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Original Article

Ultra-small phospholipid nanoparticles in the treatment of combined hyperlipidemia: a randomized placebo-controlled clinical trial

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Abstract

Background and purpose: Combined hyperlipidemia is associated with an increased risk of cardiovascular events. This clinical trial investigated phospholipovit (essential phospholipids, Institute of Biomedical Chemistry, Moscow, Russia), an ultra-small phospholipid nanoparticle (micelles), targeted to phospholipids of HDL in lowering non-HDL-cholesterol (non-HDL-C) and triglycerides (TG) levels in patients with combined hyperlipidemia and moderate cardiovascular risk.
Experimental approach: A randomized, double-blinded, placebo-controlled phase II trial was conducted on 100 patients. Phospholipovit or placebo was randomly administered orally (500 mg) 2 times a day for 12 weeks. The primary endpoint was the percent change of non-HDL-C from baseline to 12 weeks of exposure.
Findings/Results: Treatment with phospholipovit resulted in a mean non-HDL-C reduction of 13.2% versus 4.3% compared with placebo. The absolute decrease in non-HDL-C was -23.2 (-48.7 - 7.0) mg/dL versus -7.3 (-17.0 - 12.0) mg/dL, significantly. The therapeutic target of non-HDL-C less than 130 mg/dL (3.4 mmol) was achieved in 15 of 39 patients (38.5%) in the phospholipovit group versus 2 of 41 patients (4.9%) in the placebo group OR 11.8 (2.4 - 116). Significant reduction in TG, apolipoprotein B, total cholesterol, and very low-density lipoprotein cholesterol levels was also observed. There were no changes in the liver and kidney functions, vital signs, or electrocardiography. There were no serious adverse events.
Conclusion and implications: Phospholipovit significantly reduced non-HDL-C, TG, and atherogenic lipoproteins in patients with combined hyperlipidemia and moderate cardiovascular risk. It can be used as an add-on therapy to statins.

Keywords: Combined hyperlipidemia; Non-HDL-C; Phospholipid nanoparticles (micelles); TG.

INTRODUCTION

The combined hyperlipidemia (former hyperlipidemia type IIB or familial combined hyperlipidemia) includes patients with mild to moderate hypertriglyceridemia and low high-density lipoproteins (HDL) cholesterol levels. Circulating triglycerides (TG) are carried by TG-rich lipoproteins (TGL), which are consistently associated with atherosclerotic cardiovascular disease (ASCVD) (1) according to epidemiological studies (2), genome-wide analyses (3), and Mendelian randomization studies (4). All TGL particles contain the apolipoprotein B (ApoB) molecule, and to estimate all atherogenic ApoB-containing lipoproteins, a non-HDL-cholesterol (non-HDL-C), as a standard in clinical practice, is now widely used. Also, there is a high positive correlation between the decrease in non-HDL-C and TG (5).

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HDL is an antiatherogenic substance and its primary function is to promote reverse cholesterol transport (RCT). The first step of RCT is cholesterol efflux from macrophages to HDL, the so-called cholesterol efflux capacity (CEC) (6). The animal study showed the inverse correlation between CEC and the size of atherosclerotic lesions (7). In human studies, the CEC was inversely associated with the incidence of ASCVD (8). Recently, to enhance CEC it was performed a new method for generating ultra-small phospholipid (PL) nanoparticles (micelles) from soybean phosphatidylcholine (PC), which had an average size of 30 nm (9). Also, the micelles were investigated *in vitro* and *in vivo* studies. The mass-spectrometric analysis showed that the micelles enriched HDL with dilinoleoyl PC (10). *In vitro* study showed a dose-dependent 8-fold increase of PL in HDL, and a 2-fold increase in CEC, as compared with native ApoB-depleted plasma, when incubated with micelles (11). *In vivo* study found that the intravenous administration of micelles in the cholesterol-fed rabbit restored lipid profiles protected the vessel wall from developing intimal lipid lesions, and showed a similar decrease in the extent of atherosclerotic lesions, as compared with atorvastatin and fenofibrate (11). For clinical trials, the water-soluble pharmaceutical form of micelles, phospholipovit, was used. Phase I trial showed the safety of oral administration of phospholipovit (1 g for 1 day) in 14 healthy volunteers. Phospholipovit was well tolerated and no serious adverse events (AE) were observed (12).

We hypothesized that the phospholipidation of HDL improves the interactions of lipoproteins, which leads to a decrease in atherogenic lipids. So, the present trial aimed to show the safety and efficacy of 1 g oral administration of phospholipovit daily versus placebo for 12 weeks in changing non-HDL-C, TG, and atherogenic lipoproteins in patients with combined hyperlipidemia and moderate cardiovascular (CV) risk (phase II trial).

MATERIALS AND METHODS

Trial design

This study was a randomized, double-blinded, and placebo-controlled phase II clinical trial for evaluating phospholipovit and conducted in 3 clinical sites in Russia including E.I. Chazov National Medical Research Center of Cardiology, Moscow; Nizhny Novgorod Medical Clinic, Nizhny Novgorod; and Medical Center for Diagnostics and Prevention Plus, Yaroslavl.

Sample size calculation was presented in the supplementary appendix 1. The randomization sequence was created by an independent biostatistician using computer-generated random numbers. Twenty-five blocks (block size of 4) of opaque sealed envelopes were numbered sequentially from 1 to 100 and opened in numerical order to receive either phospholipovit (n = 50) or placebo (n = 50). Investigators and patients were unaware of their assigned treatment. Phospholipovit or placebo was administered in a dose of 500 mg orally 2 times a day. The composition of the placebo was maltose used as an excipient in phospholipovit. The treatment period was 12 weeks, with a post-treatment follow-up for 4 weeks, and the screening period lasted 1-3 days. Monitoring and stopping rules were related to liver and renal function, platelet count, other parameters of blood count, biochemical blood analysis, blood pressure (systolic and diastolic), and electrocardiography. The threshold limits were prespecified in the protocol.

The current study was conducted according to the guidelines of the Declaration of Helsinki (World Medical Association, 1964 and amended by the 64th General Assembly, Fortaleza, Brazil, 2013), and approved by the Ethics Council of the Ministry of Health of Russia (No. 8414 dated of July 10, 2015) and by the Ministry of Health (No. 05F of March 23, 2016). Informed consent was obtained from all subjects involved in the study. Independent statisticians did the statistical analysis and data interpretation.

