ELSEVIER

Nutrition Research 22 (2002) 393–403

NUTRITION RESEARCH

www.elsevier.com/locate/nutres

Experimental and clinical study on the hypolipidemic and antisclerotic effect of Lactobacillus Bulgaricus strain GB N 1 (48)

Nadezhda Iv. Doncheva[a,]*, Georgi P. Antov[a], Ekaterina B. Softova[a], Yuri P. Nyagolov[b]

[a]*Lipid Research Laboratory, Laboratory of Toxicology, Laboratory of Hystopathology, National Center of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria*

[b]*Department of Physiology, Medical University, Sofia, Bulgaria*

Received 27 June 2001; received in revised form 8 December 2001; accepted 10 December 2001

Abstract

The study aims to perform experimental and clinical testing of the effect of the milk product "Bulgaricum" containing Lactobacillus Bulgaricus (LB) strain GB N 1 (48) on the progress and severity of experimental model of hyperlipidemia and sclerotic processes serum lipids and blood flow in aa. carotides in persons with dyslipoproteinemia (DLP). Histological and electron microscopic examinations of the components of the major intercellular matrix of rat myocardium and aorta slices evidence the combination of atherogenic diet and the milk product "Bulgaricum" leads to a delayed synthesis of collagen proteins, a tendency of decreased deposition of insoluble collagen, and improved ratio of unsulfated and sulfated glucoseaminoglycanes. Doppler sonography, and serum lipids studies in 26 individuals with DLP and ischaemic heart disease after 3 months supplementation with the product indicated substantial decrease of TC and LDL-Chol. The milk product "Bulgaricum" has hypolipidemic and antiatherosclerotic effect in humans and animals.

Keywords: Lactobacillus Bulgaricus; Atherosclerosis; Dyslipoproteinemia; Sonography; Probiotic

1. Introduction

The dietetic and curative properties of original Bulgarian fermented milk are well known from ancient times. Its specific features are due to its microflora in which the Bulgarian

* Corresponding author. Tel.: +359-2-5812-546.

E-mail address: n.doncheva@nchmen.government.bg (N.I. Doncheva).

0271-5317/02/$ – see front matter
PII: S0271-5317(01)00397-9

physician Stamen Grigorov discovered Lactobacillus Bulgaricus (LB) in Geneva in the beginning of the XXth century [1]. The Russian scientist Ilya Mechnikov revealed the antagonistic effect of LB against intestinal putrefactive flora as a reason for the beneficial effect of Bulgarian fermented milk on human health [2]. Literature data indicate differences in type, amount and effect potential between the different strains isolated from different sources [3,4,5]. Hypolipidemic effect of whole fat and defatted milks, fermented milk and other milk products were reported by a number of researchers [6,7,8,9,10,11]. Considerable research advances concerning the favorable health effects of probiotics as a component of various milk products are reported recently [12,13]. The putative role of the probiotics for mitigating the risk of atherosclerosis associated with dyslipoproteinemia (DLP), obesity and diabetes type II requires new comprehensive research [14]. Investigation of the effects of various LB strains for diminishing the risk of atherosclerotic cardiovascular disease, respectively eventual delay of the progress, new formation or regression of existing plaques is interesting from a scientific and a practical point of view.

The aim of this study is to test experimentally and clinically the effects of liquid milk product "Bulgaricum" containing Lactobacillus Bulgaricus strain GB N 1 (48) on the development and severity of experimentally modeled hyperlipidemia and sclerotic process, serum lipids and blood flow in aa. carotides communes in DLP patients.

2. Methods and materials

The study comprises an experimental and a clinical parts.

