CLINICAL STUDY

One-year application of probiotic strain Enterococcus faecium M-74 decreases serum cholesterol levels

Hlivak P, Odraska J, Ferencik M, Ebringer L, Jahnova E, Mikes Z

*Clinic of Geriatric Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia.***hlivak@susch.sk**

Abstract

Objectives: **To investigate the impact of long-term orally administered probiotic strain Enterococcus faecium (EF) M-74 enriched with selenium on lipid profile (total cholesterol, LDH, HDL, and triglycerides) in humans.**
Background: **The discovery that hypercholesterolemia plays a major role in the development of atherosclerosis has led to a number of pharmacological and non-pharmacological (including dietary) approaches resulting in its elimination. Up to now, the question of supposed hypocholesterolemic effect of probiotics has not been definitely established.**
Methods: **43 volunteers were randomized into two groups. Participants were given single capsule a day containing $2x10^9$ of EF M-74 plus 50 µg of organically bound selenium (E-group) or placebo (P-group). The study was double-blind and lasted 60 weeks. Peripheral blood was analyzed for lipid parameters before intervention, after 6, 12, 23, 44, and 56 weeks of capsule administration, and four weeks following interruption of administration.**
Results: **After 56 weeks of application, decrease in total cholesterol in E-group (17/3 women/men, mean age 75.4±1.5 year) was observed (5.94±0.29 mmol/l at week 0 vs 5.22±0.25 mmol/l after 56 weeks, $p<0.001$). This reduction was achieved mainly due to a fall in LDL cholesterol (3.85±0.27 vs 3.09±0.21 mmol/l, $p<0.001$), as no significant alterations in HDL and triglycerides were noted. In placebo group (14/4, 78.1±1.7 year), no statistically important changes were observed after one-year capsule administration.**
Conclusions: **In our study, the administration of E. faecium M-74 probiotic strain was associated with reduction of serum cholesterol concentration by 12 % after 56 weeks. The crescent amount of facts on this issue gives a solid reason to assume that probiotics will find their place as a therapeutic alternative in human medicine.** ***(Tab. 4, Fig. 4, Ref. 36.)***
Key words: probiotic, cholesterol.

The value of managing atherosclerosis risk factors has been confirmed in the past few decades. Hypercholesterolemia, hypertension and smoking are still considered to be the "classical" risk factors. Intervention trials have generally shown that lowering "risk factors" reduces the subsequent rate of coronary heart disease, stroke, and other cardiovascular diseases. The Framingham Study was the major study, which verified that cholesterol increases the risk of developing heart disease. Data output was clear: the risk of clinically apparent coronary heart disease is a continuous nonlinear function of blood cholesterol levels (1). Each increase in the serum cholesterol concentration by 1 % results in 2–3 % increase in the risk of CHD (2, 3). Discovery that hypercholesterolemia plays a major role in the formation and development of atherosclerosis has led to number of pharmacological and non-pharmacological (including dietary) approaches resulting in its elimination. Fact that the serum cholesterol concentration is influenced by food has been known for a long time ago. Later, the first evidence of affecting cholesterol level by influencing intestinal microflora was found. One way to do this is by using probiotics.

Clinic of Geriatric Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia

Address for correspondence: P. Hlivak, MD, Dept of Arrhytmias, Slovak Cardiovascular Institute, Pod Krasnou horkou 1, SK-833 48 Bratislava 37, Slovakia.
Phone: +421.2.59320398

Tab. 1. Baseline subject characteristics.

	E-group	P-group	p-value
Number	20	18	
Gender (men/women)	3/17	4/14	
Age (years)	75.35±1.49	78.05±1.68	0.11
BMI (kg/m^2)	29.40±0.86	29.08±1.14	0.41
Waist-hip ratio (cm)	0.90 ±0.02	0.92±0.01	0.23
BP systolic (mmHg)	150.25±5.79	154.03±4.67	0.31
BP diastolic (mmHg)	85.25±2.60	84.58±1.94	0.42

Values are n (%) or mean±SD (standard error of mean), as indicated, BMI represents body mass index, BP – blood pressure, p-value level of significance, significant if $p<0.05$.

Tab. 2. The presence of diseases in both groups at baseline.