Eligibility

Inclusion criteria

Patients who were 30-75 years of age; had no history of diseases such as coronary heart disease (CHD), acute myocardial infarction (MI), stroke, transient ischemic attack, peripheral artery disease, or heart failure; were at moderate CV risk and had TG level of 151 - 399 mg/dL (1.7 - 4.5 mmol/L),

HDL-C < 39 mg/dL (< 1 mmol/L) for men, and < 46 mg/dL (< 1.2 mmol/L) for women were eligible for enrollment. Patients were screened in the outpatient department of the E.I. Chazov National Medical Research Center of Cardiology (Moscow, Russia) and other clinical sites.

Exclusion criteria

Exclusion criteria included diseases or metabolic disorders that can increase low-density lipoproteins (LDL) cholesterol, total cholesterol, and TG (secondary dyslipidemia); patients receiving high doses of statins (rosuvastatin > 40 mg and atorvastatin > 80 mg) and any hypotriglyceridemic drugs, such as omega-3, fibrates, and niacin; type 1 diabetes mellitus (DM); patients with kidney decompensation defined as increase in serum creatinine ≥ 0.3 mg/dL (26.5 μmol/L) within 48 h or ≥ 50% from baseline value known or presumed to have occurred within the prior 7 days and/or urine output ≤ 0.5 mL/Kg for ≥ 6 h; patients with liver decompensation defined by acute development of one or more major complications of liver disease (*i.e.*, ascites, hepatic encephalopathy, gastrointestinal hemorrhage, and bacterial infection), and can be further complicated by organ failures and high short-term mortality; porphyria, myopathy, diseases of the central nervous system, with exacerbation of chronic infections diseases, with acute conditions (infection, injuries, and operations) less than 2 months before screening; patients of a positive human immunodeficiency virus test and positive hepatitis B, C, and syphilis test results; oncology during the last 5 years, hypothyroidism, or thyroid-stimulating hormone levels (TSH) exceeding > 1 upper limit of normal (ULN) during screening; glomerular filtration rate less than 30 mL/min/1.73 m^2; alanine aminotransferase (ALT) or aspartate aminotransferase (AST) level exceeding twice the ULN lower than the lower limits of normal range in blood and urine tests; alcohol abuse more than 5 units/week (Table S1).

Laboratory measurements

All laboratory parameters including total cholesterol, HDL-C, ApoB, ApoA, lipoprotein (a) Lp(a), high-sensitivity C-reactive protein (hs-CRP), AST, ALT, creatinine, complete blood count were measured by commercially available kits (Vital Diagnostics, St. Petersburg, Russia and Erba Lachema s.r.o., Brno, Czech Republic). Non-HDL cholesterol was calculated as follows: total cholesterol minus HDL-C. Low-density lipoproteins cholesterol (LDL-C) was calculated according to the Friedewald formula (13). Very LDL-C (VLDL-C) in mmol/L was calculated as TG level divided by 2.2.

Endpoints

The primary efficacy endpoint was the percentage change in non-HDL-C from baseline to the time point of 12 weeks of treatment with phospholipovit, as compared with placebo.

The secondary efficacy endpoints were the percentage change from baseline in TG, total cholesterol, HDL-C, LDL-C, VLDL-C, ApoB, ApoA, Lp(a), and hs-CRP levels. All endpoints were listed in Table S2.

Statistical analysis

Statistical analyses were performed using R statistical software version 4.1 (R Core Team, Vienna, Austria). Continuous variables were presented as mean ± standard deviation (SD) or as the median and interquartile range (median (Q1 - Q3)). Categorical variables were presented as n (%). In the unpaired case, the Mann-Whitney U test was used to compare the distributions of continuous variables, and the two-sided Fisher's exact test was used to compare categorical variables. In the paired case, the Wilcoxon test was used to compare the distributions of continuous variables, and McNemar's exact test was used to compare categorical variables. The confidence interval for the median was calculated using the MedianCI function from DescTools R-package version 0.99.46 (Zurich, Switzerland). The odds ratio (OR) was calculated using the Fisher test function from the R-package based on the conditional maximum likelihood estimate, which depends on the sample sizes (14). *P*-values < 0.05 were considered statistically significant.

RESULTS

Patients

In total, 325 patients were screened, of whom 100 were randomized to receive either phospholipovit or a placebo. One hundred seventy patients had no inclusion criteria; the most common of them were TG level less than 150 mg/dL (1.7 mmol/L) and HDL-C more than 39 mg/dL (1.0 mmol/L) for men and 46 mg/dL (1.2 mmol/L) for women. Fifty-five patients were excluded, most receiving high doses of statins and type 1 DM. The first patient was enrolled in April 2016 and the last in November 2017. Three patients in the phospholipovit group and 2 patients in the placebo group discontinued the trial on the 4th visit. Eight patients in the phospholipovit group and 7 patients in the placebo group were excluded because of non-compliance to the recommended diet (5 versus 4) and enhanced alcohol consumption (3 versus 3). These 20 patients were excluded from the efficacy analysis but were included in the safety analysis (Fig. 1).

All included patients had a diet that modified plasma TG and HDL-C levels, according to Russian Atherosclerosis Society Guidelines (15); some of the patients used statins (rosuvastatin in a dose of $\leq$ 40 mg and atorvastatin in a dose of $\leq$ 80 mg) for the treatment. The average compliance (percent of the total scheduled volume of drug received) was 78% in the phosholipovit group and 82% in the placebo group. Demographics and baseline characteristics of patients were similar in both groups (Table 1). In the phospholipovit group, at baseline, the mean non-HDL-cholesterol level was 177.4 ± 32.6 mg/dL (4.6 ± 0.8 mmol/L), and the median level was 170.9 mg/dL (156.3 - 206.4) or 4.4 mmol/L (4.0 - 5.3). In the placebo group, at baseline, the mean non-HDL-cholesterol level was 177.0 ± 27.7 mg/dL (4.6 ± 0.7 mmol/L), and the median level was 175.0 mg/dL (155.2 - 199.9) or 4.5 mmol/L (4.0 - 5.2) (Table 1).