2.1. Animal experiment (design and methods)

The experiments were performed on 50 albino male "Wistar" rats, aged 4 months with initial body mass 150–170 g, divided into groups of 10 as follows: I group—control, administered physiological solution; II group—positive control, treated with sunflower oil (0.7 ml/100 g b.m.); III group—subjected to atherogenic diet [15], (modified by Antov [16]) (1.5% cholesterol, 0.5% holic acid and Vitamin D_2 (30000 IU/kg b.m.), fed with the diet each third day in the course of two weeks); IV group—treated with LB strain GB N1 (48)—0.7 ml/100 g b.m.; V group—receiving both atherogenic diet and LB strain GB N1 (48). The following fibrillar structure components were studied in myocardium and aorta: alkaline-, neutral- and acid-soluble collagen (AlSC, NSC and ASC) after L. Slutskii [17] and insoluble collagen (IC) after M. Chrapil [18]), biopolymers of the basic intercellular matrix: total, nonsulfated and sulfated glucoseaminoglycanes (tGAG, nsGAG and sGAG) after L. Franson [19]. Histological (hemalaun-eosin, Sudan III, Sudan-Schwartz) and electron microscopic studies were performed on 5 μm thick slices from the myocardium and aorta.

2.2. Clinical study (design and methods)

The effect of 200 ml milk product "Bulgaricum" supplemented to a hypolipidemic diet on serum lipids was studied in 26 individuals (16 women and 10 men) with DLP (mean age 53.5

years). The criterion for inclusion in the study was total cholesterol (TC) > 5.2 mmol/L and clinically manifested atherosclerosis: 14 patients with ischaemic heart disease (IHD) and/or Doppler sonographic data for stenosis of aa. carotides (5 persons) and/or family DLP (7 individuals). For patients with moderate and severe DLP (14 persons), corrected after drug treatment, the criterion for administering the product with LB strain GB N1 (48) was reaching TC levels of 5.2–6.7 mmol/L. TC was determined with enzyme colorimetric method—CHOD-PAP test of POINTE Scientific Inc. [20]. Triglycerides (TG) were determined with enzyme colorimetric method—GPO test with kits of POINTE Scientific Inc. [21]. Cholesterol in high density lipoproteins (HDL-Chol) was determined after precipitation and sedimentation of apo B-containing LP with 20% polyethylene glycol with test kits of POINTE Scientific Inc. Cholesterol in low density lipoproteins (LDL-Chol) was determined by Friedewald's formula [22]. The ratio TC/LDL-Chol was calculated. Using continuous wave ultrasonic Doppler sonography [23] at optimal angle of 45° between the probe and the arterial axis and frequency 4 MHz at 1.5 cm before the bifurcation of the left and right artery using Sonovit SV (Schiller—Switzerland) the following indicators of blood flow in aa. carotides communes in all subjects were recorded or calculated: maximal linear velocity of blood flow (V_{max}); minimal velocity (V_{min}); mean velocity (V_{mean}); Pourcelot's resistance index (RI); Gosling's pulse index (PI); systolic-diastolic ratio (V_{max}/V_{min}); Diastolic-systolic ratio (V_{min}/V_{max}). The normal indices values for brain arteries with low resistance are respectively: RI—0.55–0.75; PI—0.7–10; V_{max}/V_{min}—1.8–2.7; V_{min}/V_{max}—1/3–2/3. The subjects were examined before and in the third month after product administration. Study protocol was approved by the Institution Ethical Committee.

2.3. Characteristics of liquid "Bulgaricum" fermented with LB strain GB N1 (48)

For the needs of the clinical testing a team of "Genesis" laboratory produced liquid milk product "Bulgaricum" containing LB (Table 1). Dry low fat cow milk was used for this product. This input material after recovery and twofold thermal processing at 96°C was fermented with LB strain GB N1 (48) at temperature 45°C and was cultivated to coagulation. At pH 5.0 or pH 5.1 the product was cooled and stored in a refrigerator. Patients' weekly dose was examined for LB live active cells count. This count varied from 250 to 950 millions per cm^3, an average of 400 millions live cells in 1 ml or an average of 80 billions cells per daily dose of 200 ml. Liquid "Bulgaricum" fermented with LB strain GB N1 (48) is a fermented milk product produced after technology similar to that of Bulgarian fermented milk. Its particularities are due to the nature of the LB pure culture.