Disorders/diseases	E-group n (%)	P-group n (%)
IHD	16 (80 %)	12 (67 %)
HLP/DYSLP	7 (35 %)	4 (22 %)
Hypertension	16 (80 %)	11 (61 %)
TIA/stroke	1 (5 %)	2 (11 %)
Diabetes mellitus	5 (25 %)	5 (28 %)
MUSD	13 (65 %)	10 (56 %)

Values are given in absolute numbers (%), IHD denotes ischemic heart disease, HLP/DYSLP – hyper/dyslipidaemia, hypertension – arterial hypertension, TIA – transient ischemic attack, MUSD – musculoskeletal diseases.

Up to now, the question of supposed hypocholesterolemic effect of probiotics has not been definitely established. While some studies documented it well (4, 5, 6), others bring contradictary findings or even doubt it (7, 8). Another unresolved issue is an existing previously mentioned effect from the long term point of view. In available sources, we have not found any report where probiotics were administrated for one year.

Some experimental and clinical studies have documented the hypocholesterolemic effect of probiotic cultures. Not all of the probiotics seem to have the same properties and same effect on serum cholesterol levels. The aim of our study was to investigate the impact of long-term orally administered probiotic strain Enterococcus faecium M-74 (EF M-74) enriched with selenium on lipid profile (total cholesterol, LDH, HDL and triglycerides) in humans.

Material and methods

43 volunteers from an elderly nursing home in Bratislava were randomized into two groups. Participants in one group were given a single capsule containing $2x10^9$ bacteria EF M-74 and 50 μg of organically bound selenium (E-group) a day. At the same time, subjects in the second – placebo group (P-group) were given a capsule containing placebo. The study was randomized, double-blind and placebo controlled lasting from April 2001 to May 2002. All individuals were properly familiarized with the study protocol, which was acknowledged by informed consent. The enrollment examination consisted of the complete history with regard to cardiovascular risk profile assessment, physical examination including antropometric measurement (to calculate BMI [body mass index] as person's weight in kilograms divided by height in meters squared – (BMI=kg/m^2), and WHR [waist:hip ratio]). We also took a blood pressure.

Peripheral blood was analyzed during the enrollment examination, i.e. before intervention, after 6, 12, 23, 44, and 56 weeks of capsule administration and four weeks following interruption of administration. Blood samples were drawn after an overnight fast and abstinence always between 8.00–9.00 A.M. Chemical parameters were determined using the „Reflotron (r) Plus Meter" (Roche Diagnostics) analyzer. We assessed the total cholesterol level, HDL, triglycerides and LDL (this value was calculated by using the Friedwald formula: LDL cholesterol (mmol/l) = total cholesterol – (HDL + triglycerides/2.2).

Statistical analyses: Categorical variables are given as counts (expressed in %), continuous variables as means±SEM, standard error of mean. Coefficient of kurtosis and skewness were used to test data distribution. Data found to have normal (Gaussian) probability distribution were analyzed by parametric methods. For statistical analyses of quantitative variables, we used Student·s t-test paired for comparisons within groups. Analyses were performed with SPSS (Statistical Package for the Social Sci ences), version 8.0 for Windows and Microsoft Office 2000 (Microsoft Excel for Windows). All significance tests were 2-tailed at the level of significance ($\alpha=0.05$).

Results

Results are presented as changes between baseline values (week 0) and values obtained at the end of application of capsules (control 6, i.e. after 56 weeks) and one month after stopping the administration (control 7, i.e. after 56 weeks). All these data are given for both of the two groups, E-group and P-group. The basic subject characteristics are summarized in Table 1. No significant differences were found among the two groups. Comparing these characteristics between baseline values and those at the end of the study, the only significant diference was improving WHR, namely in both groups (from 0.90±0.02 to 0.87±0.01, $p<0.01$ in the E-group, from 0.92±0.01 to 0.89±0.01, $p<0.0001$ in the placebo group). Other parameters did not change significantly. Morbidity of participants verified according to their his-

Tab. 3. Lipid parameters in both groups at baseline

Parameter	E-group	P-group	p-value
Total cholesterol (mmol/l)	5.94±0.29	5.44±0.25	0.20
HDL (mmol/l)	1.17±0.07	1.17±0.08	0.47
LDL (mmol/l)	3.85±0.27	3.6±0.23	0.42
Triglycerides (mmol/l)	1.96±0.32	1.48±0.14	0.057

Values are expressed as means±SEM, standard error of mean, p-level of significance, significant when * $p<0.05$.

