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Treatment with PCSK9 inhibitors reduces atherogenic VLDL remnants in a real-world study

Tim Hollstein[a], Anja Vogt[g], Thomas Grenkowitz[a], Tatjana Stojakovic[b], Winfried März[b,d,e], Ulrich Laufs[c,f], Bediha Bölükbasi[c], Elisabeth Steinhagen-Thiessen[a], Hubert Scharnagl[b,*], Ursula Kassner[a]

[a] Department of Endocrinology, Campus Virchow-Klinikum, Charité Universitätsmedizin Berlin, Berlin, Germany
[b] Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
[c] Klinik für Innere Medizin III, Universitätsklinikum des Saarlandes, Homburg, Germany
[d] Department of Internal Medicine 5, Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany
[e] Synlab Academy, Synlab Holding Germany GmbH, Mannheim, Germany
[f] Klinik und Poliklinik für Kardiologie, Universitätsklinikum Leipzig, Leipzig, Germany
[g] Medizinische Klinik und Poliklinik IV, Klinikum der Universität München, München, Germany

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ABSTRACT

Background: Proprotein convertase subtilisin-kexin type 9 inhibitors (PCSK9-I) reduce low-density lipoprotein (LDL) cholesterol in human studies. Previous studies suggest that PCSK9-I may also affect very-low-density lipoproteins (VLDL). We therefore studied VLDL size and composition in a "real-world" study population with the use of β-quantification.

Subjects and methods: 350 patients (62 ± 11 years old, 58% men, 22% with diabetes mellitus) with different concomitant lipid lowering therapies, and in whom PCSK9-I treatment was indicated, received either evolocumab (140 mg) or alirocumab (75 or 150 mg). The major lipoprotein fractions were separated by β-quantification and lipid and apolipoprotein compositions were determined before and 4 weeks after initiation of PCSK9-I treatment.

Results: After 4 weeks of PCSK9-I treatment, the ratio of triglycerides to apolipoprotein B in VLDL particles (VLDL-TG/apoB ratio) increased by 40% ($p < .0001$). VLDL-associated apolipoproteins E, CII, and CIII were reduced by 29.4%, 16.4%, and 12.4%, respectively (all $p < .0001$).

Conclusion: PCSK9-I treatment increased VLDL size (estimated by an increased VLDL-TG/apoB ratio) and reduced VLDL-associated apolipoproteins in a heterogeneous "real-world" study-population, reflecting a higher clearance of small atherogenic VLDL remnant particles by PCSK9-I. This may potentially lower cardiovascular risk in clinical routine patients beyond low-density cholesterol (LDL-C) reduction.

1. Introduction

Proprotein convertase subtilisin-kexin type 9 inhibitors (PCSK9-I) represent the newest class of low-density lipoprotein cholesterol (LDL-C) lowering drugs aiming to reduce cardiovascular disease (CVD) risk [1–3]. Currently, two PCSK9-I are available for prescription: evolocumab and alirocumab, human monoclonal antibodies of the IgG2 and IgG1 isotype, respectively. Both antibodies increase LDL receptor density on hepatocytes by binding to PCSK9 and inhibiting LDL-receptor degradation, which leads to 50–60% lower LDL-C levels and better

Abbreviations: apoAI, apolipoprotein AI; apoAII, apolipoprotein AII; apoB, apolipoprotein B; apoCII, apolipoprotein CII; apoCIII, apolipoprotein CIII; apoE, apolipoprotein E; BMI, body mass index; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; HDL-TG, high-density lipoprotein triglycerides; Lp(a), Lipoprotein(a); LDL-apoB, low-density lipoprotein apolipoprotein B; LDL-C, low-density lipoprotein cholesterol; LDL-C/apoB ratio, ratio of cholesterol to apolipoprotein B in low-density lipoprotein particles; LDL-TG, low-density lipoprotein triglycerides; LLT, lipid lowering therapy; PCSK9-I, Proprotein convertase subtilisin-kexin type 9 inhibitor; TC, Total-Cholesterol; TG, triglycerides; VLDL-apoB, very-low-density lipoprotein apolipoprotein B; VLDL-C, very-low-density lipoprotein cholesterol; VLDL-TG, very-low-density lipoprotein triglycerides; VLDL-TG/apoB ratio, ratio of triglycerides to apolipoprotein B in very-low-density lipoprotein particles.

* Corresponding author at: Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria.
E-mail address: hubert.scharnagl@medunigraz.at (H. Scharnagl).

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cardiovascular outcomes [1,3].

Whereas the impact of PCSK9-I on common lipid parameters is well characterized, only few studies investigated their effects on lipoprotein composition and particle size. This may be relevant, however, as smaller LDL and very-low-density lipoprotein (VLDL) particles are associated with greater cardiovascular risk [4–7]. Both lipoproteins are part of the endogenous pathway of lipid metabolism, where VLDL particles are first secreted from the liver and mainly carry triglycerides (TG) and apolipoproteins (apo) E, CII, and CIII. VLDL undergo lipolysis, which converts them to smaller VLDL remnant or intermediate-density lipoproteins. They can be taken up by hepatocytes or convert to even smaller LDL particles, which mainly carry cholesterol and apoB.

Our primary aim of the present study was to determine VLDL and LDL composition and estimate their particle sizes after 4 to 6 weeks of treatment with alirocumab or evolocumab in a heterogeneous multicenter "real-world" study population. We used β-quantification for lipoprotein analysis, which combines ultracentrifugation with precipitation and is often considered the "gold standard" method [8,9].

