ISSN 1996-353X
ISSN 2311-6951

DOI: 10.32345/USMYJ.3(117).2020.36-45

Neverovskyi Artem
PhD student of Department of internal medicine №1, Bogomolets National Medical University, Kyiv, Ukraine

Chernyavskyi Volodymyr
MD, PhD, professor of Department of internal medicine №1, Bogomolets National Medical University, Kyiv, Ukraine

Shypulin Vadim
MD, PhD, professor, Head of Department of internal medicine №1, Bogomolets National Medical University, Kyiv, Ukraine

Gvozdecka Lesya
MD, PhD, associate professor of Department of internal medicine №1, Bogomolets National Medical University, Kyiv, Ukraine

Mikhn`ova Nataliya
MD, PhD, associate professor of Department of internal medicine №1, Bogomolets National Medical University, Kyiv, Ukraine

MODIFICATION OF GUT BACTERIAL BILE SALT HYDROLASE ACTIVITY AND CARDIOVASCULAR RISK: A RANDOMIZED STUDY

Abstract. *Introduction: secretion of bacterial bile salt hydrolase (BSH) is one of the main mechanisms by which gut microbiota play role in cholesterol metabolism. There are limiting and controversial data regarding the clear effect of gut BSH activity correction on modification of serum cholesterol and cardiovascular risk (CVR). Aim of investigation was to evaluate the relationship between modification of the gut bacterial BSH relative activity (RA) by probiotic L. plantarum and serum cholesterol with CVR levels. Methods: the study was conducted as open, comparative, randomized, parallel and included 26 almost healthy participants (healthy control group) and 77 patients with dyslipidemia and without anamnesis of major cardiovascular events, that were divided in two groups: main treatment group (n=41) received combination therapy (capsules with Lactobacillus plantarum in the amount of 2*109 CFU one time a day and tablets simvastatin 20 mg one time a day) and control treatment group (n=36) received monotherapy (simvastatin 20 mg one time a day) during 12 weeks. Before and after 12 weeks of treatment the assessment of total RA of gut BSH, lipid profile and CVR level according to 5 risk scores were performed. Results: at baseline the RA of BSH was higher in healthy adults comparing to participants with dyslipidemia (p<0,001); after 12 weeks of treatment there wasn`t difference between healthy control and only main treatment groups (p=0,45). It was found that with increasing of RA of gut bacterial BSH, the risk of failure of treatment efficacy endpoints achievement (≥20% reduction of values) decreased regarding: total cholesterol (TC) (p=0,0306), OR=0,00133 (95% CI; 3,28*10-6-0,538); low-density lipoproteins (LDL) (p<0,001), OR=5,65*10-14(95% CI; 6,38*10-20-5*10-8); CVR level according to Framingham score (p=0,0035), OR=4,09*10-5(95% CI; 4,66*10-8-0,0359); CVR level according to 2013 ACC/AHA algorithm (p=0,0135), OR=3,8*10-4(95% CI; 7,34*10-7-0,197); CVR level according to PROCAM score (p=0,00125), OR=8,38*10-6 (95%; CI; 6,93*10-9-0,0101). Conclusions: additional supplementation with BSH-producing bacteria L. plantarum was more effective in increasing of BSH activity compared to simvastatin monotherapy. Increasing of BSH RA by L. plantarum was associated with higher chances to achieve treatment efficacy goals regarding reduction of TC, LDL and CVR levels according to Framingham, 2013 ACC/AHA algorithm and PROCAM scores.*

Key words: bile salt hydrolase activity, dyslipidemia, Lactobacillus plantarum, probiotic.

Introduction. Cardiovascular diseases (CVD) play the main role in global morbidity and mortality in the world (Mensah, Roth, & Fuster, 2019; WHO CVD Risk Chart Working Group [WCRCWG], 2019). Evolution and progression of CVD depends on different risk factors, like elevated blood pressure, diabetes mellitus, smoking, dyslipidemia etc. Hypercholesterolemia due to increasing of total cholesterol (TC) and low-density lipoproteins (LDL) is the significant cardiovascular risk (CVR) factor (Ference et al., 2017; Mach et al., 2020), reduction of which allow to decrease the risk of fatal and non-fatal CVD events (Baigent et al., 2010; Cholesterol Treatment Trialists' (CTT) Collaboration [CTTC], Silverman et al., 2016).

Regulation of lipids levels in the blood has different aspects, main are the de novo synthesis and absorption in intestines from food (Luo, Yang & Song, 2020). Cholesterol takes part in a lot of metabolic processes, including the producing of bile acids (BA), that requires quite big amount of cholesterol (Charach et al., 2017; Chiang, 2009; Luo et al., 2020). After entering the gut, BA salts undergo the deconjugation by bile salt hydrolase (BSH) synthesized by gut microbiome (Lau et al., 2017; Reis, Conceição, Rosa, Siqueira & Peluzio, 2017; Urdaneta & Casadesús, 2017) to secondary BA that are less soluble and evacuated from entero-hepatic circulation. This serves as a signal to liver to enhance the de novo producing of new BA from free serum cholesterol (Geng & Lin, 2016; Lau et al., 2017; Reis et al., 2017; Urdaneta & Casadesús, 2017). Hence, BSH may have lipid lowering effect.

With the lipid lowering aim, some investigations proposed to use the isolated form of BSH enzyme (Bi, Fang, Lu, Du &Chen, 2013; Sridevi, Vishwe & Prabhune, 2009) with promising results, but such approach is technically and economically inconvenient and has not been widely developed. But a lot of investigations on animal models (Kim et al., 2017; Yao, Tian, Song & Wang, 2020) and human trials (Costabile et al., 2017; Sharma, Kurpad & Puri, 2016; Wang et al., 2018; Wu, Zhang, Ren & Ruan, 2017) have revealed the hypocholesterolemic effect of different BSH-producing probiotic bacteria, for example different strains of *Lactobacilli* (Costabile et al., 2017; Huang, Ho, Chen, Hsu & Lin, 2019; Wang, Huang, Xia, Xiong & Ai, 2019; Wu et al., 2017; Yao et al., 2020). Our previous trial also shown that probiotic *Lactobacillus plantarum* may have effect in reducing levels of TC and LDL (Шипулін, Чернявський, Неверовський & Парунян, 2018).