2.4. Statistical analysis

The results from the biochemical studies in the experimental design and the instrumental sonographic examination were processed with variation analysis. The significance of the results was evaluated with Student-Fischer's t-criterion at $p < 0.05$. The results were presented as mean values and standard deviation (mean $\pm$ SD). The results concerning the effect of "Bulgaricum" intake by subjects with DLP were processed with pair analysis for

Table 1
Characteristics of LB strain GB N1(48)

Indicators	Strain GB N1 (48)
origin	natural
colonies type	RS
microscopic picture	short, solid bars without volutine particles
development at	
15°C	no growth
37°C	10 hours
45°C	9 hours
lactic acid %	1.7
configuration of lactic acid	L/+/
development of milk with 1% methylene blue	−
development in hydrolysed milk broth (HMB) with 2% NaCl	+
development in HMB with 4% bile	−
gas from glucose	−
NH2 formation from arguinine	−
development in esculin broth	−
thermal resistance at 63°C—30 min.	+
development in nutritional media MRS broth with 3% phenol	−
sugar fermentation	lactose and glucose

comparison of samples of dependent populations for assessment of the treatment effect. The rate (%) of index changes was calculated.

3. Results and discussion

3.1. Experimental part

In the serum of the animals from the positive control group (II group) on the background of unchanged content of TC and TG significant elevation of HDL-Chol and LDL-Chol in relation to the control values was found (Table 2).

No deviations in the components building the fibrillar structures, basic intercellular matrix of the cellular tissue and in the normal structure of the myocardium, intramural branches of coronary arteries and aorta were found (Table 3).

The III group (atherogenic diet) revealed elevated serum levels of TC, LDL-Chol and HDL-Chol compared to Groups I and II (Table 2). The content of NSC was significantly diminished, and that of AlSC, ASC and IC—significantly elevated. The deviations in collagen fractions were accompanied by significant changes of the amount of GAG building the basic intercellular matrix. Increase of tGAG resulted from increase of sGAG and decrease of nsGAG (Table 3). Similar was the character of the observed deviations in the cardiac muscle. Increase of soluble collagen fractions (NSC, AlSC and ASC) was recorded. The increase of tGAG was a consequence from increased sGAG and decreased nsGAG. The histological structure of the myocardium was preserved and scarce lymphoid cell infiltrates

Table 2
Serum lipids at modeled sclerotic process

Indicator (mmol/L)	Group	Number (n)	Value (mean ± SD)
TC	I (control)	5	1.81 ± 0.124
	II (positive control)	4	1.72 ± 0.197
	III (atherogenic diet)	9	3.26 ± 0.769*,**
	IV (LB)	6	1.74 ± 0.692
	V (atherogenic diet + LB)	8	1.56 ± 0.512*
TG	I (control)	5	1.22 ± 0.362
	II (positive control)	4	0.79 ± 0.209
	III (atherogenic diet)	9	0.97 ± 0.270
	IV (LB)	6	1.00 ± 0.418
	V (atherogenic diet + LB)	8	0.85 ± 0.218
HDL-Chol	I (control)	5	0.77 ± 0.178
	II (positive control)	4	0.22 ± 0.085*
	III (atherogenic diet)	9	0.64 ± 0.379
	IV (LB)	6	0.67 ± 0.319**
	V (atherogenic diet + LB)	8	0.70 ± 0.283**
LDL-Chol	I (control)	5	0.49 ± 0.165
	II (positive control)	4	1.13 ± 0.200*
	III (atherogenic diet)	9	1.47 ± 0.489*
	IV (LB)	6	0.58 ± 0.476**
	V (atherogenic diet + LB)	8	0.32 ± 0.222**,**

** —significant in relation to control.
**** —significant in relation to positive control.
****** —significant in relation to atherogenic diet.

were present. In some animals slight thickening of the aortic wall was found, as well as swelled endothelium with vacuolated cytoplasm, under which there were deposited lipid granules and accumulated "foamy" cells (Fig. 1A, 1B). In some areas the endothelium was desquamated baring the underlying layer. Lipid substances "incrusting" the visceral muscle cells situated focally in the media were observed. These observations were confirmed by electron microscopic examinations.