2. Patients and methods

2.1. Patients

This is a prospective, open-label study in patients receiving PCSK9-I (alirocumab 75 or 150 mg sc. once every 2 weeks or evolocumab 140 mg sc. once every 2 weeks) in clinical routine. Patients were recruited 2016 through 2017 at the Outpatient Lipid Clinic of the Charité Berlin (Berlin, Germany), at the Department of Cardiology of the University Hospital Homburg Saar (Homburg, Germany), and at the Outpatient Lipid Clinic of the University Hospital Munich. Inclusion criteria were age ≥ 18 years, prescription of PCSK9-I, and ability to understand the purpose of the study. Patients receiving alirocumab or evolocumab in line with current guidelines were eligible for inclusion into the study [1,10]. Patients not able to participate in the follow-up visit were excluded. There were 2 study visits, the first before the start of the treatment with PCSK9-I and the second 4 to 6 weeks after initiation of treatment with PCSK9-I (all patients received at least 2 injections of a PCSK9-I, some received 3 injections). At the first visit, all patients filled out a standard questionnaire on their medical history. In addition, they underwent routine clinical investigation. Before treatment initiation and after 4 weeks of treatment, patients underwent blood sampling. Diabetes mellitus, hypertension, and CVD (coronary artery disease and/or cerebral artery disease and/or peripheral artery disease) were diagnosed based on medical records. Written informed consent was obtained from each patient included in the study; the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the institution's ethics committees on research on humans (EA4/178/15 and 162/15).

2.2. Laboratory analyses

Laboratory analyses were performed in fasting blood samples. Lipoproteins (VLDL, LDL, HDL) were separated using a combined ultracentrifugation precipitation method (β-quantification) [11,12]. Plasma was ultracentrifuged at a density of d = 1.0063 kg/l (30.000 rpm for 18 h) using a Beckman rotor type 50.4. The VLDL fraction was removed, LDL were precipitated with phosphotungstic acid/$MgCl_2$. Total cholesterol (TC), free cholesterol, TG, and phospholipids were measured using enzymatic reagents from Diasys (Holzheim, Germany) and were calibrated using secondary standards from Roche Diagnostics (Mannheim, Germany; TC, TG) and Diasys (Holzheim, Germany; free cholesterol, phospholipids). Esterified cholesterol was calculated as the difference between TC and free cholesterol. Total apolipoproteins, apoB in VLDL and LDL, and Lipoprotein(a) (Lp(a)) were determined by immunoturbidimetry using reagents from DiaSys (Holzheim, Germany) and standards from Siemens (Marburg, Germany; apoAI, apoB, apoE), Kamiya Biomedical (Seattle, WA, USA; apoAII, apoCII, apoCIII), and Diasys (Lp(a)). All measurements were performed on an Olympus AU640 automatic analyzer. The coefficients of variation (between day) were < 5%. ApoCII was not detectable in one patient at baseline. These data were therefore excluded from the analysis of apoCII changes. VLDL particle size was estimated by calculating the ratio of TG to apoB in the VLDL fraction (termed VLDL-TG/apoB ratio), as previously showed [13]. LDL particle size was estimated by calculating the ratio of cholesterol to apoB in the LDL fraction (termed LDL-C/apoB ratio), as previously showed [14]. ApoE genotype was determined with reverse transcription polymerase chain reaction (RT-PCR) in a local laboratory after additional written informed consent of patients was obtained. Lipid parameters are reported in mg/dl and can be converted to mmol/l (International System of Units) by multiplying by 0.02586 (Cholesterol), 0.0115 (TG) and 0.0129 (PL), respectively.

2.3. Statistical analysis

Baseline clinical and biochemical characteristics are presented for the entire cohort using means and standard deviations for normally distributed continuous variables, medians with upper and lower quartile in parentheses for skewed variables (TG, Lp(a), VLDL-C), and numbers and percentages for categorical data. Changes in lipid parameters in response to PCSK9-I treatment are presented as arithmetic mean, geometric mean, or median with its 95% confidence interval (CI) as appropriate. The change (Δ) in lipid parameters from baseline to 4-week PCSK9-I treatment was assessed by paired *t*-test for normally-distributed values and by Wilcoxon signed-rank test for skewed values. The Chi-Square test was used to compare categorical data. Pearson correlation coefficients were used to examine associations between variables. The changes in lipid parameters were also shown stratified for concomitant lipid-lowering therapy (LLT) and PCSK9-I drug. Comparisons among the groups were made with ANalysis Of Variance and Kruskal-Wallis Test, the former for parametric data, the latter for non-parametric data. The SAS Enterprise Guide V7.15 (SAS Institute, Cary, NC, USA) statistical package was used.

3. Results

Baseline characteristics and lipid profile were available from 350 patients, of which 73% were recruited in Berlin, Germany (Table 1). The study population consisted of 97% Caucasians, 58% men and had a mean ± standard deviation age of 62 ± 11 years. 22% and 82% of the subjects reported a history of diabetes mellitus or CVD, respectively. 36% did not receive any concomitant LLT, whereas 40% received a combination of statin and ezetimibe. 46% were prescribed evolocumab, 54% alirocumab. Briefly, baseline mean LDL-C and HDL-C were 151 and 45 mg/dl, respectively. Median VLDL-C, TG and Lp(a) concentrations were 38, 148, and 26 mg/dl, respectively. There were gender differences regarding age, CVD, use of concomitant LLT with statin/ezetimibe, and Lp(a). Absolute lipid values at baseline and after 4 weeks of PCSK9-I therapy as well as absolute changes are reported in Supplementary Table 1. None of the subjects reported any serious adverse event.

