



Probiotic *Lactobacillus plantarum* may reduce cardiovascular risk: An experimental study

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Original Article

Abstract

BACKGROUND: Reduction of cardiovascular risk (CVR) is based on the correction of risk factors, especially dyslipidemia. Due to the limiting factors of conventional lipid-lowering medications, the investigation of alternative approaches is necessary.

METHODS: The present open, comparative, randomized, and parallel investigation was conducted on 77 patients. Participants were of both sexes, 40-74 years-of-age, and had dyslipidemia. The participants were divided into 2 groups; the treatment group (n = 41) received a combination of *Lactobacillus plantarum* and simvastatin 20 mg once a day, and the control group (n = 36) received simvastatin 20 mg once a day. The trial included 5 visits; screening on the first 2, and treatment on the next 3 (on weeks 4, 8, and 12). On visits 1, 3, 4, and 5, the lipid profile was evaluated and CVR was calculated using 5 tools.

RESULTS: The combination treatment led to a more pronounced decrease in total cholesterol (TC) and low-density lipoproteins (LDL) after 8 weeks (P = 0.002 and 0.016, respectively), that persisted after 12 weeks (P < 0.001 and 0.002, respectively). Reduction in TC and LDL by ≥ 20% was observed more predominantly in the treatment group. A significant reduction was observed in CVR in the treatment group according to the Prospective Cardiovascular Münster (PROCAM) score (P = 0.004). Reduction of CVR by ≥ 20% was mostly observed as a result of prescribing combination therapy according to the Framingham Risk Score (70.7%; P = 0.003), 2013 ACC/AHA ASCVD Risk Calculator (51.2%; P = 0.035), PROCAM (65.9%; P < 0.001), and WHO CVD risk chart (56.1%; P = 0.012).

CONCLUSION: Additional supplementation with *Lactobacillus plantarum* was more effective in the reduction of TC, LDL, and CVR according to PROCAM and the attainment of treatment goals regarding lipid profile and CVR levels.

Keywords: Dyslipidemias; Heart Disease Risk Factors; *Lactobacillus Plantarum*

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Introduction

Cardiovascular diseases (CVDs) are the leading causes of morbidity and mortality from non-communicable diseases in the world.¹⁻⁵ Atherosclerotic CVD (ASCVD) has numerous risk factors. Dyslipidemia, obesity, diabetes mellitus (DM), arterial hypertension, and smoking have different impacts on cardiovascular risk (CVR) level. Dyslipidemia due to high total cholesterol (TC) and low-density lipoproteins (LDL) is one of the most influential risk factors of CVD.^{6,7} Many CVR assessing scales are available that consider

the levels of different types of lipoproteins and evaluate their impact on CVR.^{2,8-10}

According to guidelines, there are different treatment options for the management of dyslipidemia.^{6,11}

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Lifestyle modification, and normalization of body weight and blood pressure should be recommended to all patients, but they may be effective in case of low to medium CVR. Patients with higher levels of CVR require the additional prescription of medication. Statins, ezetimibe, and PCSK9 inhibitors are commonly used to decrease the level of LDL. However, the possible side effects of statins and other drugs, and the high cost of new medications limit their practical application. Therefore, there is a need for the investigation of new lipid-lowering remedies.

The latest *in vitro*^{12,13} and *in vivo* investigations on animal^{14,15} and human models^{16,17} have revealed the possible lipid-lowering effect of different probiotic bacteria. Of particular interest are bile salt hydrolase (BSH) expressing bacteria, like Lactobacilli, the effect of which is due to the enzyme activity of BSH.^{18,19} Through the deconjugation of primary bile acids (BA) in the intestines to secondary BA, bacterial BSH decreases the reabsorbed pool of BA that subsequently stimulates the *de novo* synthesis of BA from free cholesterol in the liver.^{20,21} Our previous preliminary investigation²² also revealed that probiotic Lactobacillus plantarum in combination with statins are more effective in decreasing TC and LDL compared with statin monotherapy. However, there are still insufficient data regarding the clear effect of BSH-positive bacteria on lipoprotein levels. In addition, we did not find any data regarding the relation between the lipid-lowering effect of probiotic bacteria and CVR assessed using risk scales.

The aim of the current investigation was to compare the effectiveness of combination therapy [capsules containing 2×10^9 colony-forming unit (CFU) of live active strain of Lactobacillus plantarum per capsule and tablets containing simvastatin 20 mg] with monotherapy (tablets containing simvastatin 20 mg) in reducing CVR assessed using risk scores in patients with dyslipidemia.

