CU, Stein J. Tributyrin, a butyrate precursor, impairs growth and induces apoptosis and differentiation in panceratic cancer cells. *Anticancer Res* 2001; 21: 2815- 2819.
26. Luhrs H, Kudlich T, Neumann M, et al. Butyrate-enhanced TNF-alpha-induced apoptosis is associated with inhibition of NF-kappaB. *Anticancer Res*. 2002; 22: 1561-1568.
27. Inan MS, Rasoulpour RJ, Yin , et al. The luminal short-chain fatty acid butyrate modulates NF-kappa B activity in a human colonic epithelial cell line. *Gastroenterology* 2000; 118: 724- 734.
28. Yin L, Laevsky G, Giardina C. Butyrate suppression of colonocyte NF-kappa B activation and cellular proteasome activity. *J Biol Chem* 2001; 276: 44641-44646.
29. Zapolska-Downar D, Siennicka A, Kaczmarczyk M, et al. Butyrate inhibits cytokine-induced VCAM-I and ICAM-I expression in cultured endothelial cells: the role of NF-KB and PPARa. *J Nutr Biochem* 2004;15: 220-228
30. Naruszewicz M, Johansson M-L, Zapolska-Downar D, Bukowska H. Effect of Lactobacillus plantarum 299v on cardiovascular disease risk factors in smokers. *Am J Clin Nutr* 2002;76: 1249-1255
31. Lencner AA, Lencner CP, Mikelsaar ME. The quantitative composition of the intestinal lactoflora before and after space flights of different lengths. *Nahrung*. 1984;70: 607-612.

Received: January 31, 2005
Accepted: February 15, 2005

Author's address: National Food and Nutrition Institute, Powsinska 61/63, 02-903 Warsaw, Poland.
E-mail: mnaruszewicz@izz.waw.pl