3.1. Changes in estimated VLDL and LDL particle size

The TG/apoB ratio of VLDL particles (which was used to estimate VLDL particle size) increased from 5.4 at baseline to 7.5 after 4 weeks of PCSK9-I treatment (+2.1, $p < .0001$, Fig. 1A). The percent increase in the VLDL-TG/apoB ratio was 40.2%. It was negatively associated with baseline VLDL-TG/apoB ratio ($r = -0.60$; $p < .0001$; Fig. 1B).

The cholesterol/apoB ratio of LDL particles (which was used to estimate LDL particle size) decreased from 1.54 at baseline to 1.43 after 4 weeks of PCSK9-I treatment (−0.11, $p < .0001$, Fig. 1C). The relative decrease in the LDL-C/apoB ratio was 7.0% and was not

Table 1
Baseline characteristics.

	Total (n = 350)	Men (n = 203)	Women (n = 147)
Age (years)	62 ± 11	60.3 ± 11.3	63.4 ± 11.4*
BMI (kg/m^2)	28 ± 5	28.6 ± 4.3	27.7 ± 5.7
Race/ethnicity			
Caucasian	341 (97.4)	195 (96.1)	146 (99.3)
Arabic	8 (2.3)	7 (3.4)	1 (0.6)
Asian	1 (0.2)	1 (0.5)	0
Clinical characteristics			
Diabetes type 2	77 (22)	46 (23)	31 (21)
CVD	286 (82)	178 (89)	108 (73)*
Smoker	82 (24)	55 (28)	27 (19)
Hypertension	217 (64)	128 (65)	69 (62)
Concomitant lipid-lowering therapy			
None	125 (36)	68 (34)	57 (39)
Statin	48 (14)	22 (11)	26 (18)
Ezetimibe	36 (10)	20 (10)	16 (11)
Statin/Ezetimibe	139 (40)	91 (45)	48 (33)*
Prescribed PCSK9-I			
Evolocumab 140 mg	161 (46)	88 (43)	73 (50)
Alirocumab 150 mg	116 (33)	71 (35)	45 (31)
Alirocumab 75 mg	73 (21)	44 (22)	29 (20)
Recruitment center			
Berlin	257 (73)	149 (73)	108 (73)
Homburg	41 (12)	28 (14)	13 (9)
Munich	52 (15)	26 (13)	26 (18)
Lipid parameters			
Total cholesterol (mg/dl)	246.2 ± 71.1	228.9 ± 59.5	270.1 ± 78.8
VLDL-C (mg/dl)	38 (19; 61)	40 (22; 62)	33 (16; 59)
LDL-C (mg/dl)	151.4 ± 58	137.5 ± 47.8	170.6 ± 65.2
HDL-C (mg/dl)	45.2 ± 14.8	41.4 ± 12.9	50.5 ± 15.7
Triglycerides (mg/dl)	148 (100; 217)	149 (104; 232)	143 (95; 215)
Lp(a) (mg/dl)	26 (9; 81)	22 (6; 64)	42 (13; 107)*
apoB (mg/dl)	121.5 ± 37.8	115.3 ± 33.1	130.0 ± 42.1

Continuous data presented as mean ± standard deviation, or median (interquartile range). Categorial data presented as number (percent). Missing data: BMI (n = 93), diabetes type 2 (n = 5), CVD (n = 3), smoker (n = 9), hypertension (n = 9), concomitant lipid-lowering therapy (n = 2).
Abbreviations: apoB, apolipoprotein B; BMI, body mass index; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; Lp(a), Lipoprotein(a); LDL-C, low-density lipoprotein cholesterol; PCSK9-I, Proprotein convertase subtilisin-kexin type 9 inhibitor; VLDL-C, very-low-density lipoprotein cholesterol.
* : $p < .05$ vs. men by Student's unpaired t-test (for normally distributed data), Wilcoxon signed-rank test (for skewed data) or $\chi 2$ test (for categorial data).

associated with the baseline LDL-C/apoB ratio ($p = .7$; Fig. 1D).

In a subgroup analysis, changes in the VLDL-TG/apoB and LDL-C/apoB ratios were comparable between male/female and diabetic/non-diabetic subjects (all $p > .15$).

3.2. Lipid-lowering effects

After 4 weeks of PCSK9-I treatment, TC was reduced by 38.6% ($p < .0001$, Fig. 2A), which was mainly driven by a reduction of LDL-C (−77.2 mg/dl, −50.7%, $p < .0001$, Supplementary Table 1) and to a lesser extent by a reduction of VLDL-C (−23.3 mg/dl, −46.0%, $p < .0001$). Compared to women, men showed a higher reduction of TC (−8.2%, $p < .0001$), LDL-C (−8.5%, $p < .003$), and VLDL-C (−9.3%, $p < .02$, data not shown). High-density lipoprotein cholesterol (HDL-C) increased by 12.9% ($p < .0001$, Fig. 2D). TG decreased by 22.7% ($p < .0001$, Fig. 2A), which was mainly attributable to a decrease of VLDL-TG (−33.7 mg/dl; −22.5%, $p < .0001$, Supplementary Table 1) and to a lesser extent to a decrease of LDL-TG (−12.4 mg/dl, −36.3%, $p < .0001$). HDL-TG slightly increased by 8.3% ($p = .0005$). Median Lp(a) decreased by 19.3% ($p < .0001$, Fig. 2A). Compared to women, men showed a higher reduction of LDL-TG (−8.1%, $p = .0004$).