Materials and Methods

This clinical investigation was conducted in accordance with the Ukrainian laws, the requirements of Good Clinical Practice, and ethical principles of the Declaration of Helsinki. Written informed consent for participation in the investigation was obtained from all participants before the trial began. The protocol was approved by the Bioethical Committee of Bogomolets National Medical University, Kyiv, Ukraine.

Participants: The study population consisted of

patients of both sexes, aged 40-74 years, with dyslipidemia, and without previous history of major cardiovascular events. The inclusion criteria were men and women aged 30-74 years, LDL level ≥ 3.0 mmol/l, TC level > 5.0 mmol/l, no previous intake of statins or intake of statins more than 6 months before screening, a negative pregnancy test result for reproductive women, and an informed written consent. The exclusion criteria included increased sensitivity to the investigational drugs, administration of any lipid-lowering drugs for 4 weeks before screening, pregnancy and lactation, previous history of major cardiovascular events [myocardial infarction (MI), or stroke], chronic liver disease (CLD) with elevation of liver enzymes to more than 3 times the upper limit of normal, any acute diseases within 2 months before the start of the investigation, myopathy, endocrine diseases, arterial hypotension, alcohol abuse, concomitant administration of active CYP3A4 inhibitors, and participation in other clinical trials.

Study design: The present open, comparative, randomized, parallel investigation was conducted in 2 phases, a screening phase of up to 5 days and the phase of treatment with investigational drugs combinations for 12 weeks. Patients were monitored on an outpatient basis and visited the research center at scheduled visit dates. Deviations of 1-2 days from scheduled visit dates were allowed.

A total of 82 patients were selected for the treatment phase, and 5 patients were eliminated from the investigation, 3 due to personal circumstances and 2 due to side effects of simvastatin (myalgia). All phases of investigation were completed by 77 patients, who were divided into the 2 groups of treatment ($n = 41$; patients received combination therapy in the form of 1 Lactobacillus plantarum capsule once a day and 1 simvastatin tablet once a day) and control ($n = 36$; patients received monotherapy in the form of 1 simvastatin tablet once a day). All participants received standard lifestyle and dietary recommendations according to European guidelines.⁶ During the study, the use of prebiotics, probiotics, antibiotics, laxatives, and other hypolipidemic agents was prohibited. Participants with arterial hypertension took hypotensive drugs according to recommendations.²³

Investigational drugs: Capsules containing 2×10^9 CFU of live active strain of Lactobacillus plantarum per capsule were manufactured by Biopharma LLC, Kyiv, Ukraine. The simvastatin tablets (Zocor 20 mg dose) were manufactured by Merck Sharp and Dohme Idea Inc., Grad Beograd, Serbia.