The aim of present trial was to find the possible relationship between the modification of total relative activity (RA) of gut bacterial BSH by probiotic *Lactobacillus plantarum* and lipid profile values with CVR assessed using validated risk scores in patients with dyslipidemia.

The objectives of the study were:

1. To evaluate and compare the total RA of gut bacterial BSH in the compared groups during study time points.
2. To evaluate the RA of BSH of probiotic *L. plantarum*.
3. To assess the statistical relationship between the modification of total RA of gut bacterial BSH and lipid profile values of the compared groups during study time points.

To assess the statistical relationship between the modification of total RA of gut bacterial BSH and CVR levels calculated using validated risk scores in the compared groups during study time points.

Materials and methods. The clinical investigation was conducted in accordance with the Ukrainian laws, the requirements of Good Clinical Practice, 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 the Bogomolets National Medical University, Kyiv, Ukraine.

Participants. The study population included participants of both sexes: 26 almost healthy participants aged 22-44 years without dyslipidemia (healthy control group) and 77 patients aged 40-74 years with dyslipidemia and without anamnesis of major cardiovascular events. For the latter inclusion criteria were: men and women aged 30-74 years; LDL level ≥ 3.0 mmol/l; a level of TC > 5.0 mmol/l; patients who have not previously received statins or received them more than 6 months before screening; for reproductive women – a nega-

tive result of the pregnancy test; informed written consent. Exclusion criteria were: 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, stroke); chronic liver disease with elevation of liver enzymes more than 3 upper limits 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; participation in other clinical trials.

Study design. The study was conducted as open, comparative, randomized, parallel and included two stages. During first stage it was selected 26 almost healthy adults (healthy control group) and 77 patients with dyslipidemia with subsequent comparison of their baseline characteristics. Then, during second stage (treatment stage), patients with dyslipidemia were randomly divided in two groups: main treatment group (n=41) receiving combination therapy (capsules containing live active strain of *Lactobacillus plantarum* in the amount of 2*109 CFU in one capsule one time a day and tablets containing simvastatin in a dose of 20 mg one time a day) and control treatment group (n=36) receiving monotherapy (tablets containing simvastatin in a dose of 20 mg one time a day). Duration of second stage was 12 weeks. The monitoring of patients was on outpatient basis by visiting research center according to study visit schedules with possible deviations of 1-2 days.

Recommendations on lifestyle modification and diet were given to all participants according to current international guidelines (Mach et al., 2020). The following drugs were strongly prohibited during the study period: prebiotics, probiotics, antibiotics, laxatives, other hypolipidemic agents. The appropriate hypotensive treatment was prescribed to patients with arterial hypertension (Williams et al., 2018).

Investigational drugs. Capsules containing live active strain of *Lactobacillus plantarum* in the amount of 2*109 CFU in one capsule were manufactured at the LLC «Pharmaceutical plant «Biopharma», Kyiv, Ukraine. Simvastatin tablets – Zocor 20 mg dose manufactured by Merck Sharp & Dohme Idea Inc.

Study visit schedules. The trial consisted of 2 stages. The first stage included screening, selection and measurement of participants baseline characteristics. Anamnesis, written informed consent were obtained during the first visit. During the second stage (randomization and 12 weeks of treatment) the participants with dyslipidemia were randomly allocated in two groups (main treatment group and control treatment group) with prescribing of investigational drugs on 12 weeks. Objective examination, anthropometric measurements, blood pressure measurements were performed at each visit; ECG - during the first and last visits.

Assessment of total RA of gut bacterial BSH. For evaluation of total RA of gut bacterial BSH the specimens of participants feces were collected immediately after defecation. The ultraperformance liquid chromatography – mass spectrometry (UPLC-MC) was used for the assessment of total enzyme activity of gut BSH of fecal samples as was described before (Huijghebaert & Hofmann, 1986; Joyce et al., 2014). Relative activity of gut BSH was expressed in units of choloylglycine hydrolase/mL (from Clostridium perfringens, EC 3.5.1.24, Sigma-Aldrich).

Assessment of BSH total RA of probiotic *Lactobacillus plantarum*. The ultraperformance liquid chromatography – mass spectrometry (UPLC-MC) was used for the assessment of total enzyme activity of BSH of samples of probiotic bacteria *Lactobacillus plantarum* (Huijghebaert & Hofmann, 1986; Joyce et al., 2014). Relative activity of BSH was expressed in units of choloylglycine hydrolase/mL (from Clostridium perfringens, EC 3.5.1.24, Sigma-Aldrich).

Biochemical blood analyzes. Blood for evaluation of TC, LDL, high-density lipoproteins (HDL), TG (triglycerides), liver tests and creatine phosphokinase (CPK) was collected during the 1 and 4 study visits. Standard enzymatical methods were used to assess the level of TC, LDL, HDL, TG.

Cardiovascular risk assessment. During the 1 and 4 study visits the levels of CVR were calculated in main and control treatment groups using five validated risk scores: Globorisk (Hajifathalian et al., 2015), Framingham 10-years CVD risk estimation (D'Agostino et al., 2008), 10-year

risk of heart disease or stroke using the ASCVD algorithm published in 2013 ACC/AHA (Goff et al., 2014), Cardiovascular Risk PROCAM Score (Assmann, Cullen & Schulte, 2002), WHO cardiovascular disease risk chart (WCRCWG, 2019).