3.3. Changes in apolipoproteins

ApoB significantly decreased by 48.2% ($p < .0001$, Fig. 2A), mostly due to reduced LDL-apoB (−47.4 mg/dl, −47.5%, $p < .0001$, Supplementary Table 1) and only slightly due to a reduction in VLDL-apoB (−11.2 mg/dl, −44.9%, $p < .0001$). The percent reduction in LDL-apoB was independent of baseline LDL-apoB ($r = -0.1$, $p = .06$, data not shown), whereas the percent reduction in VLDL-apoB was inversely associated with baseline VLDL-apoB ($r = -0.53$, $p < .0001$, data not shown). ApoB, LDL-apoB, and VLDL-apoB were more reduced in men compared to women (−8.3%, $p < .0001$, −7.7%, $p = .01$, and − 8.9%, $p = .02$, respectively). ApoCII, apoCIII, and apoE were reduced by 16.4%, 12.4%, and 29.4%, respectively (all $p < .0001$, Fig. 2C), and percent reductions of all three apolipoproteins were inversely associated with their respective baseline value (all $r < -0.13$, all $p < .01$, data not shown). The apoE genotype did not influence reductions of apoE or major lipid parameters (data not shown). ApoE was more reduced in men compared to women (−5.3%, $p = .006$, data not shown). ApoAI increased by 4.4%, $p < .0001$, Fig. 2D), whereas apoAII did not change during PCSK9-I therapy.

3.4. Subgroup: concomitant lipid lowering therapy

We further compared whether the effects of PCSK9-I treatment differed according to concomitant LLT (no therapy, statin, ezetimibe, or both). Baseline characteristics stratified by LLT showed no differences in sex, body mass index (BMI), CVD history, diabetes history, PCSK9-I drug, or VLDL-TG/apoB ratio, whereas significant differences were detected when comparing age, smoking status, use of alirocumab 150 mg, TC, VLDL-C, LDL-C, TG, Lp(a), apoB, and LDL-C/apoB ratio (Supplementary Table 3).

After 4-week PCSK9-I treatment, ApoCIII did not decrease in patients without concomitant LLT ($p = .6$, Table 2), VLDL-TG did not decrease in patients receiving statins only ($p = .08$), and HDL-TG did not increase in patients receiving ezetimibe only ($p = .34$, Table 2).

In patients receiving both statin and ezetimibe, apoB and the LDL-C/apoB ratio decreased more and the VLDL-TG/apoB ratio increased more, compared to patients without concomitant LLT (all $p < .05$, Table 2). The LDL-C/apoB ratio also decreased more in patients received statins only, compared to patients without concomitant LLT ($p < .05$, Table 2).

3.5. Subgroup: prescribed PCSK9-I drug

We tested if alirocumab (pooled low-dose and high-dose, n = 189) and evolocumab (n = 161) had different effects on lipoprotein composition and estimated particle sizes. All baseline characteristics except HDL-C (evolocumab: 46.9 mg/dl; alirocumab: 43.8 mg/dl; $p = .048$) were similar in the groups treated with alirocumab or evolocumab (data not shown). After 4 weeks of treatment, all lipid parameters except apoAII changed significantly with both therapy regimes and no difference was detected between both drugs (Supplementary Table 2).

4. Discussion

Treatment for 4 weeks with alirocumab and evolocumab increased VLDL size (as estimated by an increased VLDL-TG/apoB ratio) and decreased VLDL-associated apolipoproteins in a heterogeneous, "real-world" study population, reflecting a reduced number of small remnant VLDL particles, which can potentially reduce cardiovascular risk in clinical routine patients beyond LDL-C reduction. Similar results were observed with different concomitant lipid lowering therapies. Major