Animals with experimental model of sclerotic processes demonstrated disturbances in the lipid components in serum, activated collagen synthesis and accumulation of insoluble collagen, elevated content of total and sulfated GAG in the basic intracellular matrix of the aortal and myocardium, initial stage of development of atherosclerotic plaques (stage of "fatty streaks" in the aortal wall).

The individual introduction of LB strain GB N1 (48) provoked in the serum of the experimental animals of IV group significant increase of HDL-Chol and decrease of LDL-Chol without deviations in TC and TG levels vs. the positive control (Table 2). No changes were recorded in the amount of cellular tissue components, structure of the aorta and cardiac muscle (Table 3).

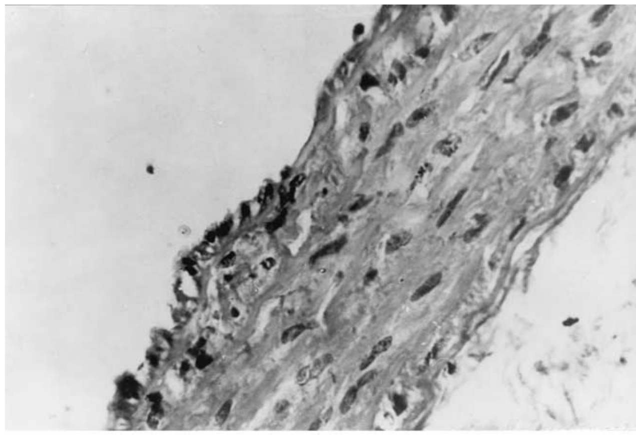
The introduction of LB strain GB N1 (48) together with atherogenic diet (V group) caused the development of substantially weaker deviations in the lipid profile of the serum compared to the effect of the atherogenic factor (Table 2). The contents of TC, TG, HDL-Chol, LDL-Chol did not differ substantially from the respective values in the controls. The significant decrease of TC and LDL-Chol vs. the respective levels in the serum of test

Table 3
Cellular tissue components in aorta and myocardium at modeled sclerotic process