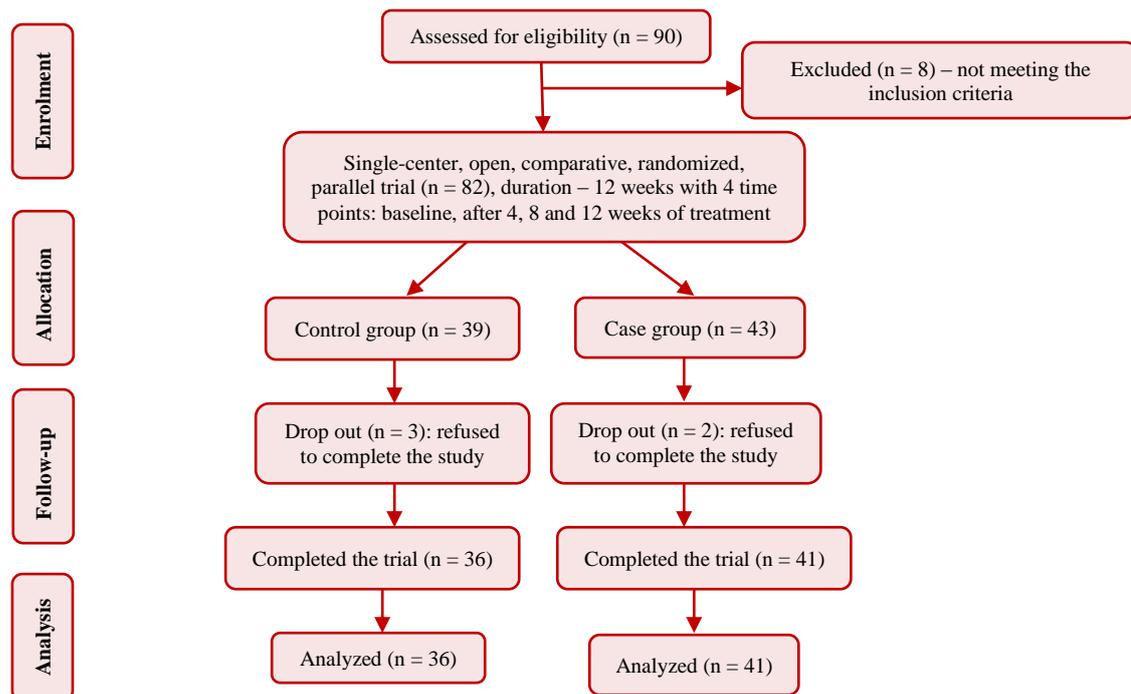


Figure 1. The flow chart of the study

Study visit schedules: The trial included 5 visits, the first 2 visits for the screening phase and the next 3 visits for the treatment phase (on weeks 4, 8, and 12 since the start of the study). Anamnesis, written informed consent forms were obtained from all participants during the first visit. The patients' were assessed in terms of the inclusion criteria and were randomly divided into groups, and the appropriate investigational drugs were administered on the second visit (2-3 days after the first visit). Objective examination, anthropometric measurements [body mass index (BMI) calculation], and blood pressure measurements were performed on the first, third, fourth, and fifth visits, and electrocardiogram (ECG) was performed during the first and fifth visits. For patients with arterial hypertension, appropriate hypotensive drug combinations were prescribed.

Biochemical blood analyzes: Blood samples were collected for the measurement of TC, LDL, high-density lipoprotein (HDL), triglyceride (TG), and creatine phosphokinase (CPK) and liver tests during study visits 1, 3, 4, and 5. Enzymatic methods were used to assess the level of TC, LDL, HDL, and TG. Analyses were performed using a biochemistry analyzer Cobas 6000 with appropriate reagent kits (Roche Diagnostics, Switzerland).

Cardiovascular risk assessment: During study visits 1, 3, 4, and 5, CVR levels were calculated using 5 validated risk scores, including the Globorisk tool,⁸ Framingham Risk Score (10-year CVD risk

estimation),⁹ [American College of Cardiology (ACC)/ American Heart Association (AHA) ASCVD] Risk Calculator (10-year risk of heart disease or stroke; algorithm published in 2013),²⁴ Prospective Cardiovascular Münster (PROCAM) Score,¹⁰ and World Health Organization (WHO) CVD risk chart.²

Efficacy endpoints: The primary efficacy endpoints were absolute changes in TC, LDL, and CVR levels calculated using validated risk scores during the investigation periods and at the completion of the study. Treatment was considered effective in case of achieving a 20% or more reduction in TC, LDL, and CVR levels compared to baseline.

Statistical analysis: The statistical analysis of the obtained data was performed using SPSS software (version 23, IBM Corp., Armonk, NY, USA). All continuous variables were checked in terms of normality using the Shapiro-Wilk test. In case of normal distribution, the data were presented as arithmetic mean and standard deviation (Mean \pm SD), and in case of non-normal distribution, as median with first and third quartiles [Median (Q1-Q3)]. To assess the difference between the means of the 2 groups, the independent (unpaired) t-test (in case of normal distribution) or Wilcoxon 2-sample test (in case of non-normal distribution) were used. The difference between the values at different study time points (baseline, and weeks 4, 8, and 12) were analyzed using one-way repeated

measures analysis of variance (ANOVA) with prior implementation of Mauchly's test of sphericity (in case of normal distribution) or the Friedman test (in case of non-normal distribution). Post-hoc analysis was performed using paired t-test with Bonferroni correction (after repeated measures ANOVA) or Wilcoxon rank test with Bonferroni correction (after Friedman test). The clinical effects were evaluated using odds ratios. The difference between the study groups was considered statistically significant at $P < 0.050$.

Results

Baseline characteristics of the compared groups: There were no statistically significant differences between the treatment and control groups at baseline (Table 1).

Body mass index and systolic blood pressure of the compared groups at different study time points: There were no significant differences in terms of BMI within groups at different study time points (one-way repeated measures ANOVA with prior performance of Mauchly's test of sphericity; $P > 0.100$ in both groups) and between the treatment and control groups (unpaired t-test; $P > 0.1$ in both groups).

SBP differed significantly in the control group at different study time points (repeated measures ANOVA with prior performance of Mauchly's test of sphericity; $P < 0.001$). The post-hoc analysis showed significant differences in SBP levels between baseline and weeks 4, 8, and 12 ($P < 0.050$ in all), but no significant differences between weeks 4, 8, and 12.