Efficacy

The primary efficacy endpoint: percent change in non-HDL-C level

After 12 weeks of treatment, a statistically significant reduction in non-HDL-C was observed in the phospholipovit group (-13.2% (-29.1% - -3.9%)) compared with the placebo group (-4.3% (-9.9% - 6.5%)) (Table 2). Also, there was a significant difference in the absolute change of non-HDL-C from baseline to 12 weeks between the phospholipovit group (-23.2 mg/dL (-48.7 - -7.0) or -0.6 mmol/L (-1.2 - -0.2)) and the placebo group (-7.3 mg/dL (-17.0 - 12.0) or -0.2 mmol/L (-0.4 - 0.3)) (Table 2). Before treatment, only one patient (in the placebo group) had a level of non-HDL-C less than 130 mg/dL (3.4 mmol/L). After 12 weeks of the treatment, the goal of non-HDL-C < 130 mg/dL (3.4 mmol/L) was achieved in 15 of 39 patients (38.5%) in the phospholipovit group versus 2 of 41 patients (4.9%) in the placebo group (P = 0.001, OR 11.8 (2.4 - 116)).

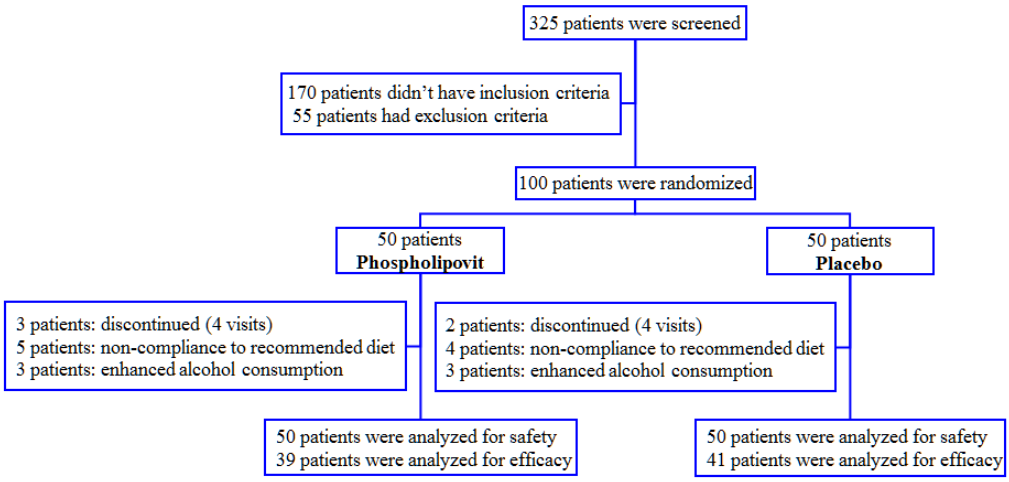

Fig. 1. Trial flow diagram. Phospholipovit or placebo (maltose) was administered orally at 500 mg, 2 times /day for 12 weeks.

Table 1. Demographic, clinical characteristics at baseline in phospholipovit and placebo groups.

Baseline characteristics	Phospholipovit group (n = 50)	Placebo group (n = 50)
Age (year)	55.6 ± 11.1	57.1 ± 9.2
Male sex	33 (66)	25 (50)
White race	50 (100)	50 (100)
BMI (Kg/m^2)	29.0 (26.1 - 33.0)	30.3 (28.1 - 34.0)
CV risk factors		
Dyslipidemia	50 (100)	50 (100)
Hypertension	48 (96)	49 (98)
Ever smoker	36 (72)	35 (70)
Current smoker	16 (32)	17 (34)
Type 2 diabetes	1 (2)	1 (2)
Impaired glucose tolerance	6 (12)	5 (10)
Statins	46 (92)	47 (94)
Platelet aggregation inhibitors	48 (96)	49 (98)
β-adreno-blockers	29 (58)	31 (62)
ACE-inhibitors	14 (28)	15 (30)
Other anti-hypertensive drugs: Ca^{++}antagonists, AT-II inhibitors	4 (8)	3 (6)
Anti-diabetic drugs	7 (14)	6 (12)
Non-HDL cholesterol (mmol/L)	4.6 ± 0.8	4.6 ± 0.7
Non-HDL cholesterol (mg/dL)	177.4 ± 32.6	177.0 ± 27.7
Non-HDL cholesterol (mmol/L)	4.4 (4.0 - 5.3)	4.5 (4.0 - 5.2)
Non-HDL cholesterol (mg/dL)	170.9 (156.3 - 206.4)	175.0 (155.2 - 199.9)
Total cholesterol (mmol/L)	5.5 (5.0 - 6.3)	5.6 (5.0 - 6.2)
Total cholesterol (mg/dL)	211.5 (192.1 - 243.3)	215.2 (195.0 - 241.3)
HDL cholesterol (mmol/L)	1.0 (0.9 - 1.0)	1.0 (0.9-1.1)
HDL cholesterol (mg/dL)	37.9 (36.0 - 39.0)	37.9 (34.8 - 44.5)
Triglycerides (mmol/L)	2.2 (1.9 - 2.7)	2.2 (1.9 - 2.7)
Triglycerides (mg/dL)	198.0 (169.4 - 239.1)	198.8 (169.4 - 238.7)
LDL cholesterol (mmol/L)	3.3 (2.9 - 4.1)	3.3 (2.9 - 3.9)
LDL cholesterol (mg/dL)	128.4 (111.2 - 157.3)	126.5 (111.1 - 149.9)
VLDL cholesterol (mmol/L)	1.0 (0.9 - 1.2)	1.0 (0.9 - 1.2)
VLDL cholesterol (mg/dL)	39.3 (33.6 - 47.5)	39.5 (33.6 - 47.4)
Apolipoprotein B (mg/dL)	110.5 (98 - 124.8)	112.5 (96.8 - 123.8)
Apolipoprotein A (mg/dL)	132.5 (122.3 - 142.8)	137 (128.5 - 149)
Lipoprotein (a) (mg/dL)	8.5 (3.1 - 33.8)	9.6 (5.2 - 16.2)
hs-CRP (mg/dL)	0.22 (0.14 - 0.4)	0.2 (0.11 - 0.43)

Phospholipovit or placebo (maltose) was administered orally at the dose of 500 mg, 2 times/day for 12 weeks. Data were expressed as mean ± SD, number (%), or median (Q1-Q3), where applicable. BMI, Body mass index; CV, cardiovascular; ACE, angiotensin-converting enzyme; AT-II, angiotensin-II; HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very-low-density lipoproteins; hs-CRP, high-sensitivity C-reactive protein.