Tab. 4. Comparison of lipoprotein levels between both groups during the study period.

Parameter (mmol/l)	P-group					E-group				
	w.0	w.56	w.60	p(0-56)	p(0-60)	w.0	w.56	w.60	p(0-56)	p(0-60)
Total cholesterol	5.44±0.25	5.14±0.25	4.66±0.23	0.194	0.005*	5.94±0.29	5.22±0.25	5.13±0.25	0.000*	0.001*
HDL	1.17±0.08	1.23±0.10	1.19±0.07	0.523	0.659	1.17±0.07	1.22±0.11	1.23±0.10	0.462	0.382
LDL	3.60±0.23	3.21±0.18	2.80±0.20	0.083	0.003*	3.85±0.27	3.09±0.21	3.14±0.24	0.000*	0.002*
TG	1.48±0.14	1.52±0.11	1.49±0.11	0.772	0.947	1.96±0.32	2.15±0.30	1.91±0.30	0.251	0.726

Values are expressed as means±SEM, standard error of mean, p-level of significance, significant when * p<0.05. w. means week HDL – high density cholesterol, LDL – low density cholesterol, TG – triglycerides.

tory, medical records, and physical examination is shown in Table 2. As shown in Table 2, the lipopproteins levels were comparable between E- and P-groups. Only difference in triglycerides is approaching statistical significance (1.96 mmol/l±0.32 in E-group vs 1.48±0.14, p<0.057 in the P-group). Overall summary of the lipid profile for active, E-group as well as the placebo, P-group, is presented in Table 4.

Total cholesterol: We observed a slight gradual reduction of TC from the baseline values (5.94±0.29 mmol/l) up to the 3rd control (week 23) in the E-group, when reduction reached statistical significance (5.45±0.24, p<0.030). Another significant decrease in comparing to input figures was noticed at the end of E. faecium M-74 administration, i.e. after 56 weeks (5.22±0.25 mmol/l, p<0.001). This drop also remained important 4 weeks after cessation of capsule application (5.13±0.25 mmol/l, p<0.001) (Tab. 4, Fig. 1). In the placebo group, after a mild initial increase in serum TCH level, a gentle decrease with its maximum after 23 weeks (5.28±0.20 mml/l) followed. In both cases, changes were not significant. During the whole study, only one statistically significant difference in comparison to initial values was noticed in this group: lower levels of TC achieved 4 weeks after discontinuation of capsules, i.e. in week 56 (4.66±0.23 mmol/l, p<0.005) (Tab. 4, Fig. 1).

LDL cholesterol: Serum levels of LDL cholesterol approximately mimicked TC concentration in the E-group during all control examinations. However, up to week 56, changes were not statistically important. The respective week, we observed a significant decrease in LDL cholesterol comparing to the baseline values (3.09±0.21 mmol/l, p<0,001). Similarly to TC, this reduction also remained important 4 weeks after termination of capsules administration (3.14±0.24, p<0.002) (Fig. 2). A trend resembling that in LDL (and also similar to the TC) has been seen in placebo group, too, but reduction after 56 weeks did not reach the significant level (3.21±0.18 mmol/l, p<0.083). A statistically important drop was noticed only one month after stopping the application, i.e. week 56 (2.80±0.20 mmol/l, p<0.003) (Tab. 4, Fig. 2).

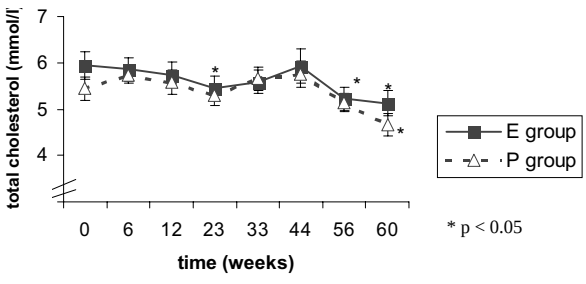

Fig. 1. Effect of E. faecium M-74 vs placebo on total cholesterol.

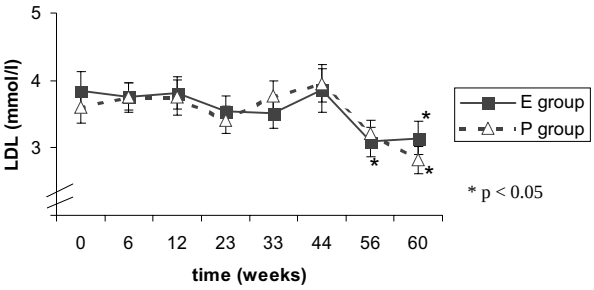

Fig. 2. Effect of E. faecium M-74 vs placebo on LDL cholesterol.