SBP levels differed significantly at different study time points in the treatment group (repeated measures ANOVA with prior performance of Mauchly's test of sphericity, $P < 0.001$). The post-

hoc analysis showed significant differences in SBP levels between baseline and weeks 4, 8, and 12 ($P < 0.050$ in all), but showed no significant differences between weeks 4, 8, and 12.

There were no statistically significant differences in SBP between the treatment and control groups at different study time points (unpaired t-test; $P > 0.100$ in both groups).

Treatment of hypertension: After 4 weeks, the percentage of participants treated with hypotensive therapy had increased in both groups, 44.4% in the control group and 51.2% in the treatment group, but this difference was not significant ($P = 0.712$), that was unchanged also after 8 and 12 weeks. There were no significant differences within the groups at different study time points.

Lipid profile of the compared groups: The TC and LDL levels in the control group were significantly different at different study time points ($P < 0.001$ and $P < 0.001$, respectively). The post-hoc analysis showed a significant difference in TC and LDL levels between baseline and weeks 4, 8, and 12 ($P < 0.050$ in all), but not between weeks 4, 8, and 12. The HDL and TG levels did not differ significantly in the control group at different study time points (Table 2).

TC and LDL levels were significantly different at different study time points in the treatment group ($P < 0.001$ and $P < 0.001$, respectively). The post-hoc analysis showed significant differences in TC and LDL levels between baseline and weeks 4, 8, and 12 ($P < 0.050$ in all), but showed no significant differences between weeks 4, 8, and 12. HDL and TG levels in the treatment group were not significantly different at different study time points (Table 2).

Table 1. Baseline characteristics of the treatment and control group participants

Variable	Control group (n = 36)*	Treatment group (n = 41)*	P**
Sex [n (%)]			
Men	8 (22.2)	13 (31.7)	0.500
DM [n (%)]	16 (44.4)	20 (41)	0.881
Smoking [n (%)]	10 (27.8)	13 (31.7)	0.897
Treatment of hypertension (%)	27.8	36.6	0.564
Age	60.9 ± 8.7	57.7 ± 10.3	0.143
BMI	27.0 ± 3.3	28.0 ± 4.0	0.223
SBP (mmHg)	135.0 ± 16.0	134.0 ± 14.0	0.619
HDL (mmol/l)	1.4 ± 0.3	1.4 ± 0.5	0.819
SBP (mmHg)	135.0 ± 16.0	134.0 ± 14.0	0.619
TC (mmol/l)	5.9 (5.6-6.5)	5.7 (5.5-6.5)	0.537
LDL (mmol/l)	4.0 (3.7-4.5)	4.0 (3.8-4.5)	0.302

BMI: Body mass index; DM: Diabetes mellitus; SBP: Systolic blood pressure; TC: Total cholesterol; LDL: Low-density lipoproteins; HDL: High-density lipoproteins; TG: Triglycerides

* Normally and non-normally distributed data were presented as mean ± standard deviation (SD) and median (Q1-Q3), respectively.

** For normal and non-normal distribution, t-test and Wilcoxon two-sample test were used, respectively.

Table 2. Lipid profile in the control and treatment groups at different study time points

Study time point	TC (mmol/l)*	LDL (mmol/l)*	HDL (mmol/l)*	TG (mmol/l)*
Control group				
Baseline	5.9 (5.6-6.5)	4.0 (3.7-4.5)	1.4 ± 0.3	1.5 (0.9-2.9)
4 weeks	4.9 (4.4-5.7)	3.2 (2.6-3.6)	1.4 ± 0.3	1.6 (1.04-2.5)
8 weeks	5.1 (4.8-6.0)	3.3 (3.1-3.7)	1.5 ± 0.3	1.3 (0.9-2.1)
12 weeks	5.3 (4.5-5.8)	3.4 (2.9-3.6)	1.4 ± 0.4	1.6 (1.0-2.2)
P**	< 0.001	< 0.001	0.600	0.338
Treatment group				
Baseline	5.8 (5.5-6.5)	4.0 (3.8-4.5)	1.4 ± 0.5	1.3 (0.7-1.8)
4 weeks	4.8 (4.4-5.0)	3.0 (2.7-3.3)	1.4 ± 0.5	1.1 (0.7-2.0)
8 weeks	4.8 (4.4-5.1)	3.1 (2.7-3.5)	1.4 ± 0.4	1.1 (0.8-1.5)
12 weeks	4.8 (4.0-4.9)	3.0 (2.6-3.3)	1.3 ± 0.4	1.1 (0.7-1.4)
P**	< 0.001	< 0.001	0.387	0.987

TC: total cholesterol; LDL: low-density lipoproteins; HDL: high-density lipoproteins; TG: triglycerides

* Normally and non-normally distributed data were presented as mean ± standard deviation (SD) and median (Q1-Q3), respectively.