Taking to attention the age limit for assessment of CVR, in healthy control group, for participants <40 years we have assumed their CVR as 1% according to Globorisk score, 2013 ACC/AHA algorithm and WHO cardiovascular disease risk chart; <35 years – as 20 points according to PROCAM score; <30 years – as 1% according to Framingham score.

Efficacy endpoints. Treatment was considered effective in case of achieving the reducing of TC and LDL by 20% or more from baseline, reducing of CVR levels calculated using 5 validated risk scores by 20% or more from baseline.

Statistical analysis. The obtained data were analyzed using IBM SPSS Statistics v23 for Windows. Shapiro-Wilk test was used to check the normality of continuous variables. The data were presented as arithmetic mean with standard deviation (Mean±SD) in case of normal distribution or as median with first and third quartiles (Median (Q1-Q3)) in case of non-normal distribution. The differences between the means of two groups were checked by the unpaired t-test (in case of normal distribution) or Wilcoxon two sample test (in case of non-normal distribution). The differences between the means of 3 groups were checked using ANOVA or Kruskal–Wallis test in case of non-normal distribution; in post-hoc analysis the Bonferroni correction or Dunn test were used. The difference between qualitive variables was checked by chi-square test. The clinical effects were assessed by odds ratios. For determining the statistical relationships between variables, the logistic regression analysis was used. The difference between the study groups was considered statistically significant with p <0.05.

Results. Baseline characteristics of the compared groups. It was revealed the significant difference between healthy control and treatment control with main treatment groups regarding the age, BMI, levels of systolic blood pressure (SBP), TC, LDL, CVR levels according to all scores (p<0,01 in all). But there were no differences between control treatment and main treatment groups regarding these characteristics (p>0,05 in all). There were no differences between the compared groups regarding other values (Tab.1.).

Anthropometric and other values of the control treatment and main treatment groups after 12 weeks of study. After 12 weeks of treatment with investigational drugs there were no significant differences between control treatment and main treatment groups in terms of the levels of BMI, SBP and hypotensive treatment rates. But it was revealed the significant reduction of TC, LDL, TG and level of CVR according to PROCAM score in main treatment group comparing with control treatment group (Tab.2).

Tab.2. Anthropometric and other values of the control treatment and main treatment groups after 12 weeks of study

RA of bacterial BSH of *L. plantarum*. It was shown that one capsule of investigational probiotic containing 2*109 CFU *L. plantarum* has a RA of BSH=1,0006±0,037 U/mL comparing with 0,033±0,009 U/mL of *L. plantarum* with eliminated BSH-genes, p<0,05.

RA of gut bacterial BSH of the compared groups during study time points. At baseline it was revealed significant difference in RA of gut bacterial BSH between healthy control group and control treatment with main treatment groups (p<0,001 and p<0,001, respectively) without difference between control treatment and main treatment groups (p>0,05) (Tab.3, Fig.1).

After 12 weeks of investigation there was no difference in RA of gut bacterial BSH between healthy control group and main treatment group (p=0,45). But there were differences in RA of gut bacterial BSH between healthy control group and control treatment group (p<0,001) and between main treatment group and control treatment group (p<0,001) (Tab.3, Fig.2).

Achievement of treatment efficacy endpoints. The rates of achieving of treatment efficacy goals (20% and more reduction of TC, LDL, CVR levels according to all validated risk scores, except Globorisk score) were higher in main treatment group comparing with control treatment group (Tab.3).

Association between the RA of gut bacterial BSH and risk of failure of treatment efficacy endpoints achievement. To analyze the associa-

Characteristic		Healthy control (n=26) **	Treatment control (n=36) **	Main treatment (n=41) **	Difference (p) ***
Age		32,5±6,1	60,8±8,6	57±9,5	p<0,001
Sex	Men	13 (50%)	8 (22,2%)	13 (31,7%)	p=0,070
	Women	13 (50%)	28 (77,8%)	28 (68,3)	
BMI*		23,3±2,4	27±3,3	28±4	p<0,001
DM*	yes	-	16 (44,4%)	20 (48,8%)	p=0,881
	no	-	20 (55,6%)	21 (51,2%)	
Smoking	yes	8 (30,8%)	10 (27,8%)	13 (31,7%)	p=0,929
	no	18(69,2%)	26 (72,2%)	28 (68,3%)	
SBP (mmHg) *		120 (110-120)	135 (120-150)	130 (120-140)	p<0,001
Treatment of hypertension	yes	-	10 (27,8%)	15 (36,6%)	P=0,564
	no	-	26 (72,2%)	26 (63,4%)	
TC (mmol/l) *		4,64 (4,19-4,83)	5,93 (5,63-6,53)	5,76 (5,45-6,52)	p<0,001
LDL (mmol/l) *		2,61 (2,17-2,84)	3,97 (3,67-4,48)	4,03 (3,82-4,52)	p<0,001
HDL (mmol/l) *		1,54±0,44	1,4±0,3	1,38±0,45	p=0,249
TG (mmol/l) *		1,15 (0,8-1,7)	1,48 (0,9-2,88)	1,27 (0,71-1,76)	p=0,127
Globorisk (%)		1 (1-1)	41,5 (15-54,5)	29 (15-60)	p<0,001
Framingham (%)		1,25 (1-2,8)	20,2 (9,2-33,2)	17 (9,7-33,5)	p<0,001
2013 ACC/AHA algorithm (%)		1 (1-1)	12,9 (3,7-22)	8,2 (3,4-21)	p<0,001
PROCAM (points)		20 (20-20)	50,5 (41-57,5)	50 (39-58)	p<0,001
WHO risk chart (%)		1 (1-1)	22,5 (10,5-31,5)	17 (8-31)	p<0,001

**BMI – body mass index; DM – diabetes mellitus; SBP – systolic blood pressure; TC – total cholesterol; LDL – low-density lipoproteins; HDL – high-density lipoproteins; TG – triglycerides; CVR – cardiovascular risk*