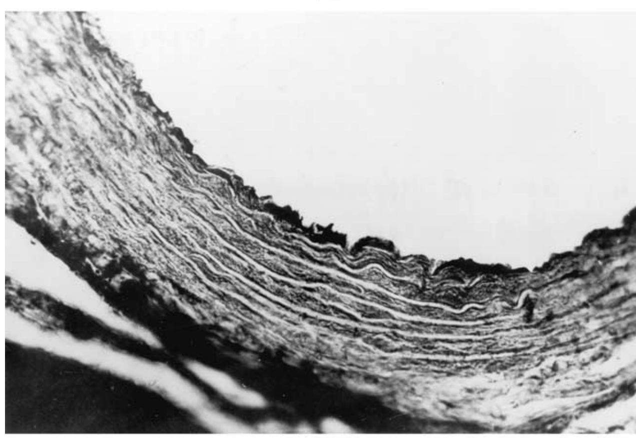
Indicator (mg/g)	Group	Aorta (mean ± SD)	Myocardium (mean ± SD)
NSC (neutral-soluble collagen)	I	14.9 ± 1.84	33.8 ± 3.72
	II	14.2 ± 2.50	39.6 ± 4.21
	III	9.7 ± 0.96*,**	60.6 ± 5.80*,**
	IV	14.1 ± 1.76	32.1 ± 2.93
	V	12.4 ± 1.03**,***	4.81 ± 4.28*,**,***
A1SC (alkaline-soluble collagen)	I	19.5 ± 2.41	25.4 ± 1.38
	II	19.8 ± 1.76	26.5 ± 2.84
	III	28.4 ± 3.11*,**	47.6 ± 3.77*,**
	IV	18.3 ± 1.39	24.3 ± 2.54
	V	25.3 ± 2.34*,**	38.7 ± 1.94*,**,***
ASC (acid-soluble collagen)	I	8.6 ± 1.26	7.4 ± 0.72
	II	9.8 ± 0.95	8.0 ± 0.82
	III	14.5 ± 1.62*,**	13.0 ± 1.27*,**
	IV	9.3 ± 0.68	7.9 ± 0.44
	V	11.2 ± 2.47*	12.5 ± 1.76*,**
IC (insoluble collagen)	I	25.8 ± 3.11	5.8 ± 0.63
	II	26.2 ± 2.17	5.2 ± 0.48
	III	38.4 ± 2.96*,**	8.4 ± 0.37*,**
	IV	24.1 ± 3.55	6.2 ± 0.55
	V	26.7 ± 3.17*,**	7.5 ± 0.82*,**
TCA (total glucoseaminoglycanes)	I	18.4 ± 0.78	11.7 ± 0.93
	II	18.7 ± 1.34	10.8 ± 1.27
	III	32.6 ± 3.09*,**	15.6 ± 2.18*,**
	IV	19.3 ± 1.57	11.3 ± 1.54
	V	25.7 ± 1.86*,**,***	13.8 ± 1.83
nsGAG (nonsulfated glucoseaminoglycanes)	I	4.2 ± 0.52	5.3 ± 0.69
	II	3.8 ± 0.64	4.8 ± 0.53
	III	2.1 ± 0.31*,**	2.9 ± 0.37**
	IV	3.6 ± 0.42	4.9 ± 0.38
	V	2.6 ± 0.20*,**	3.9 ± 0.45*,**,***
sGAG (sulfated glucoseaminoglycanes)	I	14.0 ± 1.18	6.9 ± 0.84
	II	14.5 ± 2.70	6.1 ± 0.72
	III	31.9 ± 2.45*,**	10.8 ± 1.34*,**
	IV	15.2 ± 1.33	6.2 ± 0.39
	V	23.1 ± 2.84*,**,***	8.7 ± 0.69*,**,***

** —significant in relation to control.
** —significant in relation to positive control.
*** —significant in relation to atherogenic diet.

animals receiving only atherogenic diet proved favorable effect. Kiyosawa et al. also found decrease of TC and LDL-Chol in experimental groups with atherogenic diet after intake of low fat milk but net after yogurt intake [11]. The combined effect of the two studied factors leads to decreased degree of damages in the collagen fractions and components of the basic intercellular matrix of the aortal and myocardial tissue (Table 3). Favorable effect of liquid "Bulgaricum" fermented with LB strain GB N1 (48) was found mainly in relation to soluble forms of collagen and biopolymers tGAG, sGAG and nsGAG which values in both tissues outlined a tendency to approximation to the corresponding values of control animals without



(a)



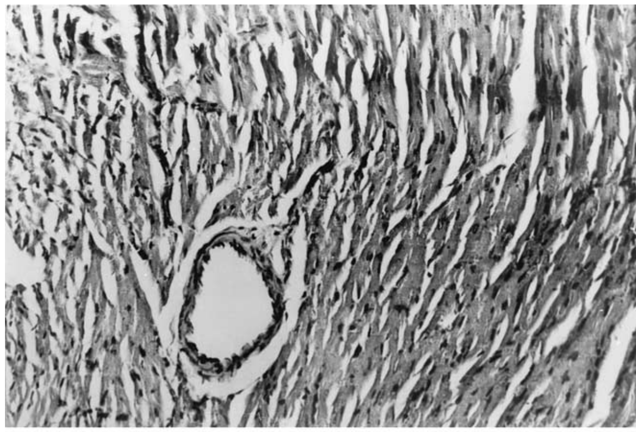
(b)

Fig. 1. **A.** Aorta from an animal subjected to atherogenic diet (30th day). Swelled, with vacuolised cytoplasm endothelial cells, "foamy" cells accumulated under them. Staining HE, magnification 10.0 × 40.0; **B.** Aorta from an animal from the same group. Areas with conjugation of lipid granules and formation of fatty streaks. Staining Sudan-Schwartz, magnification 10.0 × 6.3.