** For normally and non-normally distributed data, one-way repeated measures ANOVA and the Friedman test were used, respectively.

The post-hoc analysis showed significant differences in TC and LDL levels between baseline and weeks 4, 8, and 12 ($P < 0.05$ in all), but showed no significant differences between weeks 4, 8, and 12. HDL and TG levels in the treatment group were not significantly different at different study time points (Table 2).

The comparison of lipid profile parameters between the groups showed that TC and LDL levels were significantly lower in the treatment group after weeks 8 and 12 of investigation ($P < 0.050$ in all). However, HDL levels did not differ significantly. TG levels were lower in the treatment group after weeks 4 and 12 of investigation ($P < 0.050$ at both times) (Table 3).

After 12 weeks of investigation, TC reduced by 20% or more in 63.4% of the participants in the treatment

group compared with 27.8% of the participants in the control group [Odds ratio (OR) = 2.28; 95% confidence interval (95%CI): 1.28-4.06; $P = 0.004$].

After 12 weeks of investigation, LDL reduced by 20% or more in 85.5% of the participants in the treatment group compared with 41.7% of the participants in the control group (OR = 2.05; 95%CI: 1.36-3.08; $P < 0.001$).

Cardiovascular risk levels of compared groups: The CVR levels calculated using the 5 validated risk scores in the control group were significantly different at different study time points ($P < 0.001$ in all comparisons) (Table 4). In the post-hoc analysis, all 5 risk scores differed significantly between baseline and weeks 4, 8, and 12 ($P < 0.050$ in all comparisons). However, the values of the 5 risk scores were not significantly different at weeks 4, 8, and 12.

Table 3. Lipid profile in the control and treatment groups at different study time points

Lipid parameter	Control group (n = 36)*	Treatment group (n = 41)*	P**
After 4 weeks			
TC (mmol/l)	4.9 (4.4-5.7)	4.8 (4.4-5.0)	0.070
LDL (mmol/l)	3.2 ± 0.8	3.0 ± 0.5	0.298
HDL (mmol/l)	1.4 ± 0.3	1.4 ± 0.5	0.676
TG (mmol/l)	1.7 ± 1.0	1.3 ± 0.8	0.043
After 8 weeks			
TC (mmol/l)	5.4 ± 0.9	4.8 ± 0.6	0.002
LDL (mmol/l)	3.5 ± 0.7	3.1 ± 0.6	0.016
HDL (mmol/l)	1.5 ± 0.3	1.4 ± 0.4	0.929
TG (mmol/l)	1.3 (0.9-2.1)	1.1 (0.8-1.5)	0.115
After 12 weeks			
TC (mmol/l)	5.3 (4.5-5.8)	4.8 (4.0-4.9)	< 0.001
LDL (mmol/l)	3.4 (2.9-3.6)	3.0 (2.6-3.3)	0.002
HDL (mmol/l)	1.4 ± 0.4	1.3 ± 0.4	0.486
TG (mmol/l)	1.6 (1.0-2.2)	1.1 (0.7-1.4)	0.007

TC: total cholesterol; LDL: low-density lipoproteins; HDL: high-density lipoproteins; TG: triglycerides

* Normally and non-normally distributed data were presented as mean ± standard deviation (SD) and median (Q1-Q3), respectively.

** For normal and non-normal distribution, t-test and Wilcoxon two-sample test were used, respectively.

Table 4. The levels of cardiovascular risk (CVR) calculated using validated risk scores in the control and treatment group at different study time points