***In case of normal and non-normal distribution, the data were presented as Mean±SD and Median (Q1-Q3) respectively*

**** ANOVA or Kruskal–Wallis test was used for normal and non-normal distribution*

Tab. 1. Baseline characteristics of the healthy control, treatment control and main treatment groups.

tion of risk of failure of treatment efficacy endpoints achievement (20% and more reduction of TC, LDL, CVR levels according validated risk scores) with RA of gut bacterial BSH, we used the method of constructing and analyzing a one-factor logistic regression model regarding each endpoint. It was found that with increasing of RA of gut bacterial BSH, the risk of failure of treatment efficacy endpoints achievement regarding:

1. 20% and more reduction of TC – decreased (p=0,0306), OR=0,00133 (95% CI; $3,28*10^{-6}$- 0,538). Fig. 4, A. shows the ROC curve of this model, AUC =0,66 (95%CI; 0,533-0,786);

Fig.1. The RA of BSH of the compared groups at baseline

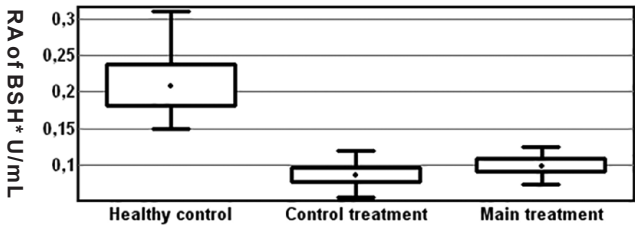
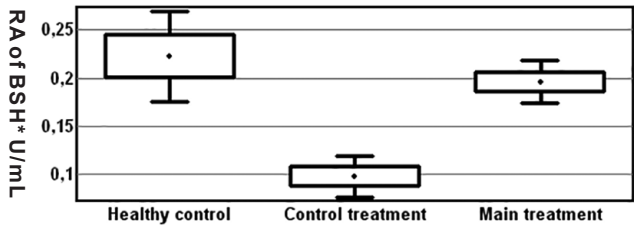


Fig.2. The RA of BSH of the compared groups after 12 weeks of investigation.



**relative activity of gut bacterial bile salt hydrolase expressed in units of choloylglycine hydrolase/mL from Clostridium perfringens, EC 3.5.1.24, Sigma-Aldrich.*

Characteristic		Treatment control (n=36) **	Main treatment (n=41) **	Difference (p) ***
BMI*		27±3,3	28±4	p=0,223
SBP (mmHg) *		131±10	128±11	p=0,242
Treatment of hypertension	yes	16 (44,4%)	21 (51,2 %)	p=0,712
	no	20 (55,6%)	20 (48,8)	
Lipid profile				
TC (mmol/l) *		5,29 (4,51-5,77)	4,84 (4,03-4,94)	p<0,001
LDL (mmol/l) *		3,35 (2,89-3,61)	2,97 (2,6-3,3)	p=0,002
HDL (mmol/l) *		1,39±0,38	1,33±0,44	p=0,486
TG (mmol/l) *		1,58 (0,99-2,2)	1,1 (0,7-1,43)	p=0,007
CVR* levels				
Globorisk (%)		35 (12-51)	19 (13-48)	p=0,244
Framingham (%)		15,9 (7,7-28,35)	11,4 (7,3-21,9)	p=0,249
2013 ACC/AHA algorithm (%)		11,05 (3,9-19)	6 (2,9-16,8)	p=0,195
PROCAM (points)		45,5 (38-50,5)	37 (31-41)	p=0,004
WHO risk chart (%)		19,39±11,91	15,83±11,96	p=0,188

**BMI – body mass index; DM – diabetes mellitus; SBP – systolic blood pressure; TC – total cholesterol; LDL – low-density lipoproteins; HDL – high-density lipoproteins; TG – triglycerides.*

***In case of normal and non-normal distribution, the data were presented as Mean±SD and Median (Q1-Q3) respectively*

**** t-test or Wilcoxon two sample test was used for normal and non-normal distribution*

Tab. 2. Anthropometric and other values of the control treatment and main treatment groups after 12 weeks of study.

RA of gut bacterial BSH, (units/mL) *	Healthy control (n=26) **	Treatment control (n=36) **	Main treatment (n=41) **	Difference (p) ***
Baseline	0,21 (0,13-0,32)	0,09 (0,05-0,13)	0,1 (0,06-0,14)	P<0,001
After 12 weeks	0,22±0,12	0,1±0,06	0,2±0,07	P<0,001

** relative activity of gut bacterial bile salt hydrolase expressed in units of choloylglycine hydrolase/mL from Clostridium perfringens, EC 3.5.1.24, Sigma-Aldrich.*

***In case of normal and non-normal distribution, the data were presented as Mean±SD and Median (Q1-Q3) respectively*

**** ANOVA or Kruskal–Wallis test was used for normal and non-normal distribution*

Tab. 3. The RA gut bacterial BSH of the compared groups during study time points

20% and more reduction of value	Treatment control, n (%)	Main treatment, n (%)	OR* (95% CI), p
TC*	10 (27,8%)	26 (63,4%)	2,28 (95%CI; 1,28-4,06), p=0,004.
LDL*	15 (41,7%)	35 (85,4%)	2,05 (95%CI; 1,36-3,08), p<0,001
Globorisk	10 (27,8%)	17 (41,5%)	p=0,311
Framingham	12 (33,3%)	29 (70,7%)	2,12 (95%CI; 1,28-3,51), p=0,003
2013 ACC/AHA algorithm	9 (25%)	21 (51,2%)	2,05 (95%CI; 1,08-3,88), p=0,035
PROCAM	9 (25%)	27 (65,9%)	2,63 (95%CI; 1,44-4,83), p<0,001
WHO risk chart	9 (25%)	23 (56,1%)	2,24 (95%CI; 1,2-4,2), p=0,012.