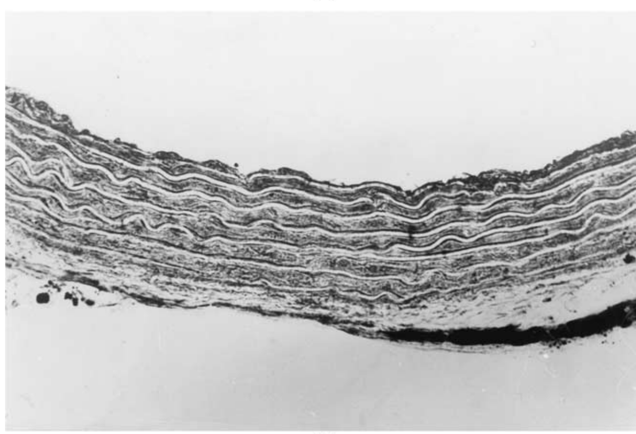
sclerotic changes. The introduction of LB strain GB N1 (48), though, did not affect significantly the amount of IC accumulated in the tissues. No significant differences between the histological structures of the myocardium and vessels of test and control animals were found (Fig. 2A). Only one animal showed fine lipid substances in the sub endothelial space and partly in the media of the aortal wall (Fig. 2B).

3.2. Clinical study

Sixteen subjects were fenotyped as DLP type IIA after Fredrikson's classification, 9—as mixed IIB type and one—with hypoalphalypoproteinemia. The three-month intake of liquid "Bulgaricum" of subjects with DLP showed significant decrease of TC and LDL-Chol at



(a)



(b)

Fig. 2. **A.** Myocardium from an animal subjected to atherogenic diet and treated with LB strain GB N 1. Preserved histological structure, unchanged vessel wall thickness. Staining HE, magnification 10.0 × 6.3; **B.** Aorta from an animal from the same group, almost complete lack of lipid substances in the wall. Staining Sudan-Schwartz, magnification 10.0 × 6.3.

unchanged levels of HDL-Chol. The triglycerides and the atherogenic lipid index showed a reduction though without significant differences (Table 4). The changed as percents were as follows: TC—8.86%, LDL-Chol—10.8%, TG—21.10% and the ratio TC/HDL-Chol—5.2%.

The values of TC and LDL-Chol in three subjects with DLP treated with "Bulgaricum" after preceding drug therapy sustained about the initial levels without a tendency to increase and 4 individuals showed slight reduction. Five of the subjects with TC $>$ 7.0 mmol/L and/or TG $>$ 2.2 mmol/L recorded decreased lipid indices. Thus certain individuals with severe DLP, IHD and/or present carotid atherosclerosis could be favorably influenced by liquid "Bulgaricum" intake. Other researchers also reported significant hypocholesterolemic effect of yogurt fermented with LB and Str. Thermophilus as well as decreased TC synthesis [7,8,24]. LB unlike other lactic microorganisms is exogenous for the organism, but has the

Table 4
Mean values and standard deviation of studied lipid indicators before and after treatment with LB stain GB N1 (48)

Indicators mmol/L	mean ± SD before	after	P
TC	6.21 ± 1.11	5.66 ± 0.83	$p < 0.01$
HDL-Chol	1.38 ± 0.41	1.32 ± 0.38	n.s.
LDL-Chol	4.00 ± 1.04	3.57 ± 0.93	$p < 0.05$
TG	1.99 ± 1.41	1.57 ± 0.82	n.s.
TC/HDL-Chol	4.97 ± 1.70	4.71 ± 1.61	n.s.

property to adapt to the conditions in the human intestinal tract for a longer time. LB, introduced as a natural ferment of Bulgarian fermented milk survives not only during consumption but is preserved up to 14 days after [3]. H. Haenel explained the absence of LB in intestinal content of test subjects with the inability of the respective strains of Middle-European strains to survive and adapt to intestinal conditions [25].