HDL cholesterol: In the group E, after initial increase in week 4 (1.226±0.07 mmol/l, p<0.10) a reduction with its maximum in week 12 followed (1.17±0.07 mmol/l in week 0 vs 1.095±0.07 mmol/l after 12 weeks, p<0.052). Subsequently, the serum HDL levels rose a little, but not significantly. In the placebo group, no significant differences during the whole study were observed when comparing to the initial levels (Tab. 4, Fig. 3).

Triglycerides: Triglycerides were maximally reduced in E-group after 23 weeks (1.696±0.22 mmol/l, p<0.059). However, this alteration likewise other readings in this group, were not statistically significant. In the placebo group, we saw only one statistically significant differerence from baseline: a reduction after 12 weeks (1.208±0.11 mmol/l, p<0.038) (Tab. 4, Fig. 4).

Completely identical results in lipoprotein spectrum were achieved during analyzing data after adjustment for lipid-lowering drug taking (3 individuals in both groups). This is why we do not present them separately.

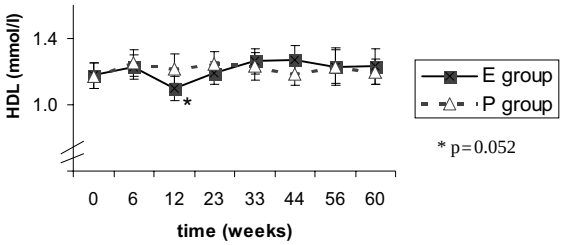

Fig. 3. Effect of E. faecium M-74 vs placebo on HDL cholesterol.

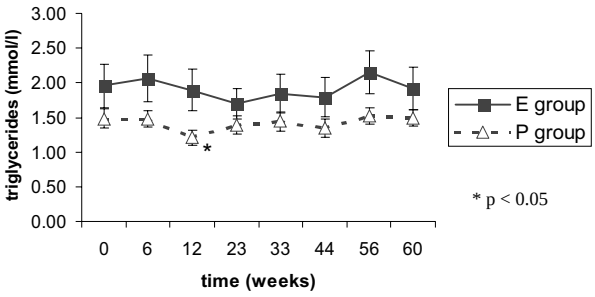

Fig. 4. Effect of E. faecium M-74 vs placebo on triglycerides.

Discussion

Originally, the term "probiotics" was used for a phenomenon, when each of both together cultivated organisms had produced substances stimulating the growth of the another one (9). The meaning of the term „probiotic" has been evolving since. Many definitions have been suggested. The most commonly used, scientifically valid and therefore acceptable one is that postulated by Fuller (10). According to him, probiotics are living microorganisms that favourably influence the health of the host by improving composition of its intestinal microflora. The FAO (Food and Agriculture Organisation for the United Nations) and WHO (World Health Organisation) refer to probiotics as living microorganisms which when ingested in sufficient amount, beneficially influence the health of the host (11, 12).

Before a certain bacterial strain can be classified as probiotic, it must meet some criteria (13, 14, 15). The most important one is its beneficial health effect. However, effective probiotics in addition:

– are non-pathogenic or toxic,
– contain large number of living cells,
– are capable of surviving and metabolic activity in the gut,
– retain their whole viability during storage (in the form of pills, capsules, powders, etc.),
– should be isolated from the same species as intended host.

The knowledge of probiotic intervention in lipid metabolism is not new. In the 1970s, Mann and later Hepner (16) observed hypocholesterolemic effect of yoghurt eaten daily in large amount in humans. Similar observations have been seen in animal studies (17, 18). Many trials aimed to proving the hypocholesterolemic effect of milk and fermented milk products come from early observations by Shaper (19) and Mann (20). They found a low incidence of clinical coronary heart disease in some African tribes (Masai, Samburu) with low cholesterol levels in spite of their diet rich in saturated fats, meat and cholesterol but abundant in milk and fermented milk products along.