** TC – total cholesterol; LDL – low-density lipoproteins; CVR – cardiovascular risk; OR – odds ratio.*

Tab. 3. Rates of treatment efficacy goals achievement (20% and more reduction of TC*, LDL* and CVR* levels from baseline).

2. 20% and more reduction of LDL – decreased (p<0,001), OR=5,65*10^{-14}(95% CI; 6,38*10^{-20}-5*10^{-8}). Fig. 4, B. shows the ROC curve of this model, AUC=0,908 (95% CI; 0,837-0,979).
3. 20% and more reduction of CVR level according to Framingham score – decreased (p=0,0035), OR=4,09*10^{-5} (95% CI; 4,66*10^{-8}- 0,0359). Fig. 5, A. shows the ROC curve of this model, AUC =0,736 (95% CI; 0,618-0,855).
4. 20% and more reduction of CVR level according to 2013 ACC/AHA algorithm – decreased (p=0,0135), OR=3,8*10^{-4} (95% CI; 7,34*10^{-7}- 0,197). Fig. 5, B. shows the ROC curve of this model, AUC =0,662 (95% CI; 0,538-0,787).

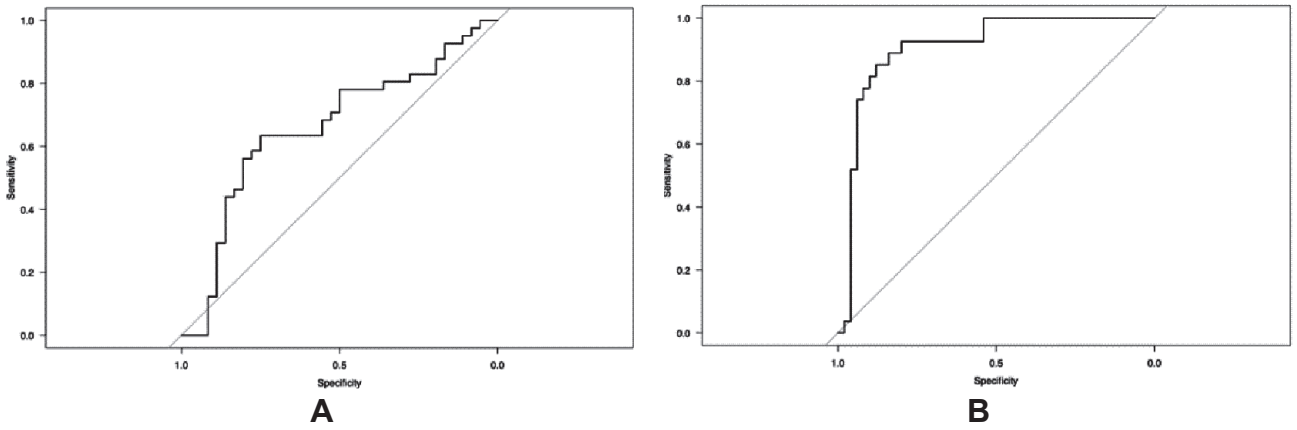


Fig. 4. The ROC-curves of the one-factor logistic regression models of association of relative activity of gut bacterial bile salt hydrolase with the risk of failure of 20% and more reduction of total cholesterol (A) and low-density lipoproteins (B).