Study time point	Globorisk (%) [*]	Framingham (%) [*]	2013 ACC/AHA ASCVD Risk Calculator (%) [*]	PROCAM (points) [*]	WHO risk chart (%) [*]
Control group					
Baseline	41.5 (15-54.5)	20.2 (9.2-33.2)	14.3 ± 10.5	48.9 ± 11.3	22.4 ± 13.1
4 weeks	36 (14-48)	15.6 (7.1-25)	12.5 ± 9.4	42.6 ± 11.2	18.8 ± 11.6
8 weeks	37.5 (14-52)	15.5 (7.4-25.3)	12.7 ± 9.1	43.4 ± 9.9	19.8 ± 12.2
12 weeks	35 (12-51)	15.9 (7.7-28.35)	12.7 ± 9.2	44.4 ± 9.6	19.4 ± 11.9
P ^{**}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Treatment group					
Baseline	29 (15-60)	17 (9.7-33.5)	8.2 (3.4-21)	50 (39-58)	20.2 ± 14.2
4 weeks	23 (11-43)	12.7 (7.1-21.9)	6.4 (2.2-16.8)	39 (31-49)	16.5 ± 11.6
8 weeks	22 (13-48)	11.9 (6.1-27)	5.6 (2.2-16.2)	38 (32-45)	16.5 ± 11.8
12 weeks	19 (13-48)	11.4 (7.3-21.9)	6 (2.9-16.8)	37 (31-41)	15.8 ± 11.6
P ^{**}	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

ACC: American College of Cardiology; AHA: American Heart Association; ASCVD: Atherosclerotic cardiovascular disease; PROCAM: Prospective Cardiovascular Münster; WHO: World Health Organization

* Normally and non-normally distributed data were presented as mean ± SD and median (Q1-Q3), respectively.

** For normally and non-normally distributed data, one-way repeated measure ANOVA and the Friedman test were used, respectively.

The levels of CVR calculated using the 5 risk scores were significantly different at different study time points in the treatment group ($P < 0.001$ in all comparisons) (Table 4). In the post-hoc analysis, the levels of all 5 risk scores differed significantly between baseline and weeks 4, 8, and 12 ($P < 0.001$ in all comparisons). The values of 4 scores, except the WHO CVD risk chart, did not differ significantly between weeks 4, 8, and 12. The value of the WHO CVD risk chart was significantly lower after 12 weeks compared to after 4 and 8 weeks

($P < 0.001$ in both comparisons), but there was no significant difference between weeks 4 and 8.

The comparison of the values of CVR calculated using the validated risk scores between the control and treatment group showed that the PROCAM score was significantly lower after 12 weeks in the treatment group compared to the control group ($P = 0.004$). There were no statistically significant differences between the groups at different study time points in terms of values of the other risk scores (Table 5).

Table 5. The levels of cardiovascular risk calculated using validated risk scores in control and treatment group at different study time points

CVR risk score	Control group (n = 36) [*]	Treatment group (n = 41) [*]	P ^{**}
Baseline			
Globorisk (%)	41.5 (15-54.5)	29 (15-60)	0.369
Framingham (%)	22.1 ± 14.2	23.0 ± 16.5	0.796
2013 ACC/AHA ASCVD Risk Calculator (%)	14.3 ± 10.5	13.3 ± 11.5	0.690
PROCAM (points)	48.9 ± 11.3	48.3 ± 12.1	0.819
WHO risk chart (%)	22.4 ± 13.3	20.2 ± 14.2	0.486
After 4 weeks			
Globorisk (%)	33.7 ± 20.6	28.1 ± 19.8	0.226
Framingham (%)	15.6 (7.1-25)	12.7 (7.1-21.9)	0.561
2013 ACC/AHA ASCVD Risk Calculator (%)	11.8 (2.8-18.7)	6.4 (2.2-16.8)	0.291
PROCAM (points)	42.6 ± 11.2	40.0 ± 11.5	0.306
WHO risk chart (%)	18.8 ± 11.6	16.5 ± 11.7	0.398
After 8 weeks			
Globorisk (%)	37.5 (14-52)	22.0 (13-48)	0.196
Framingham (%)	15.5 (7.4-25.3)	11.9 (6.1-27)	0.366
2013 ACC/AHA ASCVD Risk Calculator (%)	10.9 (4.45-20)	5.6 (2.2-16.2)	0.176
PROCAM (points)	42.5 (35-51)	38.0 (32-45)	0.050
WHO risk chart (%)	19.8 ± 12.2	16.5 ± 11.8	0.230
After 12 weeks			
Globorisk (%)	35.0 (12-51)	19.0 (13-48)	0.244
Framingham (%)	15.9 (7.7-28.4)	11.4 (7.3-21.9)	0.249
2013 ACC/AHA ASCVD Risk Calculator (%)	11.1 (3.9-19)	6.0 (2.9-16.8)	0.195
PROCAM (points)	45.5 (38-50.5)	37.0 (31-41)	0.004
WHO risk chart (%)	19.4 ± 11.9	15.8 ± 12	0.188

ACC: American College of Cardiology; AHA: American Heart Association; ASCVD: Atherosclerotic cardiovascular disease; WHO: World Health Organization

* Normally and non-normally distributed data were presented as mean ± standard deviation (SD) and median (Q1-Q3), respectively.