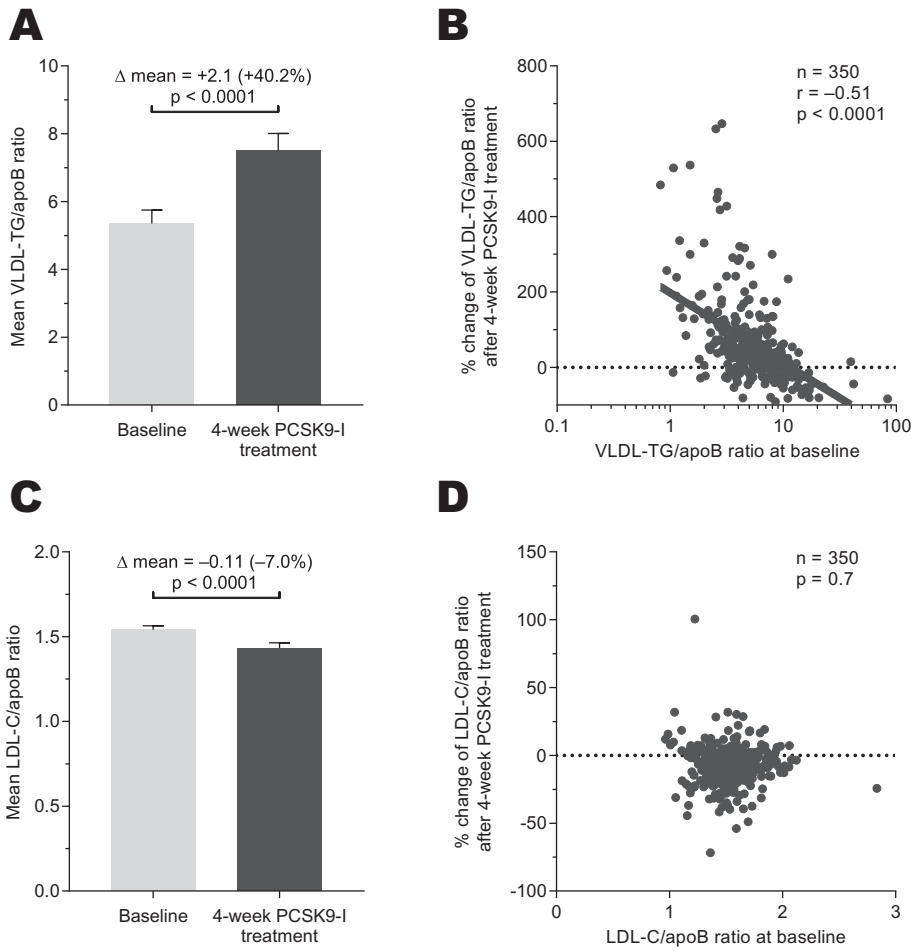

Fig. 1. Changes in VLDL-TG/apoB ratio and LDL-C/apoB ratio after 4 weeks of PCSK9-I treatment.
(A) Mean VLDL-TG/apoB ratio and (C) mean LDL cholesterol/apoB ratio at baseline and after 4 weeks of PCSK9-I treatment. VLDL-TG/apoB ratio is expressed as geometric mean, LDL-C/apoB ratio is expressed as arithmetic mean. Error bars indicate 95% confidence interval (CI). Absolute changes in both ratios (Δ) are reported as arithmetic mean with its 95% CI. Percent changes in both ratios are reported as the ratio of the respective geometric/arithmetic means (post-treatment/baseline). *P*-values were calculated by Student's paired *t*-test for normally distributed data and by Wilcoxon signed-rank test for skewed data. (B) Association between VLDL-TG/apoB ratio at baseline and percent change in VLDL-TG/apoB ratio after 4 weeks of PCSK9-I treatment. (D) Association between LDL cholesterol/apoB ratio at baseline and percent change in LDL cholesterol/apoB ratio after 4 weeks of PCSK9-I treatment.
Abbreviations: apoB, apolipoprotein B; LDL, low-density lipoprotein; PCSK9-I, Proprotein convertase subtilisin-kexin type 9 inhibitor; TG, triglycerides; VLDL, very-low-density lipoprotein. VLDL-TG/apoB ratio, ratio of triglycerides to apolipoprotein B in very-low-density lipoprotein particles; LDL-C/apoB ratio, ratio of cholesterol to apolipoprotein B in low-density lipoprotein particles.

differences between alirocumab and evolocumab were not detectable.

4.1. Increased clearance of small VLDL remnant particles due to PCSK9-I therapy

Our data show that PCSK9-I treatment decreased VLDL-apoB more than VLDL-TG. As TG are the most abundant component of VLDL particles and one VLDL particle only contains one apoB protein [15], these results reflect a reduced number of VLDL particles, and at the same time, an increased VLDL particle size (as estimated by a 40.2% increased VLDL-TG/apoB ratio). We hypothesize that this mirrors an accelerated degradation of smaller VLDL particles (remnant VLDLs). This can be clinically relevant as a reduced number of small VLDL remnants might potentially lower CVD risk [4].

Our results are supported by two previous studies, which examined the change in lipoprotein particle sizes during treatment with evolocumab and alirocumab [16,17]. Both studies found reduced concentrations of small VLDL as well as an increased average VLDL particle size of 10.1% with alirocumab and of 8.7% with evolocumab using nuclear magnetic resonance spectroscopy. These were, however, post-hoc analyses of randomized control trials, and only few patients with alirocumab were studied.

Previous studies already reported that PCSK9-I treatment does not increase VLDL secretion [18,19]. Our data support this as we observed, besides the reduction in VLDL-apoB, a distinct reduction in VLDL-associated apolipoproteins apoE, apoCII, and apoCIII. As no evidence exists that PCSK9-I decreases their production, we assume that this is caused by accelerated VLDL clearance due to better interaction of smaller VLDL with the LDL-receptor. ApoE is higher concentrated in remnant VLDL and plays a pivotal role in mediating their hepatic VLDL uptake [20]. Thus, reduced apoE likely reflects an increased clearance of small VLDL remnants. A previous study found that evolocumab reduced apoE to a similar extent as reported here [18]. Moreover, lower apoCII and -apoCIII concentrations can facilitate hepatic VLDL uptake, which can also lead to an increased average VLDL particle size [21]. Another explanation of the increase in average VLDL size might be an accelerated conversion of smaller VLDL to LDL due to an increased removal of LDL particles by PCSK9-I treatment.

Interestingly, our data indicates that patients with a lower VLDL-TG/apoB ratio at baseline (≙ smaller VLDL particles) were able to increase their VLDL-TG/apoB ratio, whereas patients with a higher VLDL-TG/apoB ratio at baseline (≙ larger VLDL particles) were not able to increase or even decreased their VLDL-TG/apoB ratio following PCSK9-I treatment. We observed a similar relationship for all other VLDL-specific apolipoproteins (VLDL-apoB, apoCII, apoCIII, apoE), that is, patients with higher baseline VLDL-specific apolipoproteins reduced these apolipoproteins more than subjects with lower baseline VLDL-specific apolipoproteins. These results suggest that PCSK9-I treatment lowered remnant VLDLs more effectively in patients who had more remnant VLDL particles before treatment initiation.