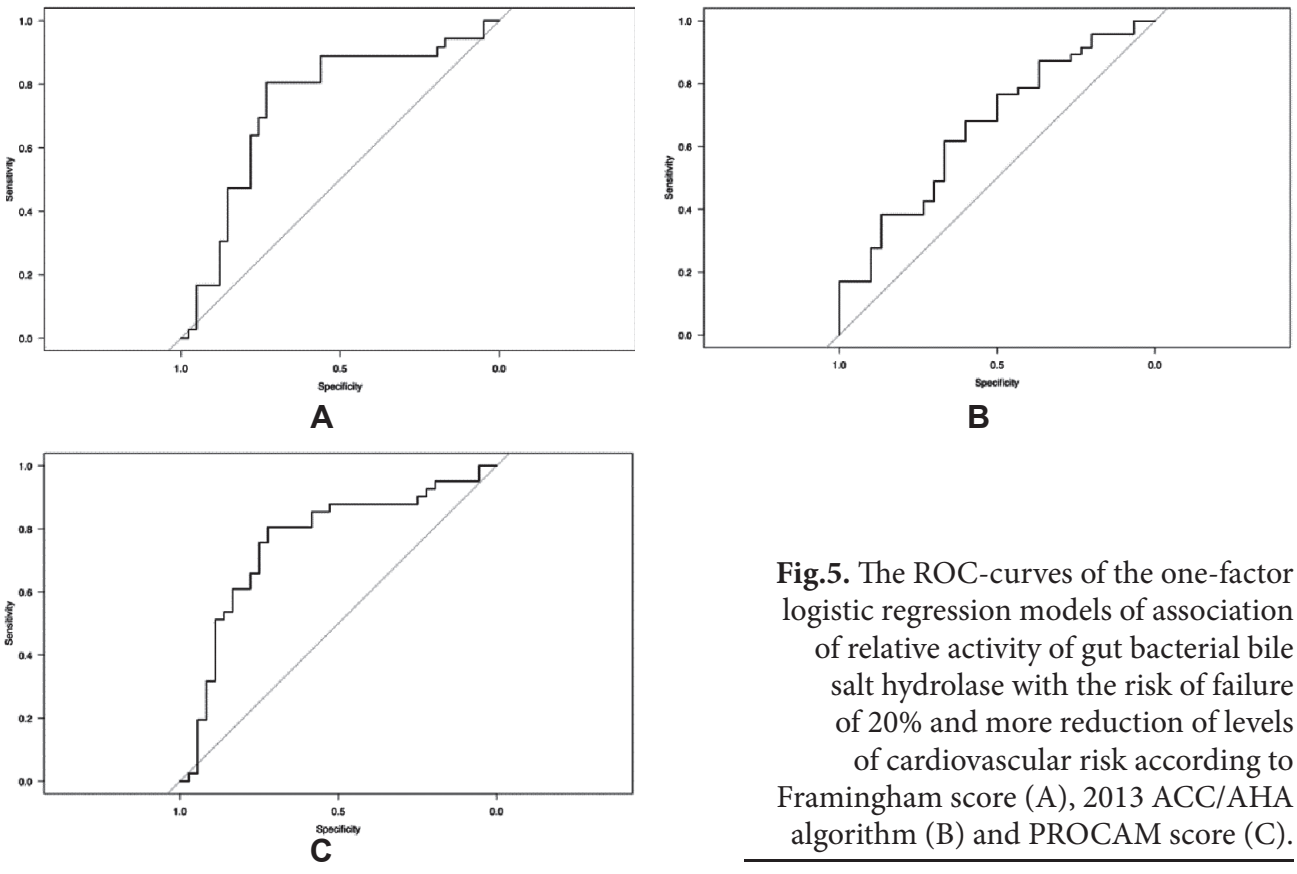


Fig.5. The ROC-curves of the one-factor logistic regression models of association of relative activity of gut bacterial bile salt hydrolase with the risk of failure of 20% and more reduction of levels of cardiovascular risk according to Framingham score (A), 2013 ACC/AHA algorithm (B) and PROCAM score (C).

5. 20% and more reduction of CVR level according to PROCAM score – decreased (p=0,00125), OR=8,38*10^{-6} (95%; CI; 6,93*10^{-9}- 0,0101). Fig. 5, C. shows the ROC curve of this model, AUC =0,662 (95% CI; 0,538-0,787).

There were no association between the RA of gut bacterial BSH and risk of failure of treatment efficacy endpoints achievement regarding the 20% and more reduction of CVR level according to Globorisk score and WHO risk chart.

Discussion. There is a small amount of literature data regarding the clear relationships between gut BSH enzyme activity with its correction and the cholesterol metabolism. Recent in vivo study (Joyce et al., 2014) revealed that enhancing of BSH activity in the mice gut was associated with decreasing of blood cholesterol. It was suggested by authors that gut microbiota may have a hypocholesterolemic effect by secretion of bile salt hydrolase (Joyce et al., 2014).

Despite the attempts of using the isolated BSH enzymes with hypocholesterolemic aim (Bi et al., 2013; Sridevi et al., 2009), most efforts of international investigators are directed on using the probiotic bacteria. A lot of animal (Kim et al., 2017; Yao et al., 2020) and human (Costabile et al., 2017; Sharma et al., 2016; Wang et al., 2018; Wu et al., 2017) investigations revealed the lipid-lowering properties of BSH-producing strains, especially different strains of *Lactobacilli*. Our previous trial also shown that additional supplementation with probiotic *L. plantarum* to low doses of simvastatin was more effective in reducing levels of TC and LDL compared with simvastatin monotherapy (Шипулін та ін., 2018).

International studies shown that *Lactobacillus plantarum* had a quite substantial BSH-activity (Huang et al., 2019; Kumar, Grover & Batish, 2012; Ren, Sun, Wu, Yao & Guo, 2011; Tsai et al., 2014). In our present trial we also revealed, using quantitively assessment method (Huijghebaert & Hofmann, 1986; Joyce et al., 2014), a quite high BSH activity of investigational capsules containing 2*109 CFU in one capsule, that confirm and expand the literature data (Huang et al., 2019; Kumar et al., 2012; Ren et al., 2011; Tsai et al., 2014). After 12 weeks of our investigation it was shown that additional supplementation with probiotic *L. plantarum* led to statistically significant increasing of RA of gut bacterial BSH in patients with dyslipidemia that was similar to healthy adults without dyslipidemia. While the participant from the control treatment group that received only simvastatin after 12 weeks had a statistically lower RA of gut bacterial BSH compared to participants additionally received probiotic *L. plantarum* and healthy adults. We revealed that there was an association between the RA of gut bacterial BSH and risk of failure of treatment efficacy endpoints achievement regarding the reduction of TC and LDL. We considered efficacy endpoint in case of achievement of 20% and more reduction of TC and LDL. On the basis of logistic regression models, we shown the statistically significant decreasing of risk of failure of achieving these treatment goals with increasing of the RA of BSH. It means that higher levels of RA of BSH were associated with higher chance to reach the treatment goals regarding the reduction of hepercholesterolemia.

In routine clinical practice several CVR assessment scores are used before prescribing hypocholesterolemic therapy, that allow to control and manage its effectiveness. (Assmann et al., 2002; D'Agostino et al., 2008; Goff et al., 2014; Hajifathalian et al., 2015; WCRCWG, 2019). All of them take to attention the levels of different types of cholesterol. In this trial we tried to find and determine the possible link between modification of BSH enzyme activity and CVR levels according to five validated risk scores. It was revealed the association with the risk of failure of treatment efficacy endpoints achievement regarding the reduction of CVR levels. We considered efficacy endpoint in case of achievement of 20% and more reduction of CVR level according to each score. Using logistic regression models, we shown that with increasing of the RA of gut bacterial BSH it was the statistically significant decreasing of risk of failure of achieving treatment aims according to 3 CVR scores: Framingham score (D'Agostino et al., 2008), 2013 ACC/AHA algorithm (Goff et al., 2014) and PROCAM score (Assmann et al., 2002). It indicates that higher levels of RA of BSH were associated with higher chance to reach the treatment goals regarding the reduction of CVR levels according to 3 above mentioned CVR scores.