The mean values of the determined indicators with Doppler sonography are presented in Table 5. None of the indices determined as mean group values showed statistically significant difference before and after "Bulgaricum" intake. Five subjects had PI > 1.0 and $V_{max}/V_{min} > 2.7$ of right a. carotis communis basically and recorded reduced indices after product supplementation. Supplementing "Bulgaricum" to the diet for only three months does not allow categorical conclusions about its effect on the blood flow in aa. carotides, respectively for mitigated progression or regression of present atherosclerotic plaques.

Subjects treated with liquid "Bulgaricum" showed very good tolerance to the product and assessed its taste as agreeable. There were no reported side effects due to intake of the product fermented with LB strain GB N1 (48).

4. Conclusions

The experimental studies evidence the milk product "Bulgaricum" containing LB strain GB N1 (48) has hypolipidemic and protective cardiovascular effect. At combining athero-

Table 5
Indices from Doppler sonography of left and right *A. carotis*

Indexes	mean ± SD left a. carotis before	after	mean ± SD right a. carotis before	after
RI	0.70 ± 0.11	0.68 ± 0.07	0.74 ± 0.14	0.75 ± 0.11
PI	1.27 ± 0.28	1.22 ± 0.20	1.41 ± 0.42	1.47 ± 0.42
V_{max}/V_{min}	3.16 ± 1.04	2.95 ± 0.53	3.54 ± 1.48	3.56 ± 1.49
V_{min}/V_{max}	0.34 ± 0.08	0.35 ± 0.07	0.33 ± 0.12	0.30 ± 0.11
V_{max}	0.75 ± 0.22	0.70 ± 0.17	0.74 ± 0.25	0.72 ± 0.19
V_{min}	0.23 ± 0.11	0.22 ± 0.07	0.19 ± 0.09	0.18 ± 0.08
V_{mean}	0.41 ± 0.13	0.38 ± 0.09	0.38 ± 0.08	0.35 ± 0.09

genic diet and "Bulgaricum" the degree of sclerotic changes in vessels and myocardium as well as hypercholesterolemia are less manifested in comparison with those at modeled sclerotic processes. The favorable effect is expressed in delayed synthesis of collagen proteins, tendency to diminished deposition of insoluble collagen, improved ratio of non-sulfated and sulfated GAG—a prerequisite for delayed development of sclerotic progress and protection of the vessel wall against plaque formation.

The milk product "Bulgaricum" has a moderate hypocholesterolemic effect and is an adequate dietetic supplement for subjects with DLP. More expressed hypolipidemic and antisclerotic effect could be expected after longer diet supplementation with this product. Although the observed stabilizing effect, in cases with severe DLP combined with IHD and/or present carotid atherosclerosis it is recommended to combine "Bulgaricum" with hypolipidemic drugs.

Acknowledgments

Nadezhda Iv. Doncheva designed the study, performed the clinical part on serum lipids and prepared the manuscript.

Georgi P. Antov executed the experimental part of the study.

Ekaterina B. Softova did the morphological part of the study.

Yuri P. Nyagolov performed Doppler sonography, prepared the bibliography and the manuscript.

The authors thank Prof. Maria Stefanova, Director of "Genesis" Laboratories, who produced and supplied liquid "Bulgaricum" for this study.