4.2. Reduction in estimated LDL particle size due to PCSK9-I treatment

PCSK9-I treatment decreased LDL-C more than LDL-apoB. As cholesterol is the most abundant component of LDL particles and one LDL particle only contains one apoB protein [15], these results reflect a reduced number of LDL particles and, at the same time, a decreased LDL particle size (as estimated by a 7% decreased LDL-C/apoB ratio). One previous study also reported a 1.7% reduced LDL particle size after evolocumab treatment, presumably due to less efficient clearance of

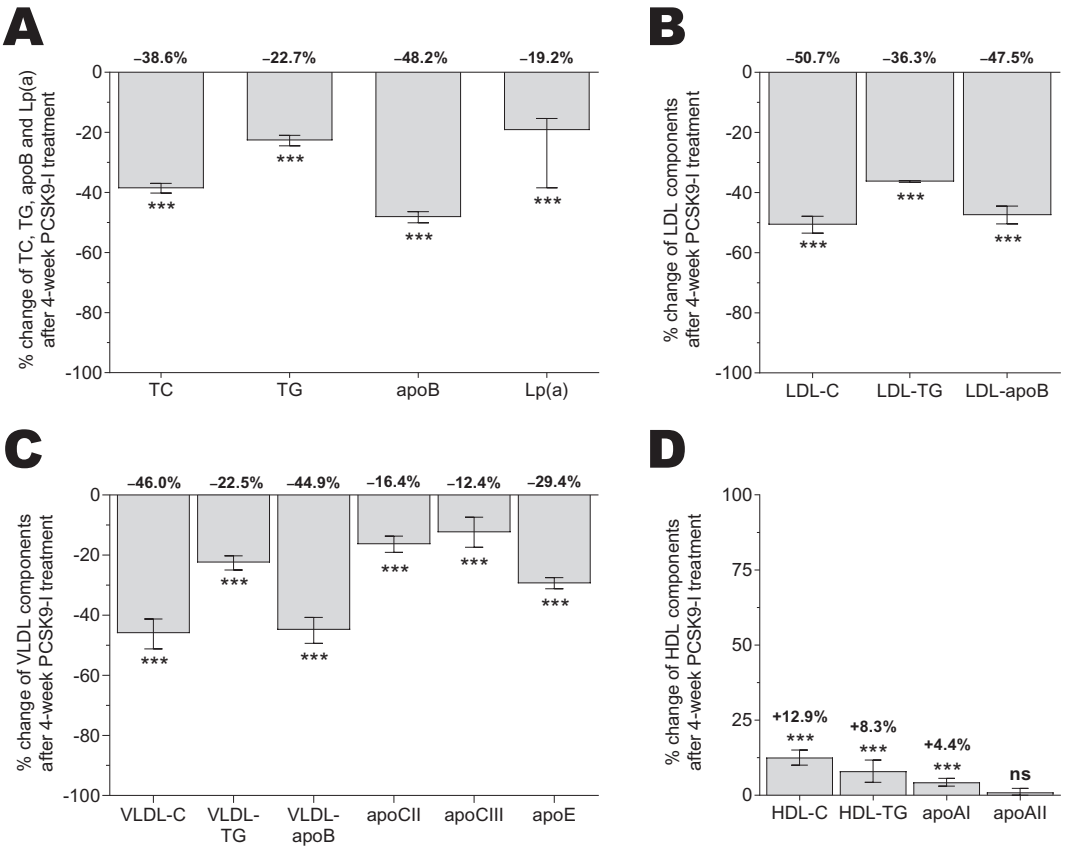

Fig. 2. Percent change in (A) TC, TG, apoB and Lp(a), (B) LDL components, (C) VLDL components, and (D) HDL components after 4 weeks of PCSK9-I therapy. Percent change was calculated as follows: mean post-treatment/mean baseline concentration × 100–100. The arithmetic mean was used for normally distributed data (TC, apoB, LDL-C, LDL-apoB, apoCII, apoCIII, apoE, HDL-C, HDL-TG, apoAI, and apoAII), the geometric mean was used for lognormally distributed data (TG, LDL-TG, VLDL-C, VLDL-TG, and VLDL-apoB), and the median was used for Lp(a). Error bars indicate 95% confidence interval. Statistical significance determined by Student's paired t-test for normally distributed data or by Wilcoxon signed-rank test for skewed data (* $p < .05$; ** $p < .01$; *** $p < .0001$).
Abbreviations: apoAI, apolipoprotein AI; apoAII, apolipoprotein AII; apoB, apolipoprotein B; apoCII, apolipoprotein CII; apoCIII, apolipoprotein CIII; apoE, apolipoprotein E; HDL-C, high-density lipoprotein cholesterol; HDL-TG, high-density lipoprotein triglycerides; Lp(a), Lipoprotein(a); LDL-apoB, low-density lipoprotein apolipoprotein B, LDL-C, low-density lipoprotein cholesterol; LDL-TG, low-density lipoprotein triglycerides; PCSK9-I, Proprotein convertase subtilisin-kexin type 9 inhibitor; TC, Total-Cholesterol; TG, triglycerides; VLDL-apoB, very-low-density lipoprotein apolipoprotein B; VLDL-C, very-low-density lipoprotein cholesterol; VLDL-TG, very-low-density lipoprotein triglycerides.

smaller LDL particles by the LDL-receptor [17]. However, it is unclear whether this slight reduction in LDL particle size is physiologically relevant.

4.3. Changes in lipoprotein components

We were also interested in the effect of PCSK9-I treatment on lipoprotein composition. Our data show that evolocumab and alirocumab reduced cholesterol across all apoB-containing lipoproteins but mainly in LDL, most likely due to their higher LDL-receptor affinity. However, we also observed a 46% reduction of VLDL-C. This might be explained by an increased LDL-receptor-mediated VLDL uptake and increased VLDL receptor activity due to PCSK9-I treatment [22]. The reduction of VLDL-C is relevant as high VLDL-C levels are associated with increased atherosclerotic risk independently of LDL-C [23]. A previous study reported a similar VLDL-C reduction with alirocumab [24], whereas another study did not find reduced VLDL-C levels after alirocumab treatment, probably due to the low sample size of 18 subjects [19].