Table 2. The primary efficacy endpoint: changes in non-HDL-cholesterol levels in phospholipovit and placebo groups.

Parameters	Phospholipovit group (n = 39)		Placebo group (n = 41)		*P*-value
	median (Q1 - Q3)	95% CI	median (Q1 - Q3)	95% CI	
Baseline non-HDL-C (mmol/L)	4.5 (4.1 - 5.3)	4.2 - 5.0	4.7 (4.2 - 5.2)	4.3 - 5.0	0.918
Non-HDL-C after 12 weeks (mmol/L)	3.8 (3.0 - 4.8)	3.1 - 4.4	4.2 (3.8 - 5.1)	3.8 - 4.8	0.008
Absolute change from baseline (mmol/L)	-0.6 (-1.2 - -0.2)	-1.0 - -0.3	-0.2 (-0.4 - 0.3)	-0.3 - 0.1	0.001
Baseline non-HDL-C (mg/dL)	172.5 (159.3 - 205.3)	161.6 - 193.7	181.7 (160.9 -201.5)	165.1 - 193.0	0.918
Non-HDL-C after 12 weeks (mg/dL)	146.6 (117.2 - 183.7)	120.7 - 171.7	161.3 (146.9 - 198.0)	147.7 - 186.4	0.008
Absolute change from baseline (mg/dL)	-23.2 (-48.7 - -7.0)	-40.6 - -12.8	-7.3 (-17.0 - 12.0)	-13.1 - 5.0	0.001
% change from baseline	-13.2 (-29.1 - -3.9)	-23.0 - -5.9	-4.3 (-9.9 - 6.5)	-8.3 - 3.1	0.001

Phospholipovit or placebo (maltose) was administered orally at the dose of 500 mg, 2 times/day for 12 weeks. Data were expressed as median (Q1 - Q3). HDL, high-density lipoproteins; CI, confidence interval.

The secondary efficacy endpoints: changes in lipids and lipoproteins level

In this study, 12-week treatment with phospholipovit resulted in a significant reduction in the levels of atherogenic lipids and lipoproteins (Table 3).

Table 3. The secondary efficacy endpoints: changes in lipids and lipoproteins levels in phospholipovit and placebo groups.