References

[1] Grigoroff St. Bacillus bulgaricus. Revue medicale de la Suisse romande 1905;25:714.
[2] Metchnikoff I. Quelgues mots sur le lactobacille. Comptes rendus de l'Academie des Sciences 1908:14.
[3] Nachev L, Antonova T, Nikoevska T. Lactic acid bacteria in Bulgarian sour milk. The effect of some chemical and physical factors on the antimicrobial activity of Lactobacterium bulgaricum strain. Izv Mikrobiol Inst (Sofiia) 1972;23:41–6.
[4] Kondratenko M, Gosheva B, Mutafova K, Shishkova A. Objective evaluation of the aroma and taste of Bulgarian sour milk. Antibiotiki 1972;17:493–6 [in Russian].
[5] Nakamura I, Anzai H. Influences of lactic acid bacteria on animal bodies. Amino acids requirement of lactic acid bacteria in culture medium. Kitasato Arch Exp Med 1971;44:21–36.
[6] Hepner G, Fried R, St. Jeor S, Fusseti L. Morin R. Hypocholesterolemic effect of yogurt and milk. Am J Clin Nutr 1979;32:19–24.
[7] Howard AN, Marks J. Hypocholesterolemic effect of milk. Lancet 1977;2:255–6.
[8] Mann GV. A factor in yogurt which lowers cholesterolemia in man. Atherosclerosis 1977;26:335–40.
[9] Rao DR, Chawan CB, Pulusani SR. Influence of milk and thermophilus milk on plasma cholesterol levels and hepatic cholesterolegenesis in rats. J Food Sci 1981;46:1339–41.
[10] Wytock DH, Dipalma JA. All yogurts are not created equal. Am J Clin Nutr 1988;47:454–7.
[11] Kiyosawa H, Sugawara C, Sugawara N, Miyake H. Effect of skim milk and yogurt on serum lipids and development of sudanophilic lesions in cholesterol-fed rabbits. Am J Clin Nutr 1984;40:479–84.

[12] Gomez-Gil B, Roque A, Turnbull JF, Inglis V. A review on the use of microorganisms as probiotics. Rev Latinoam Microbiol 1998;40:166–72.
[13] Korshunov VM, Efimov BA, Pikina AP. The characteristics of biological preparations, and food additives for functional nutrition, and the correction of the intestinal microflora. Zh Mikrobiol Epidemiol Immunobiol 2000;3:86–91 [in Russian].
[14] Roberfroid MB. Prebiotics and probiotics: are they functional foods? Am J Clin Nutr 2000;71(Suppl. 6):1682S–7S.
[15] Anbert D. Atherogenese experimentale chez le rat Wistar. Atherosclerosis 1974;20:263–80.
[16] Antov G. Hygienic significance of immediate and remote effects of exposure to toxic compounds on the cardiovascular system. D.Sc. Thesis, Sofia, 1986;156 [in Bulgarian].
[17] Slutskii LI. Method for studying soluble collagen. In: Sluzkii L, editor. Biochemistry of normal and pathologically changed cellular tissue. Leningrad: Meditzina Publ., 1969. p. 89–92 [in Russian].
[18] Chrapil M. The metabolic synthetic function: connective tissue metabolism. In: Chrapil M, editor. Physiology of connective tissue. Prague: Czechos. Med. Press, 1967. p. 206–9.
[19] Franson L. Structure of dermatansulfate. Carbohydr Res 1970;15:73–82.
[20] Allain C, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470–5.
[21] Megrow R, Dunn D, Biggs HG. Manual and continuous-flow colorimetry of triacyglyceroles by a fully enzymic method. Clin Chem 1979;25:273–8.
[22] Friedewald WT, Levy RJ, Fredrickson DS. Estimation of the concentration of the low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifige. Clin Chem 1972;18:499–502.
[23] Shotekov P. Doppler sonography of extracranial arteries and veins. In: Schotekov P, editor. Sofia: Medical Institute “Lider Press”, 1998. p. 57–140 [in Bulgarian].
[24] Bazzare T, Shih-min Liu Wu, Yuhas JA. Total and HDL-cholesterol concentrations following yogurt and calcium supplementation. Nutrition Reports International 1983;28:1225–32.
[25] Haenel HA. Milchwissenschaft 1963;18:5, 215–21.