The observed 51% LDL-C reduction from baseline matches the magnitude seen in previous large clinical trials with alirocumab and evolocumab [25,26], suggesting that the LDL-C-lowering effects of both PCSK9-I are similar in clinical practice. Interestingly, we could not detect any differences in LDL-C reduction between alirocumab and evolocumab, which is in contrast to a recently published meta-analysis [27] and might be due to the heterogeneity of our study population.

4.4. Effect of PCSK9-I according to concomitant lipid lowering therapy

Our data shows that the VLDL-TG/apoB ratio increased more in subjects receiving a concomitant LLT with statin and ezetimibe, compared to subjects without concomitant LLT. Based on current knowledge, we hypothesize that this might be due to the use of high-dose statins in the statin/ezetimibe group. It is known that statins decrease hepatic VLDL production [28] and increase PCSK9 expression [29]. In high dosages, they might hamper their own effectiveness in reducing VLDL particle numbers as PCSK9 impairs hepatic VLDL uptake [30]. Additional treatment with PCSK9-I might counteract this effect, leading to a synergy of both drugs, and consequently to a higher clearance of small VLDL particles than without statins.

4.5. Comparison of alirocumab and evolocumab

All lipid parameters were equally reduced by alirocumab and evolocumab. However, previous studies showed that apoCIII was not changed by alirocumab and evolocumab, and that VLDL-apoB only decreased with evolocumab but not with alirocumab [18,19]. The divergent results may be due to differences in sample size ($n = 350$ in our

Table 2
Relative changes in lipid parameters after PCSK9-I treatment, by concomitant LLT.

	No LLT (n = 125)	Statin (n = 48)	Ezetimibe (n = 36)	Statin/Ezetimibe (n = 139)	p-value between groups
	% change	% change	% change	% change	
Plasma lipids					
TC	**−36.3 (−38.9 to − 33.7)**	**−37.5 (−41.9 to − 33)**	**−38 (−41.6 to − 34.4)**	**−41 (−43.7 to − 38.3)**	0.08
TG	**−22.6 (−27.6 to − 17.2)**	**−18.3 (−25.6 to − 10.3)**	**−24.3 (−32.9 to − 14.7)**	**−23.7 (−28.6 to − 18.4)**	0.81
Lp(a)	**−10.7 (−6.6 to − 8.3)**	**−22.1 (−12.2 to − 7.1)**	**−20.7 (−13.5 to − 4.5)**	**−34.6 (−10.3 to − 4.7)**	0.08
ApoB	**−44.4 (−47.4 to − 41.4)**	**−49.2 (−54.1 to − 44.3)**	**−48.4 (−53 to − 43.8)**	**−51.1 (−54.3 to − 47.9)**	**0.02**†
VLDL components					
VLDL-C	**−44.3 (−50.3 to − 37.7)**	**−35 (−50.3 to − 14.9)**	**−41.2 (−55.2 to − 22.9)**	**−51.2 (−58.2 to − 43)**	0.15
VLDL-TG	**−22.4 (−29.7 to − 14.4)**	−12.5 (−24.5 to 1.5)	**−26.2 (−37.7 to − 12.6)**	**−24.6 (−32.6 to − 15.6)**	0.60
VLDL-apoB	**−38.4 (−45.3 to − 30.7)**	**−41.8 (−54.9 to − 25)**	**−43 (−53.8 to − 29.6)**	**−50.6 (−57.5 to − 42.7)**	0.09
ApoCII	**−14.1 (−18 to − 10.2)**	**−17.6 (−24 to − 11.2)**	**−17.2 (−24 to − 10.4)**	**−17.7 (−22.8 to − 12.6)**	0.15
ApoCIII	−3.6 (−16.7 to 9.5)	**−12.8 (−18.5 to − 7.2)**	**−15.8 (−21.3 to − 10.2)**	**−19.1 (−23 to − 15.2)**	0.06
ApoE	**−27.9 (−30.4 to − 25.3)**	**−27.3 (−32.9 to − 21.7)**	**−29 (−33.7 to − 24.3)**	**−31.2 (−34.7 to − 27.7)**	0.39
VLDL-TG/apoB ratio	**25.7 (13.5 to 39.3)**	**52.3 (22.8 to 88.8)**	**29.4 (6.9 to 56.6)**	**52.7 (33.6 to 74.6)**	**0.01**†
LDL components					
LDL-C	**−46.3 (−50.4 to − 42.2)**	**−53 (−60.2 to − 45.9)**	**−52.6 (−57.8 to − 47.5)**	**−53.3 (−58.6 to − 47.9)**	0.16
LDL-TG	**−34.8 (−38.5 to − 30.9)**	**−37.5 (−42.5 to − 32)**	**−35.2 (−41.6 to − 28.1)**	**−37.4 (−42.3 to − 32.1)**	0.75
LDL-apoB	**−45 (−48.6 to − 41.5)**	**−48 (−55.2 to − 40.8)**	**−49.3 (−54.8 to − 43.8)**	**−48.8 (−55 to − 42.6)**	0.71
LDL-C/apoB ratio	**−3.6 (−5.5 to − 1.6)**	**−11.2 (−14.8 to − 7.7)**	**−6.1 (−10.1 to − 2.1)**	**−9 (−12 to − 6)**	**0.003**†,††
HDL components					
HDL-C	**12.5 (9.4 to 15.5)**	**15.9 (8.6 to 23.1)**	**16.9 (3.7 to 30.2)**	**11.3 (7.6 to 15.1)**	0.47
HDL-TG	**9.8 (4.1 to 15.6)**	**11.2 (2.6 to 19.8)**	5.2 (−5.8 to 16.3)	**7.2 (0.6 to 13.8)**	0.34
ApoAI	**5.4 (3.4 to 7.4)**	**6.1 (1.7 to 10.5)**	3.8 (0 to 7.7)	**3.1 (1 to 5.2)**	0.34
ApoAII	1.8 (−0.3 to 4)	−0.9 (−4.4 to 2.7)	1.4 (−2.1 to 4.9)	0.9 (−1.5 to 3.3)	0.65