Parameters	Phospholipovit group (n = 39)		Placebo group (n = 41)		*P*-value
	median (Q1 - Q3)	95% CI	median (Q1 - Q3)	95% CI	
Triglycerides					
Baseline triglycerides, mmol/L	2.5 (2.0 - 2.8)	2.0 - 2.7	2.3 (1.9 - 2.7)	2.0 - 2.6	0.382
Triglycerides after 12 weeks, mmol/L	1.5 (1.3 - 2.3)	1.4 - 2.3	2.2 (1.7 - 2.8)	1.9 - 2.6	0.014
Absolute change from baseline, mmol/L	-0.7 (-1.2 - -0.3)	-1.0 - -0.3	-0.1 (-0.5 - 0.4)	-0.4 - 0.1	0.001
Baseline triglycerides, mg/dL	221.4 (177.6 - 244.9)	181.6 - 239.1	202.8 (168.3 -	175.4 - 230.3	0.382
Triglycerides after 12 weeks, mg/dL	136.4 (115.6 - 206.4)	119.6 - 201.1	194.9 (150.6 -	165.6 - 232.1	0.014
Absolute change from baseline, mg/dL	-62.0 (-103.6 - -26.6)	-90.3 - -28.3	-8.9 (-48.7 - 31.9)	-35.4 - 10.6	0.001
% change from baseline	-23.8 (-48.2 - -12.0)	-46.8 - -18.2	-3.7 (-20.0 - 15.4)	-16.9 - 5.6	0.000
TC					
Baseline TC, mmol/L	5.5 (5.1 - 6.3)	5.2 - 6.1	5.6 (5.1 - 6.3)	5.3 - 6.1	0.884
TC after 12 weeks, mmol/L	4.8 (4.1 - 5.9)	4.2 - 5.5	5.6 (4.8 - 6.2)	5.0 - 6.1	0.019
Absolute change from baseline, mmol/L	-0.6 (-1.0 - 0.1)	-0.8 - -0.1	-0.1 (-0.4 - 0.3)	-0.3 - 0.0	0.011
Baseline TC, mg/dL	214.2 (195.5 - 242.3)	199.5 - 235.9	216.6 (197.6 -	205.0 - 237.0	0.884
TC after 12 weeks, mg/dL	186.8 (158.7 - 227.2)	161.6 - 212.7	216.2 (185.6 -	193.7 - 235.9	0.019
Absolute change from baseline, mg/dL	-23.2 (-38.9 - 3.3)	-31.7 - -2.7	-5.4 (-15.5 - 10.1)	-11.6 - 0.0	0.011
% change from baseline	-10.8 (-18.8 - 1.4)	-15.6 - -1.4	-2.7 (-7.1 - 5.9)	-4.3 - 0.0	0.008
HDL-C					
Baseline HDL-C, mmol/L	1.0 (0.9 - 1.0)	1.0 - 1.0	1.0 (0.9 - 1.2)	0.9 - 1.1	0.779
HDL-C after 12 weeks, mmol/L	1.1 (1.0 - 1.3)	1.0 - 1.2	1.0 (0.9 - 1.2)	1.0 - 1.2	0.574
Absolute change from baseline, mmol/L	0.1 (0.0 - 0.2)	0.0 - 0.2	0.0 (0.0 - 0.2)	0.0 - 0.1	0.388
Baseline HDL-C, mg/dL	37.9 (36.2 - 39.4)	37.1 - 38.7	37.9 (34.8 - 45.6)	36.3 - 42.5	0.779
HDL-C after 12 weeks, mg/dL	42.5 (37.3 - 49.5)	37.5 - 46.4	39.8 (35.2 - 48.3)	37.9 - 46.4	0.574
Absolute change from baseline, mg/dL	4.3 (-0.6 - 8.7)	0.0 - 7.7	0.8 (-0.4 - 7.3)	0.0 - 3.9	0.388
% change from baseline	10.0 (-1.5 - 21.8)	0.0 - 18.5	2.0 (-1.0 - 22.2)	0.0 - 10.0	0.327
LDL-C					
Baseline LDL-C, mmol/L	3.4 (3.0 - 3.9)	3.0 - 3.7	3.5 (2.9 - 3.9)	3.1 - 3.9	0.987
LDL-C after 12 weeks, mmol/L	3.0 (2.3 - 3.7)	2.4 - 3.5	3.1 (2.8 - 3.8)	3.0 - 3.6	0.206
Absolute change from baseline, mmol/L	-0.3 (-0.8 - 0.1)	-0.7 - 0.0	-0.2 (-0.6 - 0.2)	-0.5 - 0.1	0.292
Baseline LDL-C, mg/dL	130.7 (114.7 - 150.6)	117.2 - 141.9	135.0 (111.0 -	118.3 - 148.9	0.987
LDL-C after 12 weeks, mg/dL	116.0 (89.9 - 144.0)	94.0 - 135.7	121.8 (107.5 -	114.1 - 138.8	0.206
Absolute change from baseline, mg/dL	-13.1 (-30.9 - 4.4)	-28.6 - 0.0	-6.4 (-24.2 - 6.2)	-18.2 - 2.3	0.292
% change from baseline	-11.9 (-23.8 - 3.9)	-21.5 - 0.0	-5.2 (-19.5 - 6.4)	-12.1 - 1.6	0.234
VLDL-C					
Baseline VLDL-C, mmol/L	1.1 (0.9 - 1.3)	0.9 - 1.2	1.0 (0.9 - 1.2)	0.9 - 1.2	0.382
VLDL-C after 12 weeks, mmol/L	0.7 (0.6 - 1.1)	0.6 - 1.0	1.0 (0.8 - 1.2)	0.8 - 1.2	0.014
Absolute change from baseline, mmol/L	-0.3 (-0.5 - -0.1)	-0.5 - -0.1	0.0 (-0.2 - 0.2)	-0.2 - 0.1	0.000
Baseline VLDL-C, mg/dL	43.9 (35.2 - 48.6)	36.0 - 47.5	40.3 (33.4 - 47.8)	34.8 - 45.7	0.382
VLDL-C after 12 weeks, mg/dL	27.1 (22.9 - 41.0)	23.7 - 39.9	38.7 (29.9 - 48.3)	32.9 - 46.1	0.014
Absolute change from baseline, mg/dL	-12.3 (-20.5 - -5.3)	-17.9 - -5.6	-1.8 (-9.7 - 6.3)	-7.0 - 2.1	0.000
% change from baseline	-23.8 (-48.2 - -12.0)	-46.8 - -18.2	-3.7 (-20.0 - 15.4)	-16.9 - 5.6	0.000
Apolipoprotein B					
Baseline Apolipoprotein B, mg/dL	111.0 (98.5 - 124.5)	105.0 - 117.0	115.0 (99.0 - 125.0)	108.0 - 122.0	0.486
Apolipoprotein B after 12 weeks, mg/dL	103.0 (88.5 - 125.5)	92.0 - 117.0	119.0 (105.5 -	109.0 - 129.0	0.011
Absolute change from baseline, mg/dL	-6.0 (-18.5 - 9.0)	-14.0 - 5.0	2.5 (-5.0 - 13.2)	-2.0 - 11.0	0.015
Baseline apolipoprotein B, g/L	1.11 (0.98 - 1.25)	1.05 - 1.2	1.15 (0.99 - 1.25)	1.08 - 1.2	0.486
Apolipoprotein B after 12 weeks, g/L	1.03 (0.88 - 1.25)	0.92 - 1.2	1.19 (1.06 - 1.35)	1.09 - 1.3	0.011
Absolute change from baseline, g/L	-0.06 (-0.18 - 0.09)	-0.14 - 0	0.03 (-0.05 - 0.13)	-0.02 - 0.1	0.015
% change from baseline	-5.8 (-18.1 - 8.1)	-12.7 - 4.3	2.3 (-4.1 - 12.2)	-1.8 - 8.0	0.009
Apolipoprotein A					
Baseline apolipoprotein A, mg/dL	134 (122.5 - 143.5)	124 - 140	137 (124 - 149)	131 - 145	0.4
Apolipoprotein A after 12 weeks, mg/dL	139 (120 - 149)	128 - 147	143.5 (131.5 - 157)	135 - 152	0.151
Absolute change from baseline, mg/dL	1 (-10 - 17)	-8 - 9	3 (-4 - 12)	-2 - 9	0.389
Baseline apolipoprotein A, g/L	1.34 (1.23 - 1.44)	1.24 - 1.4	1.37 (1.24 - 1.49)	1.31 - 1.4	0.4
Apolipoprotein A after 12 weeks, g/L	1.39 (1.2 - 1.49)	1.28 - 1.5	1.44 (1.31 - 1.57)	1.35 - 1.5	0.151
Absolute change from baseline, g/L	0.01 (-0.1 - 0.17)	-0.08 - 0.1	0.03 (-0.04 - 0.12)	-0.02 - 0.1	0.389
% change from baseline	0.9 (-7.3 - 12.9)	-5 - 8.2	2.3 (-2.8 - 9.4)	-1.5 - 6.6	0.422

Table 3. Continued

Parameters	Phospholipovit group (n = 39)		Placebo group (n = 41)		*P*-value
	median (Q1 - Q3)	95% CI	median (Q1 - Q3)	95% CI	
Lipoprotein (a)					
Baseline lipoprotein (a), mg/dL	11.2 (3.7 - 38.8)	5 - 21.9	8.4 (5.2 - 13.7)	5.5 - 12.8	0.32
Lipoprotein (a) after 12 weeks, mg/dL	9.1 (3.9 - 22.7)	5 - 14.4	8.8 (5.3 - 12)	6.1 - 11.2	0.596
Absolute change from baseline, mg/dL	-0.2 (-3.3 - 0.5)	-1.5 - 0.3	-0.6 (-2.1 - 0.9)	-0.8 - 0.5	0.794
% change from baseline	-3.5 (-19.6 - 14.3)	-11.7 - 8.6	-4.6 (-21.1 - 29.3)	-12.7 - 14.7	0.756
hs-CRP					
Baseline hs-CRP, mg/dL	0.21 (0.13 - 0.38)	0.15 - 0.3	0.19 (0.13 - 0.38)	0.15 - 0.3	0.994
hs-CRP after 12 weeks, mg/dL	0.21 (0.11 - 0.43)	0.14 - 0.3	0.22 (0.12 - 0.38)	0.14 - 0.3	0.746
Absolute change from baseline, mg/dL	0 (-0.06 - 0.06)	-0.05 - 0	0 (-0.06 - 0.08)	-0.05 - 0	0.91
Baseline hs-CRP, mg/L	2.1 (1.3 - 3.85)	1.5 - 2.7	1.9 (1.3 - 3.8)	1.5 - 3	0.994
hs-CRP after 12 weeks, mg/L	2.1 (1.05 - 4.3)	1.4 - 3.3	2.15 (1.2 - 3.83)	1.4 - 3.2	0.746
Absolute change from baseline, g/L	0 (-0.65 - 0.6)	-0.5 - 0.5	0.05 (-0.62 - 0.8)	-0.5 - 0.3	0.91
% change from baseline	0 (-40 - 35.9)	-26.9 - 21.7	3.4 (-31 - 45)	-14.3 - 26.1	0.557