Within all probiotic strains investigated for cholesterol-lowering potential, Lactobacillus acidophilus has been most widely studied. Lin et al. (4) conducted two studies. In the pilot study (without a placebo control), persons receiving tablets containing $3x10^7$ colony forming units Lactobacillus acidophilus and L. bulgaricus achieved a decrease of serum cholesterol from 5.7 to 5.3 mmol/l after 16 weeks ($p<0.05$). In the second, placebo-controlled, double-blind study, a significant effect of Lactobacilli on cholesterol was not found. Schaafsma et al observed a decrease of cholesterol levels by 0.23 mmol/l in 30 volunteers who had been consuming yoghurt enriched with a specific strain of L. acidophilus and fructooligosaccharides (21).

Other studies dealt with cholesterol-lowering effects of Enterococcus faecium. Mikes et al performed a pilot study with 12 subjects who were daily given E. faecium in lyophilized form as capsule containing $5x10^9$ bacteria. As for cholesterol, the mean levels of total and LDL cholesterol showed biphasic effect: the elevation was followed by a sharp decrease on the day 64 (6).

In a Danish study, Agerbaek et al demonstrated the LDL cholesterol-lowering effect in 58 healthy, non-obese, normocholesterolemic men whose diet was supplemented with biologically fermented milk (containing Enterococcus faecium and Streptococcus thermophilus). Serum cholesterol levels were decreased by 0.37 mmol/l after 6 weeks, while no changes were observed in the placebo group. This reduction in total cholesterol was completely ascribed to the fall in LDL cholesterol (22).

There is only a small number of trials that tested the long-term effect of probiotics. Richelsen et al compared the effect of milk products fermented with either probiotic bacteria (Enterococcus faecium and Streptococcus termophilus) or chemically (placebo). Serum LDL concentrations decreased throughout the study, with a larger decrease in the Entereococcus group at weeks 4 and 12 than in the placebo group (5). However, during long-term intake (6 month), the reduction of LDL was similar to the placebo product. The authors suggest that both chemically and biologically (with probiotics) fermented milk lower serum cholesterol, with a more rapid effect by Enterococcus faecium.

In our study, we observed a gradual decrease of serum cholesterol concentration in subjects receiving capsules with E. faecium M-74 from baseline up to week 23 when the decrease exceeded significant level ($p<0.001$). This reduction remained important also one month after the follow-up, i.e. 4 weeks after cessation of capsules application (-0.81 mmol/l, i.e. by 13.6 %). Biphasic shape of cholesterol curve was noticed in the placebo group during capsule administration, however, changes are not statistically siginificant. Throughout the study, only one statisti-

cally significant difference comparing to initial values was noticed in this group: lower levels of TC achieved 4 weeks after discontinuation of capsules, i.e. in week 56 (4.66±0.23 mmol/l, $p<0.005$). One of possible explanations why the reduction of cholesterol levels was seen in both groups at the end of the study may ground in the fact of "changing diet". Despite the fact that all the participants were instructed not to change their dietary habits, after the initial session (which also included a lecture concerning nonpharmacological treatment of cardiovascular diseases including information on healthy diet) it turned towards "healthier". Another interpretation might come from Katan and Cox (23, 24). They say that responses to dietary alterations result both in elevation as well as reduction in blood lipids and are unevenly distributed among people. Some of them are responders while the others non-responders, however, the biological genetic?) background for the phenomenon is still unknown (5).

Serum levels of LDL cholesterol approximately copied TC concentration in both groups throughout the study (actually vice versa). Changes in triglycerides, comparing to baseline, were not statistically significant during the study.

Since in-vitro and animal studies have demonstrated the possibility of cholesterol removing from culture media (25, 26), much attention has been drawn to the cholesterol-lowering potential of probiotics in humans (27). There exist several explanations of this effect, namely at the level of synthesis (inhibition of hepatic synthesis), circulation, redistribution or degradation and excretion of cholesterol from the organism. Today it is recognized that the effect observed in cultured media results from the intervention to entero-hepatic circulation of bile acids and increased bacterial activity in the large intestine. This leads to enhanced bile acids deconjugation owing to the activity of the bacterial enzyme: bile salt hydrolase (28, 29). Deconjugated bile acids are not well absorbed by mucosa of the gut and are excreted. Consequently, cholesterol as a precursor of bile acids, is used to a greater extent for the de novo bile acid synthesis (30). This mechanism would make a more precise explanation than just precipitation of cholesterol with free bile acid and subsequently its enhanced excretion.