Percent change was calculated as follows: mean post-treatment/mean baseline concentration × 100–100. The arithmetic mean was used for normally distributed data (TC, apoB, LDL-C, LDL-apoB, apoCII, apoCIII, apoE, HDL-C, HDL-TG, apoAI, and apoAII), the geometric mean was used for lognormally distributed data (TG, LDL-TG, VLDL-C, VLDL-TG, and VLDL-apoB), and the median was used for Lp(a). Numbers in parentheses indicate 95% confidence interval. Statistical significance within group was determined by Student's paired t-test for normally distributed data or by Wilcoxon signed-rank test for skewed data (significant results highlighted in bold). Statistical significance between groups was determined by ANalysis Of Variance for normally distributed data and Kruskal-Wallis Test for non-normally distributed data ($^{\dagger}p < .05$ between no therapy and statin/ezetimibe group; $^{\dagger\dagger}p < .05$ between no therapy and statin group).
Abbreviations: apoAI, apolipoprotein AI; apoAII, apolipoprotein AII; apoB, apolipoprotein B; apoCII, apolipoprotein CII; apoCIII, apolipoprotein CIII; apoE, apolipoprotein E; HDL-C, high-density lipoprotein cholesterol; HDL-TG, high-density lipoprotein triglycerides; Lp(a), Lipoprotein(a); LDL-apoB, low-density lipoprotein apolipoprotein B, LDL-C, low-density lipoprotein cholesterol; LDL-C/apoB ratio, ratio of cholesterol to apolipoprotein B in low-density lipoprotein particles; LDL-TG, low-density lipoprotein triglycerides; PCSK9-I, Proprotein convertase subtilisin-kexin type 9 inhibitor; TC, Total-Cholesterol; TG, triglycerides; VLDL-apoB, very-low-density lipoprotein apolipoprotein B; VLDL-C, very-low-density lipoprotein cholesterol; VLDL-TG, very-low-density lipoprotein triglycerides; VLDL-TG/apoB ratio, ratio of triglycerides to apolipoprotein B in very-low-density lipoprotein particles.

study vs. n = 18/81) and study population (patients with high blood lipids and CVD in our study vs. healthy subjects).

4.6. Strengths and limitations

The present study is the largest and most comprehensive effort so far to investigate the effects of the PCSK9-I alirocumab and evolocumab on lipoprotein composition in a "real-world" population. However, our study has several limitations. First, the effects we report here may be not ascribed uniquely to PCSK9-I, as this is an uncontrolled longitudinal study. Second, we observed a high number of subjects without concomitant LLT (39%) in our study population, indicating a high prevalence of statin intolerant subjects, a fact that could have skewed the results.

5. Conclusion

Our data show that treatment with PCSK9-I significantly increased VLDL size (as estimated by an increased VLDL-TG/apoB ratio), and reduced VLDL-associated lipoproteins apoE, apoCII, and apoCIII in a large heterogeneous "real-world" study population. These results reflect a higher clearance of small atherogenic VLDL remnant particles, which might contribute to cardiovascular risk reduction beyond LDL-C lowering in clinical routine. Our data also suggests that VLDL remnant particle clearance was more effective in patients who had more VLDL remnants at baseline and in patients with statin therapy, most likely indicating a synergistic effect of statins and PCSK9-I.

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Conflict of interest

We wish to draw the attention of the editor to the following facts which may be considered as.

potential conflicts of interest and to significant financial contributions to this work:

- **Tim Hollstein** reports travel fees from Sanofi and Amgen.
- **Winfried März** reports other from Synlab Services GmbH, other from Synlab Holding GmbH, grants and personal fees from Siemens Diagnostics, grants and personal fees from Aegerion Pharmaceuticals, grants and personal fees from AMGEN, grants and personal fees from AstraZeneca, grants and personal fees from Danone Research, grants and personal fees from Sanofi, personal fees from Roche, personal fees from MSD, grants and personal fees from Pfizer, personal fees from Synageva, grants and personal fees from BASF, grants from Abbott Diagnostics, and grants and personal fees from Numnares, outside the submitted work.
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Tatjana Stojakovic and Bediha Bölükbasi have nothing to disclose.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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Signed by all authors as follows:

Tim Hollstein, Anja Vogt, Tatjana Stojakovic, Winfried März, Ulrich Laufs, Thomas Grenkowitz, Bediha Bölükbasi, Elisabeth Steinhagen-Thiessen, Hubert Scharnagl, Ursula Kassner.

Author contributions

Dr. Hollstein designed the study, collected the data, wrote the manuscript, interpreted the results and approved the final manuscript as submitted.

Dr. Kassner designed the study, collected the data, interpreted the results, revised the manuscript and approved the final manuscript as submitted.

Dr. Vogt collected the data, interpreted the results, revised the manuscript and approved the final manuscript as submitted.

Dr. Stojakovic analyzed the data, interpreted the results and approved the final manuscript as submitted.

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Appendix A. Supplementary data

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