Phospholipovit or placebo (maltose) was administered orally at the dose of 500 mg, 2 times/day for 12 weeks. Data were expressed as median (Q1 - Q3). CI, Confidence interval; TC, total cholesterol; hs-CRP, high-sensitivity C-reactive protein; HDL-C, high-density lipoproteins-cholesterol; LDL-C, low-density lipoproteins-cholesterol; VLDL-C, very-low-density lipoproteins-cholesterol.

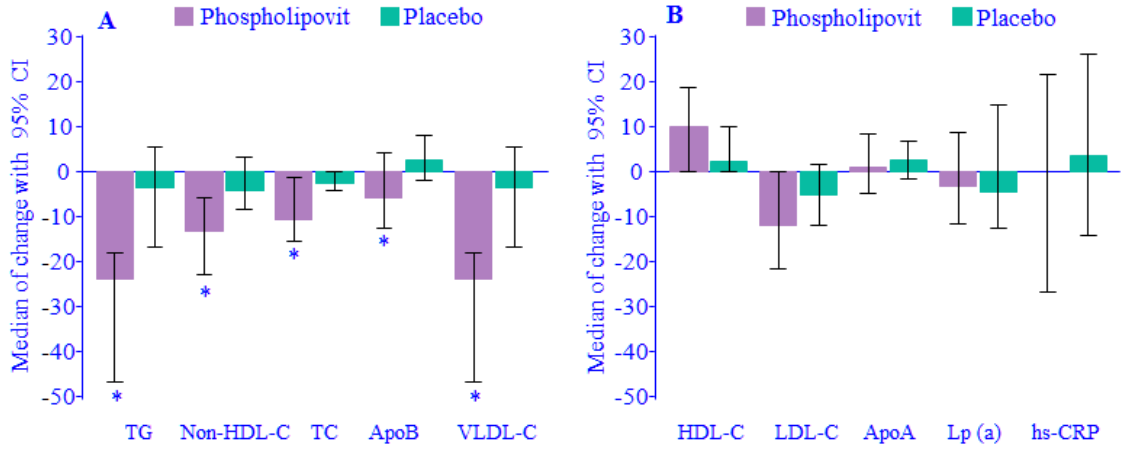

Fig. 2. The changes of biochemical parameters including (A) TG, non-HDL-C, TC, ApoB, and VLDL-C and (B) HDL-C, LDL-C, ApoA, Lp (a), and hs-CRP in phospholipovit and placebo groups. Phospholipovit or placebo (maltose) was administered orally at 500 mg, 2 times/day for 12 weeks. Data were presented as median and its confidence intervals. $^{*}P < 0.05$ indicated a significant difference compared with the placebo group. TG, Triglycerides; TC, total cholesterol; ApoB, apolipoprotein B; VLDL-C, very-low-density lipoproteins-cholesterol; HDL-C, high-density lipoproteins-cholesterol; LDL-C, low-density lipoproteins-cholesterol; ApoA, apolipoprotein A ; Lp (a), lipoprotein (a); hs-CRP, high-sensitivity C-reactive protein.

The percentage change of TG between the phospholipovit group (-23.8%) and the placebo group (-3.7%) was significant (Table 3). The percent of patients achieving a fasting TG level < 150 mg/dL (< 1.7 mmol/L) was 56.4% (22 patients) in the phospholipovit group versus 22% (9 patients) in the placebo group ($P = 0.003$, OR 4.51 (1.58 - 13.8)). There was a significant change in VLDL-C between phospholipovit and placebo groups similar to TG (Fig. 2A).

There was a non-significant tendency in the change of LDL-C between the phospholipovit (-11.9%) and the placebo (-5.2%) groups. In addition, non-significant change was observed in HDL-C between the phospholipovit (10%) and the placebo (2.0%) groups (Fig. 2B and Table 3). The percentage reduction of total cholesterol was significant between the phospholipovit (-10.8%) and the placebo (-2.7%) groups (Fig. 2A and Table 3).

Table 4. Adverse events and laboratory measurements during 12 weeks of phospholipovit treatment.

Parameters, n (%)	Phospholipovit group (n=50)	Placebo group (n=50)	*P*-value
Serious AE	0	0	-
Any AE	14 (28)	17 (34)	0.910
Mild AE	13 (93)	17 (100)	0.452
Moderate AE	1 (7)	0	0.452
Severe AE	0	0	-
Upper respiratory tract infection	5 (10)	5 (10)	0.693
Dyspepsia	5 (10)	4 (8)	0.477
Platelet count < 150,000 mm^3	0	0	-
ALT level ≤ 2ULN	0	0	-
AST level ≤ 2ULN	0	0	-
Serum creatine increase more 1 mmol/L	0	0	-
CKD-EPI > 10% decrease from baseline	0	0	-

Phospholipovit or placebo (maltose) was administered orally at the dose of 500 mg, 2 times/day for 12 weeks. Data were expressed as a number (%). AE, Adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of normal; CKD, chronic kidney disease; EPI, epidemiology collaboration formula.

The percentage change of ApoB level showed a significant difference between the phospholipovit (-5.8%) and placebo (2.3%) groups (Fig. 2A and Table 3). There were no significant changes in ApoA, Lp(a), and hs-CRP levels between phospholipovit and placebo groups (Fig. 2B and Table 3).