** For normally and non-normally distributed data, t-test or Wilcoxon two-sample test were used, respectively

After 12 weeks of investigation, CVR level was decreased by 20% or more:

- 1) according to the Framingham Risk Score (10-year CVD risk estimation) in 70.7% of the participants in the treatment group compared with 33.3% in the control group (OR = 2.12; 95%CI: 1.28-3.51; P = 0.003).
- 2) according to the 2013 ACC/AHA ASCVD Risk Calculator in 51.2% of the participants in the treatment group compared with 25.0% in the control group (OR = 2.05; 95%CI: 1.08-3.88; P = 0.035).
- 3) according to the PROCAM score in 65.9% of participants in the treatment group compared with 25.0% in the control group (OR = 2.63; 95%CI: 1.44-4.83; P < 0.001).
- 4) according to the WHO CVD risk chart in 56.1% of participants in the treatment group compared with 25.0% in the control group (OR = 2.24; 95%CI: 1.2-4.2; P = 0.012).

Safety assessment: During the investigation, several cases of liver enzymes elevation that did not exceed the upper 2 normal limits and muscle pain episodes were reported. Nevertheless, these were expected side effects of simvastatin that did not require their elimination. Other side effects such as different gastrointestinal disorders were not reported.

Discussion

The results of the present study confirm the preliminary data from our previous studies in terms of the lipid-lowering capacity of *Lactobacillus plantarum*.²² We found that the combination of simvastatin with probiotic bacteria *L. plantarum* leads to a statistically more pronounced decrease in TC and LDL after 8 weeks, which persists after 12 weeks of treatment compared with simvastatin monotherapy. The primary efficacy endpoints (reduction of TC and LDL by 20% and more) were achieved statistically in more cases when prescribing combination therapy with *L. plantarum* (63.4%, OR = 2.28; 95%CI: 1.28-4.06, and 85.5%, OR = 2.05; 95%CI: 1.36-3.08, respectively).

These results correlate with data presented in international literature^{25,26} and complement them. Our findings indicate that additional supplementation with *L. plantarum* may have an additional hypocholesterolemic effect in patients receiving small doses of statins. The effect of this kind of bacteria is most likely due to the production of bile salt hydrolase,¹⁹ which removes secondary bile acids from entero-hepatic circulation and in this way stimulates the de-novo synthesis of bile acids in

the liver from free plasma cholesterol. However, other mechanisms are also possible like production of propionic and butyric acid,²⁷ and reduction of cholesterol absorption in the intestines.²⁷

Furthermore, we found a statistically significant decrease in TG after 4 and 12 weeks of treatment with combination therapy compared with simvastatin monotherapy, but this effect was not seen after 8 weeks of treatment. Therefore, future investigations are needed to clarify the relations between TG and *L. plantarum* supplementation. The combination treatment with *L. plantarum* did not have any effect on HDL levels in comparison with simvastatin monotherapy.

Many investigations, systemic reviews, and meta-analyses have shown that modification of LDL and TC has an impact on decreasing potentially fatal and non-fatal cardiovascular events, and decreasing general and cardiovascular mortality.^{6,8,12,28,29} In addition, a more pronounced and intense decline in LDL is associated with a more pronounced decrease in mortality and CVR.²⁹

In clinical practice, different CVR scores are used to assess individual CV risk level and allow clinicians to prescribe a treatment and control its efficacy.^{2,8-10,24} To evaluate the impact of the lipid-lowering potency of additional supplementation of *L. plantarum* in our investigation we have chosen different validated risk scores, including the Globorisk tool,⁸ Framingham Risk Score (10-year CVD risk estimation),⁹ 2013 ACC/AHA ASCVD Risk Calculator (10-year risk of heart disease or stroke),²⁴ PROCAM Score,¹⁰ WHO CVD risk chart.² The 10-year risk of CVD mortality, 10-year risk of fatal and non-fatal CVD, 10-year risk of ASCVD (heart disease or stroke), the risk of an acute coronary event in the following 10 years, and 10-year risk of fatal and non-fatal CVD (MI and stroke) were, respectively, assessed.