Conclusions. In summary, additional supplementation with BSH-producing probiotic bacteria *L. plantarum* to low doses of simvastatin was statistically more effective in increasing of gut bacterial BSH enzyme relative activity in patients with dyslipidemia compared to simvastatin monotherapy. It was revealed that increasing of total relative activity of gut bacterial bile salt hydrolase by *L. plantarum* supplementation was associated with statistically higher chances to achieve treatment efficacy goals regarding reduction of serum total cholesterol, low-density lipoproteins and cardiovascular risk levels according to validated risk assessment tools: Framingham, 2013 ACC/AHA algorithm and PROCAM scores.

Funding. None declared.

Conflict if interests. None declared.

Acknowledgements. We are grateful to LLC "Pharmaceutical plant "Biopharma" for manufacturing of capsules with investigational probiotics and to Laboratory of Bioorganic Chemistry and Molecular Imaging (LCBIM), Institute of Chemical Sciences and Engineering (ISIC), Lausanne, Switzerland for performing of quantitively assessment of RA of gut bacterial BSH.

Authors contribution.

A. Neverovskyi: concept and design of research; investigation of participants, data collection; analyzing and interpretation of the obtained data; statistical analysis, writing the text of article; final approval.

V. Chernyavskyi: concept and design of research; analyzing and interpretation of the obtained data; critically revising of the article; final approval.

V. Shypulin: concept and design of research; critically revising of the article; final approval.

L. Gvozdecka: investigation of participants, data collection; critically revising of the article; final approval.

N. Mikhn`ova: investigation of participants, data collection; critically revising of the article; final approval.ння.

REFERENCES

Assmann, G., Cullen, P., & Schulte, H. (2002). Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Münster (PROCAM) study. Circulation, 105(3), 310–315. https://doi.org/10.1161/hc0302.102575.

Bi, J., Fang, F., Lu, S., Du, G., & Chen J. (2013) New insight into the catalytic properties of bile salt hydrolase. J Mol Catal B-Enzym 96:46–51.

Charach, G., Argov, O., Geiger, K., Charach, L., Rogowski, O., & Grosskopf, I. (2017). Diminished bile acids excretion is a risk factor for coronary artery disease: 20-year follow up and long-term outcome. Therapeutic advances in gastroenterology, 11, 1756283X17743420. https://doi.org/10.1177/1756283X17743420.

Chiang J. Y. (2009). Bile acids: regulation of synthesis. Journal of lipid research, 50(10), 1955–1966. https://doi.org/10.1194/jlr.R900010-JLR200.

Cholesterol Treatment Trialists' (CTT) Collaboration, Baigent, C., Blackwell, L., Emberson, J., Holland, L. E., Reith, et al. (2010). Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. Lancet (London, England), 376(9753), 1670–1681. https://doi.org/10.1016/S0140-6736(10)61350-5.

Costabile, A., Buttarazzi, I., Kolida, S., Quercia, S., Baldini, J., Swann, J. R., et al. (2017). An in vivo assessment of the cholesterol-lowering efficacy of Lactobacillus plantarum ECGC 13110402 in normal to mildly hypercholesterolaemic adults. PloS one, 12(12), e0187964. https://doi.org/10.1371/journal.pone.0187964.

D'Agostino, R. B., Sr, Vasan, R. S., Pencina, M. J., Wolf, P. A., Cobain, M., Massaro, J. M., et. al. (2008). General cardiovascular risk profile for use in primary care: the Framingham Heart Study. Circulation, 117(6), 743–753. https://doi.org/10.1161/CIRCULATIONAHA.107.699579.

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., et al. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. European heart journal, 38(32), 2459–2472. https://doi.org/10.1093/eurheartj/ehx144.

Geng, W., & Lin, J. (2016). Bacterial bile salt hydrolase: an intestinal microbiome target for enhanced animal health. Animal health research reviews, 17(2), 148–158. https://doi.org/10.1017/S1466252316000153.

Goff, D. C., Jr, Lloyd-Jones, D. M., Bennett, G., Coady, S., D'Agostino, R. B., Gibbons, R., et al. American College of Cardiology/American Heart Association Task Force on Practice Guidelines (2014). 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation, 129(25 Suppl 2), S49–S73. https://doi.org/10.1161/01.cir.0000437741.48606.98.

Hajifathalian, K., Ueda, P., Lu, Y., Woodward, M., Ahmadvand, A., Aguilar-Salinas, C. A., et al. (2015). A novel risk score to predict cardiovascular disease risk in national populations (Globorisk): a pooled analysis of prospective cohorts and health examination surveys. The lancet. Diabetes & endocrinology, 3(5), 339–355. https://doi.org/10.1016/S2213-8587(15)00081-9.

Huang, C. H., Ho, C. Y., Chen, C. T., Hsu, H. F., & Lin, Y. H. (2019). Probiotic BSH Activity and Anti-Obesity Potential of Lactobacillus plantarum Strain TCI378 Isolated from Korean Kimchi. Preventive nutrition and food science, 24(4), 434–441. https://doi.org/10.3746/pnf.2019.24.4.434.

Huijghebaert, S. M., & Hofmann, A. F. (1986). Influence of the amino acid moiety on deconjugation of bile acid amidates by cholylglycine hydrolase or human fecal cultures. Journal of lipid research, 27(7), 742–752.

Joyce, S. A., MacSharry, J., Casey, P. G., Kinsella, M., Murphy, E. F., Shanahan, F., et al. (2014). Regulation of host weight gain and lipid metabolism by bacterial bile acid modification in the gut. Proceedings of the National Academy of Sciences of the United States of America, 111(20), 7421–7426. https://doi.org/10.1073/pnas.1323599111.

Kim, S. J., Park, S. H., Sin, H. S., Jang, S. H., Lee, S. W., Kim, S. Y., et al. (2017). Hypocholesterolemic Effects of Probiotic Mixture on Diet-Induced Hypercholesterolemic Rats. Nutrients, 9(3), 293. https://doi.org/10.3390/nu9030293.