A surprising finding in our study was the decrease of HDL cholesterol in subjects given E. faecium M-74 after 12 weeks ($p<0.052$). The levels of HDL have increased gradually later on but changes were not statistically important. If we speculate about the possibility of living probiotic bacteria to activate some inflammation processes and reactions (31, 32, 33, 34), it is important to mention that inflammation can alter also HDL particles. One of the mediators of this process could be the secretory ph ospholipase A_2. This enzyme has the ability to remodel HDL. It hydrolyses HDL phospholipids and reduces HDL particle size, although unlike cholesteryl ester transfer protein and phospholipids transfer protein, it does not generate lipid-poor apolipoprotein apoA-I (35). Discovery that HDL particles could be altered by inflammation is of great importance because remodeling of HDL plays a major role in regulating the concentration and subpopulation distribution of HDL. Hence, the remodeling of HDL may be responsible for substantial changes to lipoproteins during states of inflammation (36).

In conclusion, the nonpharmacological approaches to the management of coronary artery disease risk factors still have not been exhausted, even in the modern pharmacological and interventional treatment era. In our study, the administration of the E. faecium M-74 probiotic strain was associated with the reduction of serum cholesterol concentration by 12 % after 56 weeks. The observed changes were mainly due to the decrease in LDL cholesterol, as no significant alterations in HDL and triglycerides were no ted. With crescent amount of facts it is reasonable to hypothesize that probiotics will find a place as a therapeutic alternative. Modification of the intestine microflora (namely by probiotics) may represents a new challenge in the treatment of various diseases, i.e. cardiovascular disorders.

References

1. Futterman LG, Lemberg L. The Framingham Heart Study: a pivotal legacy of the last millennium. Amer J Crit Care 2000; 9 (2): 147—151.

2. Davis CE, Rifkind BM, Brenner H, Gordon DJ. A single cholesterol measurement underestimates the risk of coronary heart disease: an empirical example form the Lipid Research Clinics mortality follow-up study. J Amer Med Ass 1990; 264: 3044—3046.

3. Manson JE, Tosteson H, Ridker PM et al. The primary prevention of myocardial infarction. New Engl J Med 1992; 326: 1406—1416.

4. Lin SY, Ayres JW, Winkler W, Sandine WE. Lactobacillus effect on cholesterol: in vitro and in vivo results. J Dairy Res 1989; 72: 2885—2899.

5. Richelsen B, Kristensen K, Pedersen SB. Long-term (6 month) effect of a new fermented milk product on the level of plasma lipoproteins-a placebo-controlled and double blind study. Europ J Clin Nutr 1996; 50: 811—815.

6. Mikeš Z, Ferenčík M, Jahnová E, Ebringer L, Čižnár I. Hypocholesterolemic and immunostimulatory effect of orally applied Enterococcus faecium M-74 in man. XIII World Congress of Cardiology. Rio de Janeiro 1998, Proceedings: 1175—1178.

7. Taylor GRJ, Williams CM. Effects of probiotics and prebiotics on blood lipids. Brit J Nutr 1998; 80 (Suppl 2): 225—230.

8. De Roos NM, Schouten G, Katan MB. Yoghurt enriched with Lactobacillus acidophilus does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. Europ J Clin Nutr 1999; 53: 277—280.

9. Ebringer L, Ferenčík M, Mikeš Z. Fortifikácia potravín probiotickými baktériami. In: Muzikár V (Ed). Aktuální problematika mikrobiologie potravin. Sborník prednášek 1999: 33—39.

10. Fuller R. A review: probiotics in man and animals. J Appl Bacteriol 1989; 66: 365—378.

11. FAO/WHO. Evalution of health and nutritional properties of powder milk and live lactic acid bacteria. Food and agriculture organisation of United nations and World Health Organisation expert consultation report. Rome: FAO, 2001.

12. FAO/WHO. Guidelines for the evaluation of probiotics in food. Food and agriculture organisation of United nations and World Health Organisation working group report. Rome: FAO, 2002.

13. Lee YK, Salminen S. The coming of age of probiotics. Trends Food Sci Technol 1995; 6: 241—254.

14. Salminen S, Laine M, von Wright A et al. Development of selection criteria for probiotic strains to assess their potential in functional foods: a Nordic and Europopean approach. Biosci Microflora 1996; 15: 61—67.