All of them take into consideration age, level of SBP, smoking, and DM. Gender is included in all except the PROCAM score. Geographical region is included in all except the Globorisk score and WHO CVD risk chart. The treatment of hypertension is taken into consideration in all except the Framingham score and 2013 ACC/AHA ASCVD Risk Calculator. Family history of MI is included in all the tools except the PROCAM score, and race is included in all except the 2013 ACC/AHA ASCVD Risk Calculator. Regarding the lipid profile, TC is included in all except the PROCAM score, and LDL and TG are only included in the PROCAM score. In addition, HDL is included in the Framingham score,

2013 ACC/AHA ASCVD Risk Calculator, and PROCAM score. Therefore, each validated score includes its own set of metrics. Some of them are unchanging, such as gender, age, DM, family history of MI, and race. Some of them may change, such as region, and smoking status. Nevertheless, during the present investigation, they have been considered as unchanging; all participants were permanent citizens of Ukraine and smoking cessation did not allow us to exclude this factor from the risk score, since this factor may be offset as a risk factor after several years.³⁰ Treatment of hypertension, SBP level, and values of lipid profile were changing factors that influenced CVR scores in our investigation at different study time points. Before the beginning of the investigation, patients with uncontrolled arterial hypertension at baseline were prescribed hypotensive medications according to guidelines.²³ However, our results revealed that there were no significant differences within and between groups at different study time points in terms of the percentage of patients treated with antihypertensive drugs. Regarding SBP, our results showed that the level of SBP within the control and treatment groups was significantly different between baseline and weeks 4, 8, and 12, but not between weeks 4, 8, and 12. We considered this to be due to our hypotensive treatment recommendations and prompt monitoring of BP after 4, 8, and 12 weeks of investigation. Therefore, considering the changes in lipid profile values between different study time points, we proposed that lipid profile values had the most pronounced impact on CVR levels after weeks 8 and 12 of the present trial. CVR level calculation according to the 5 validated risk scores revealed a significant reduction in absolute levels of all scores within the control and treatment groups after 4, 8, and 12 weeks of investigation compared to baseline. This was probably due to both the correction of SPB and dyslipidemia. In the treatment group, the WHO CVD risk chart score after 12 weeks was significantly lower than after 4 and 8 weeks; this was most probably due to the treatment of dyslipidemia. During the subsequent comparisons between groups, a significant reduction was observed in PROCAM score after 12 weeks as a result of the combination therapy with *L. plantarum* compared with simvastatin monotherapy. In the absolute level, it was reduced from 45.5 (38-50.5) to 37 (31-41) points, which means a reduction in the 10-year risk of an acute coronary event from 10-20% to 5-10%.¹⁰ Therefore, additional supplementation with probiotic *L. plantarum* may lead to a reduction in the risk of an

acute coronary event in the following 10 years.

It was also revealed that primary efficacy endpoints regarding the reduction of CVR level by 20% or more were achieved in a significantly high number of participants who had received additional supplementation with *L. plantarum* according to 4 of the risk scores, except Globorisk tool score. Therefore, probiotic bacteria *L. plantarum* additionally supplemented with low doses of simvastatin may reduce CVR in a statistically larger number of cases compared to treatment with only low doses of simvastatin.

Nevertheless, the present study had some limitations such as lack of previous research studies regarding the relation between CVR levels and use of probiotics, and lack of use of placebo in the control group. Moreover, the obtained data allows us to draw conclusions for the Ukrainian population but cannot be generalized to other populations. Cultural food habits might influence individual response to prescribed investigational drugs even with the recommended standard diet. Moreover, the duration of the study only allows us to draw conclusions on a limited time period. Therefore, future investigations with a placebo-control group, greater sample size with the involvement of participants from other populations with different cultural food habits, and longer study duration and assessment of long-term effects are needed to confirm and expand the data of the present trial.

Conclusion

In summary, treatment with probiotic bacteria *Lactobacillus plantarum* in combination with low doses of simvastatin may lead to a more pronounced decrease in TC and LDL and attainment of treatment efficacy goals compared with simvastatin monotherapy in patients with dyslipidemia. These probiotic bacteria may also cause a more significant reduction in the absolute level of the 10-year risk of an acute coronary event from 10-20% to 5-10% according to the PROCAM score and a 20% or more reduction in CVR levels from baseline compared with simvastatin monotherapy in patients with dyslipidemia. Therefore, probiotic bacteria *L. plantarum* can be recommended as an additional hypolipidemic remedy. It reduces the side effects, costs, and other negative impacts of traditional lipid-lowering medications by lowering their dose.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contribution

AN, VC, and VS formulated the concept and designed the research. AN, VC, LH, VT, TN, and NM assessed the participants, and collected data. AN and VC analyzed and interpreted the obtained data. AN performed statistical analysis, and wrote the text of the article. VC, VS, LG, VT, TN, and NM critically revised the article. All authors approved the final manuscript.

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