Kumar, R., Grover, S., & Batish, V. K. (2012). Bile Salt Hydrolase (Bsh) Activity Screening of Lactobacilli: In Vitro Selection of Indigenous Lactobacillus Strains with Potential Bile Salt Hydrolysing and Cholesterol-Lowering Ability. Probiotics and antimicrobial proteins, 4(3), 162–172. https://doi.org/10.1007/s12602-012-9101-3.

Lau K, Srivatsav V, Rizwan A, Nashed A, Liu R, Shen R, et al. (2017). Bridging the Gap between Gut Microbial Dysbiosis and Cardiovascular Diseases. Nutrients, 9(8), 859. https://doi.org/10.3390/nu9080859.

Luo, J., Yang, H., & Song, B. L. (2020). Mechanisms and regulation of cholesterol homeostasis. Nature reviews. Molecular cell biology, 21(4), 225–245. https://doi.org/10.1038/s41580-019-0190-7.

Mach, F., Baigent, C., Catapano, A. L., Koskinas, K. C., Casula, M., Badimon, L., et al. ESC Scientific Document Group (2020). 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. European heart journal, 41(1), 111–188. https://doi.org/10.1093/eurheartj/ehz455.

Mensah, G. A., Roth, G. A., & Fuster, V. (2019). The Global Burden of Cardiovascular Diseases and Risk Factors: 2020 and Beyond. Journal of the American College of Cardiology, 74(20), 2529–2532. https://doi.org/10.1016/j.jacc.2019.10.009.

Reis, S. A., Conceição, L. L., Rosa, D. D., Siqueira, N. P., & Peluzio, M. (2017). Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotics. Nutrition research reviews, 30(1), 36–49. https://doi.org/10.1017/S0954422416000226.

Ren, J., Sun, K., Wu, Z., Yao, J., & Guo, B. (2011). All 4 bile salt hydrolase proteins are responsible for the hydrolysis activity in Lactobacillus plantarum ST-III. Journal of food science, 76(9), M622–M628. https://doi.org/10.1111/j.1750-3841.2011.02431.x.

Sharma, S., Kurpad, A. V., & Puri, S. (2016). Potential of probiotics in hypercholesterolemia: A meta-analysis. Indian journal of public health, 60(4), 280–286. https://doi.org/10.4103/0019-557X.195859.

Silverman, M. G., Ference, B. A., Im, K., Wiviott, S. D., Giugliano, R. P., Grundy, S. M., et al. (2016). Association Between Lowering LDL-C and Cardiovascular Risk Reduction Among Different Therapeutic Interventions: A Systematic Review and Meta-analysis. JAMA, 316(12), 1289–1297. https://doi.org/10.1001/jama.2016.13985.

Sridevi, N., Vishwe, P., & Prabhune, A. (2009). Hypocholesteremic effect of bile salt hydrolase from Lactobacillus buchneri ATCC 4005. Food Research International, 42(4), 516-520.

Tsai, C. C., Lin, P. P., Hsieh, Y. M., Zhang, Z. Y., Wu, H. C., & Huang, C. C. (2014). Cholesterol-lowering potentials of lactic acid bacteria based on bile-salt hydrolase activity and effect of potent strains on cholesterol metabolism in vitro and in vivo. The Scientific World Journal, 2014, 690752. https://doi.org/10.1155/2014/690752.

Urdaneta, V., & Casadesús, J. (2017). Interactions between Bacteria and Bile Salts in the Gastrointestinal and Hepatobiliary Tracts. Frontiers in medicine, 4, 163. https://doi.org/10.3389/fmed.2017.00163.

Wang, G., Huang, W., Xia, Y., Xiong, Z., & Ai, L., (2019). Cholesterol-lowering potentials of Lactobacillus strain overexpression of bile salt hydrolase on high cholesterol diet-induced hypercholesterolemic mice. Food & function, 10(3), 1684–1695. https://doi.org/10.1039/c8fo02181c.

Wang, L., Guo, M. J., Gao, Q., Yang, J. F., Yang, L., Pang, X. L., et al. (2018). The effects of probiotics on total cholesterol: A meta-analysis of randomized controlled trials. Medicine, 97(5), e9679. https://doi.org/10.1097/MD.0000000000009679.

WHO CVD Risk Chart Working Group (2019). World Health Organization cardiovascular disease risk charts: revised models to estimate risk in 21 global regions. The Lancet. Global health, 7(10), e1332–e1345. https://doi.org/10.1016/S2214-109X(19)30318-3.

Williams, B., Mancia, G., Spiering, W., Agabiti Rosei, E., Azizi, M., et al. (2018). 2018 ESC/ESH Guidelines for the management of arterial hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension: The Task Force for the management of arterial hypertension of the European Society of Cardiology and the European Society of Hypertension. Journal of hypertension, 36(10), 1953–2041. https://doi.org/10.1097/HJH.0000000000001940.

Wu, Y., Zhang, Q., Ren, Y., & Ruan, Z. (2017). Effect of probiotic Lactobacillus on lipid profile: A systematic review and meta-analysis of randomized, controlled trials. PloS one, 12(6), e0178868. https://doi.org/10.1371/journal.pone.0178868.

Yao, C., Tian, W., Song, J., & Wang, J. (2020). Antihyperlipidaemic effect of microencapsulated Lactobacillus plantarum LIP-1 on hyperlipidaemic rats. Journal of the science of food and agriculture, 100(5), 2007–2017. https://doi.org/10.1002/jsfa.10218.

Шипулін, В. П., Чернявський, В. В., Неверовський, А. В., & Парунян, Л. М. (2018). Гіполіпідемічна ефективність пробіотика Lactobacillus plantarum у пацієнтів з ішемічною хворобою серця. Український терапевтичний журнал, (3-4), 27-33.