15. McFarland LV, Elmer GW. Pharmaceutical probiotics for the treatment of anaerobic and other infections. Anaerobe 1997; 3: 73—78.

16. Hepner G, Fried R, Jeor SS, Fusetti L, Morin R. Hypocholesterolaemic effect of yoghurt and milk. Amer J Clin Nutr 1979; 32: 19—24.

17. Rao DR, Chawan CB, Pulusani SR. Influence of milk and Thermophilus milk on plasma cholesterol levels on hepatic cholesterologenesis in rats. J Fd Sci 1981; 46: 1339—1341.

18. Pulusani SR, Rao DR. Whole body, liver and palsma cholesterol levels in rats fed Thermophilus, Bulgaricus and Acidophilus milks. J Fd Sci, 1983; 48: 280—281.

19. Shaper AG, Jones KW, Jones M, Kyobe J. Serum lipids in three nomadic tribes of northern Kenya. Amer J Clin Nutr 1963; 13: 135—146.

20. Mann GV, Spoerry A. Studies of a surfactant and cholesterolaemia in the Massai. Amer J Clin Nutr 1974; 27: 464—469.

21. Schaafsma G, Meuling WJA, Van Dokkum W, Bouley C. Effects of a milk product, fermented by Lactobacillus acidophilus and with fructo-oligosaccharides added, on blood lipids in healthy men volunteers. Europ J Clin Nutr, 1998; 52: 436—440.

22. Agerbaek M, Gerdes LU, Richelsen B. Hypocholesterolaemic effect of a new fermented milk product in healthy middle-aged men. Europ J Clin Nutr 1995; 49: 345—352.

23. Katan MB, De Vries JH, Nobels A. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Amer J Epidemiol 1986; 123: 221—234.

24. Cox C, Mann J, Sutherland W, Ball M. Individual variation in plasma cholesterol response to dietary saturated fat. Brit Med J 1995; 311: 1260—1264.

25. Gilliland SE, Nelson CR, Maxwell C. Assimilation of cholesterol by Lactobacillus acidophilus. Appl Environ Microbiol 1985; 49: 377—381.

26. Gilliland SE, Walker DK. Cholesterol uptake by culture of Lactobacillus acidophilus used for nonfermented acidophilus milk. Anim Sci Res Rep 1989; 11: 258—262.

27. De Roos NM, Schouten G, Katan MB. Yoghurt enriched with Lactobacillus acidophilus does not lower blood lipids in healthy men and women with normal to borderline high serum cholesterol levels. Europ J Clin Nutr 1999; 53: 277-80.

28. Klaver FAM, Van der Meer R. The assumed assimilation of cholesterol by Lactobacilli and Bifidobacterium bifidum is due to their bile salt-deconjugating activity. Appl Environ Microbiol 1993; 59: 1120—1124.

29. Tahri K, Ballongue J, Schneider F. Effect of three strains of bifidobacteria on cholesterol. Lett Appl Microbiol 1995; 21: 149—151.

30. Jones ML, Chen H, Ouyang W, Metz T, Prakash S. Microencapsulated Genetically Engineered Lactobacillus plantarum 80 (pCBH1) for Bile Acid Deconjugation and Its Implication in lowering Cholesterol. J Biomed Biotechnol 2004; 1: 61—69.

31. Mikeš Z, Ferenčík M, Jahnová E, Ebringer L, Čižnár I. Hypocholesterolemic and Immunostimulatory Effect of Orally Applied Enterococcus faecium M-74 in man. Folia Microbiol 1995; 40: 639—646.

32. Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. Infect Immun 1996; 64: 5403—5405.

33. Majarmaa H, Isolauri E. Probiotics: a novel approach in the management of food alergy. J Allergy Clin Immunol 1997; 99: 179—186.

34. Ferenčík M, Ebringer L, Mikeš Z, Jahnová E, Čižnár I. Prospešná modifikácia črevnej mikroflóry človeka perorálne podávanými baktériami mliečneho kysnutia. Bratisl Lek Listy 1999; 100: 238—245.

35. Rye KA, Duong MN. The influence of phospholipid depletion on the size, structure and remodelling of reconstitued high density lipoproteins. J Lipid Res 2000; 41: 1640—1650.

36. Barter P. Effect of inflammation on High-Density Lipoproteins. Arterioscler Thromb Vasc Biol 2002; 22: 1062—1063.

Received December 18, 2004.
Accepted January 28, 2005.