Safety analyses

There were no serious AE in both groups. The analysis of serious AE was based on good clinical practice rules, which included events that resulted in death, were life-threatening (an event in which the patient was at risk of death at the time of the event), required inpatient hospitalization, and resulted in persistent or significant disability or incapacity. AE was similar in phospholipovit and placebo groups, 14 (28%) versus 17 (34%), with the majority being mild (93% versus 100%). There was no severe AE in both groups (Table 4). The most frequent AE in phospholipovit versus placebo groups were upper respiratory tract infection (5/14 (12%) versus 5/17 (10%)) and gastrointestinal AE (dyspepsia) (5/14 (10%) versus 4/17 (8%)) (Table 4).

No one patient showed platelet count less than 150.000 mm^3 and in other blood cell count in general blood test, which was performed every 4 weeks. No differences were observed in liver biochemical tests, renal function, or significant changes in other laboratory measures, such as glucose. Also, no changes were found in vital signs and electrocardiographic measurements (Table 4).

DISCUSSION

This study demonstrated that phospholipovit (ultra-small phospholipid nanoparticles-micelles) resulted in a significant decrease in percentage change from baseline of non-HDL-C and TG after 12 weeks of treatment with 1 g daily in patients with combined hyperlipidemia at moderate CV risk.

According to the PubMed database and https://clinicaltrials.gov/, it was the first randomized placebo-controlled clinical trial, which evaluated the effects of micelles in modifying non-HDL-C and TG levels.

The reduction of non-HDL-C after 12-week treatment in the phospholipovit group was 2 times higher, as compared with this parameter in a total of 24 non-statin (fibrates, niacin, and omega 3) trials. In 49 non-statin and statin trials, the mean reduction of non-HDL-C was -24 mg/dL (-0.6 mmol/L) (5). This finding was similar to the mean reduction of non-HDL-C in the phospholipovit group (-28.3 mg/dL or -0.73 mmol/L).

The reduction of TG after 12 weeks of treatment in the phospholipovit group was 2 times higher than that in other 24 non-statin trials, including the biggest one, REDUCE-IT trial, and 3 times higher than that in 25 statin trials (16). In total, the mean reduction of TG in 49 non-statin and statin trials was -25 mg/dL (-0.3 mmol/L) (5), which was comparable with -62.0 mg/dL (-0.7 mmol/L) in the phospholipovit group.

The risk ratio of lowering TG and the risk of major vascular events in 49 non-statin and statin trials was 0.84 per 1 mmol/L (P = 0.0026). In contrast, a STRENGTH study performed on the effects of omega-3-fatty acids eicosapentaenoic acid and docosahexaenoic acid showed that the 19% reduction of TG level in statin-treated patients was not associated with reduction in the primary endpoint of significant CV events. Therefore, the STRENGTH study was stopped (17). Recently, a reduction in TG levels (-184 mg/dL or -2.08 mmol/L) was observed in the olerzasen (inhibitor of ApoC-III) phase II study after 6-12 months of treatment (18). Despite the established TG-lowering strategies, therapies that alter the function of HDL particles and reduce the risk of ASCVD, are unknown (19).

Strategies for increasing HDL-C through the reconstituted HDL nanoparticles were performed, but most of them were stopped because of serious AE and low efficacy (20-23). Only one trial included more than 18,000 patients was completed in 2024, but its results have not yet been published. (24). Nevertheless, Day's study reported that only one agent, lecithin as PC, had demonstrated the ability to reverse experimental atherosclerosis (25). *In vitro* studies showed that super-phospholipidation by PC may increase the CEC (26). The authors speculated that the increase of PL in HDL can promote the enhancement of CEC in patients with metabolic syndrome and early stages of CHD. It was found that these patients had a low CEC, which was associated with low HDL-C and high TG. The small amount of PL in HDL positively correlated with low CEC ($r = 0.62$, $P < 0.001$) (27).

Recently, a PL nanoparticle, named miNano, was developed (15 ± 1 nm), which directly binds to cholesterol crystals, dissolves them, and enhances CEC. According to experimental data, miNano prevented foam cell formation and stabilized the formation of atherosclerotic plagues. The authors speculated that these findings can provide a novel approach for atherosclerosis treatment by phospholipid nanoparticles miNano (28). These findings were correlated with the results of many different classes of PL and their action in HDL in stable ischemic heart disease (IHD) patients and with acute coronary syndrome (ACS). In patients with ACS, the PC/phosphatidylethanolamine ratio in HDL was less than in stable IHD patients. The authors speculated that the phospholipidation by PC in ACS patients could stabilize the atherosclerotic plaques and prevent them from disruption, leading to future thrombosis and myocardial infarction (29).

The phospholipovit phase II trial had some advantages. Micelles were produced without detergents and apoproteins, using only the ultrasonic homogenizer. We made a water-soluble pharmaceutical form of phospholipovit, which was safe and effective for 12-week treatment *via* oral administration and will be convenient for long-term usage.

The phospholipovit phase II trial was not without limitations. The trial was not powered for clinical outcomes as the primary efficacy endpoints. In this study, patients with the clinical features of CV diseases were not included, but only those with risk factors within the age limit of 30 - 75 years. The duration of the treatment was short-term and lasted for 12 weeks. So, the authors suggest that future trials associated with phospholipovit are performed during long-term periods, including patients with combined hyperlipidemia, and the primary endpoint will be the composite of ASCVD events.

In summary, we developed water-soluble micelles for oral administration to decrease non-HDL-C and TG in combined hyperlipidemia patients and moderate CV risk. Phospholipovit with daily administration of 1 g for 12 weeks was shown to be safe, well tolerated, and efficacious.

CONCLUSION

Phospholipovit significantly reduced non-HDL-C, TG, and atherogenic lipoproteins in patients with combined hyperlipidemia and moderated CV risk. Targeting the PL of HDL with phospholipovit is a unique and potent approach for reducing non-HDL-C, TG, and atherogenic lipoproteins. Phospholipovit may have a variety of applications; one of them is the add-on therapy to statin for residual risk reduction of CV events.

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Conflict of interest statement

The authors declared no conflict of interest.

Authors' contributions

A. Archakov contributed to the conceptualization, review, and editing of the manuscript and was a guarantor; A. Lisitsa contributed to the conceptualization, review, and editing; V. Kukharchuk was responsible for the investigation and data curation; E. Ponomarenko, Y. Romashova, and T. Pleshakova were responsible for a project administration and supervision; E. Yarovaya and V. Kutsenko performed statistical analysis; M. Guseva, V. Beregovykh, O. Ipatova, M. Zubareva, and E. Tikhonova were investigators; S. Ivanov and F. Bedretdinov performed the review and editing of the manuscript; S. Markin contributed to the conceptualization, writing, review, and editing of the manuscript. The finalized version was read and approved by all authors. The requirements for authorship as stated earlier in this document have been met. Each author believes that the manuscript represents honest work.

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SUPPLEMENTARY APPENDIX 1

Sample size calculation

Taking into account the trial design (randomized parallel group placebo-controlled trial), the following hypotheses were tested for the planned comparison:

H0: $\varepsilon \leq 0$,

where ε is the difference in the values of the efficacy indicator (decrease in the concentration of non-HDL cholesterol, expressed as a percentage of the initial level) in the compared groups ($\varepsilon = \mu_2 - \mu_1$, where μ_2 is the value of the indicator in the study drug group, μ_1 is the average value of the indicator in the control group).

An alternative hypothesis would be the following hypothesis (it is planned to test the superiority hypothesis):

Ha: $\varepsilon > 0$

If, based on the results of the statistical test, the null hypothesis is rejected, it can be argued that for a given level of significance, the alternative hypothesis of efficacy difference of combination therapy in the phospholipovit group compared to the comparison group (placebo) is valid.

For a given study power (at least 80%), the sample size will be calculated using the following equation (for equal group sizes):

$$n1 = n2 = \frac{2 \cdot (Z\alpha + Z\beta)^2 + \sigma^2}{(\varepsilon - \delta)^2}$$

where α is the permissible value of type I error when testing the primary endpoint, β is the permissible value of type II error when testing the main endpoint (1 - β is defined as the power of the study); Zα and Zβ are values of the standard normal distribution for a given level of α and β, respectively; μ1 and μ2 are the expected average values of answers in the compared groups; ε is the expected difference in answers ($\varepsilon = \mu_2 - \mu_1$) in the compared groups.

According to the results of large-scale RCTs, during statin therapy, there is a decrease in non-HDL cholesterol by 17-39% from the initial level (meta-analysis by J.G. Robinson *et al*., 2009), with an estimate of the standard deviation not exceeding 17%[1].

For $\alpha = 0.05$, study power of at least 80% ($\beta = 0.2$), the expected difference in the study and control groups for the main efficacy indicator of at least 15%, and a superiority margin of 5%, the sample size is calculated using the following equation:

$$n1 = n2 = \frac{2 \cdot (1.64 + 0.84)^2 + 0.17^2}{(0.15 - 0.05)^2} = 36$$

Therefore, the planned statistical test requires results (efficacy estimates) for at least 72 participants. Assuming a dropout rate of approximately 30%, it is recommended to recruit at least 36/(1-0.15) ~ 50 patients into the study groups (per group), with a total of 100 patients for inclusion.

[1] Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk. J Am Coll Cardiol. 2009;53(4): 316-322.
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Table S1. Inclusion and exclusion criteria.

Inclusion criteria	Men and women aged 30-75 years
	Availability of signed and dated informed consent of the patient to participate in the study
	Patients with moderate combined hyperlipidemia, defined as:
	TG 151 - 399 mg/dL (1.7 - 4.5 mmol/L)
	HDL-C < 39 mg/dL (< 1 mmol/L) for men and < 46 mg/dL (< 1.2 mmol/L) for women
	Patient consent to use reliable contraceptive methods throughout the study
	The patient's ability to adequately cooperate
Exclusion criteria	Diseases or metabolic disorders that can cause an increase in LDL-C, total cholesterol, and TG (secondary dyslipidemia)
	Patients receiving high doses of statin drugs (rosuvastatin > 40 mg and atorvastatin > 80 mg) and any hypotriglyceridemic drugs such as omega-3, fibrates, and niacin
	Type 1 diabetes mellitus
	Acute kidney injury, defined as an increase in serum creatinine ≥ 0.3 mg/dL (26.5 μmol/L) within 48 h or ≥ 50% from baseline value known or presumed to have occurred within the prior 7 days and/or urine output ≤ 0.5 mL/Kg for ≥ 6 h according to the acute disease quality initiative
	Liver decompensation, defined by the acute development of one or more major complications of liver disease (*i.e.*, ascites, hepatic encephalopathy, gastrointestinal hemorrhage, and bacterial infection), and can be further complicated by organ failures and high short-term
	Severe dysfunction of other vital organs, accompanied by decompensation of their functions; diseases of the central nervous system, with severe impairment of cognitive functions
	Oncological disease during the last 5 years
	Diagnosed porphyria
	Diagnosed myopathy
	Acute conditions (infections, injuries, and operations) in the period less than 2 months before the start of the study
	Glomerular filtration rate less than 30 mL/min/1.73 m^2
	Persistent increase in liver enzymes activity (AST and ALT) of unclear etiology or increased liver enzymes activity by 2 or more times from the upper limit of the norm
	Alcohol abuse more than 5 units of alcohol for week (1 unit alcohol is equivalent to 0.325 L beer, 130 mL wine, and 30 mL alcohol)
	Drug use
	Positive HIV test result
	Hepatitis B and C, syphilis
	Any other diseases or conditions that, in the opinion of the investigator, may distort the results of the study and limit the patient's participation in the study.

ALT, Alanine aminotransferase; AST aspartate aminotransferase; HDL-C, high-density lipoproteins-cholesterol; HIV, human immunodeficiency viruses; LDL, low-density lipoproteins.

Table S2. Efficacy endpoints.

The primary efficacy endpoint	Percentage change from baseline in non-HDL-C values on week 12
Secondary efficacy endpoint	Dynamics of change of TG level compared with the baseline on week 12
	Dynamics of change of total cholesterol level compared with the baseline on week 12
	Dynamics of change of HDL-C level compared with the baseline on week 12
	Dynamics of change of LDL-C level compared with the baseline on week 12
	Dynamics of change of VLDL-C level compared with the baseline on week 12
	Dynamics of change of ApoB level compared with the baseline on week 12
	Dynamics of change of ApoA level compared with the baseline on week 12
	Dynamics of change of Lp (a) level compared with the baseline on week 12
	Dynamics of average hs-CRP level compared with the baseline on week 12

HDL-C, High-density lipoproteins-cholesterol; LDL-C, low-density lipoproteins-cholesterol; VLDL-C, very-low-density lipoproteins-cholesterol; Lp (a), lipoproteins (a); hs-CRP, high-sensitivity C-reactive protein.