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Effect of atorvastatin, cholesterol ester transfer protein inhibition, and diabetes mellitus on circulating proprotein subtilisin kexin type 9 and lipoprotein(a) levels in patients at high cardiovascular risk

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KEYWORDS:
Atorvastatin;
Diabetes mellitus;
Lipoprotein(a);
PCSK9;
CETP

BACKGROUND: Proprotein subtilisin kexin type 9 (PCSK9) and lipoprotein (a) [Lp(a)] levels are causative risk factors for coronary heart disease.

OBJECTIVES: The objective of the study was to determine the impact of lipid-lowering treatments on circulating PCSK9 and Lp(a).

METHODS: We measured PCSK9 and Lp(a) levels in plasma samples from Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events trial patients with coronary heart disease and/or type II diabetes (T2D) mellitus. Patients received atorvastatin, which was titrated (10, 20, 40, or 80 mg/d) to achieve low-density lipoprotein cholesterol levels <100 mg/dL (baseline) and were subsequently randomized either to atorvastatin + torcetrapib, a cholesterol ester transfer protein inhibitor, or to atorvastatin + placebo.

Clinical Trial Registration: NCT00134264.

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RESULTS: At baseline, both plasma PCSK9 and Lp(a) were dose-dependently increased with increasing atorvastatin doses. Compared with patients without T2D, those with T2D had higher PCSK9 (357 ± 123 vs 338 ± 115 ng/mL, $P = .0012$) and lower Lp(a) levels (28 ± 32 vs 32 ± 33 mg/dL, $P = .0005$). Plasma PCSK9 levels significantly increased in patients treated with torcetrapib (+13.1 ± 125.3 ng/mL [+3.7%], $P = .005$), but not in patients treated with placebo (+2.6 ± 127.9 ng/mL [+0.7%], $P = .39$). Plasma Lp(a) levels significantly decreased in patients treated with torcetrapib (−3.4 ± 10.7 mg/dL [−11.1%], $P < .0001$), but not in patients treated with placebo (+0.3 ± 9.4 mg/dL [+0.1%], $P = .92$).

CONCLUSION: In patients at high cardiovascular disease risk, PCSK9 and Lp(a) are positively and dose-dependently correlated with atorvastatin dosage, whereas the presence of T2D is associated with higher PCSK9 but lower Lp(a) levels. Cholesterol ester transfer protein inhibition with torcetrapib slightly increases PCSK9 levels and decreases Lp(a) levels.

Introduction

Statin therapy decreases low-density lipoprotein (LDL) cholesterol levels and thereby reduces cardiovascular disease (CVD) risk.[1] By inhibiting intracellular cholesterol synthesis, statins increase the expression of the LDL receptor (LDLR), thus promoting an enhanced clearance of LDL particles. However, statins also increase the expression of proprotein convertase subtilisin kexin type 9 (PCSK9), a natural circulating inhibitor of the LDLR.[2] PCSK9 binds to the LDLR and after endocytosis targets the LDLR that normally recycles back to the cell surface, for lysosomal degradation. The efficacy of statins in reducing LDL cholesterol levels appears to be partially offset by a concomitant rise in PCSK9.[3,4] Pharmacologic inhibition of PCSK9 with monoclonal antibodies lowers circulating LDL cholesterol further in patients at high CVD risk and not at LDL cholesterol therapeutic goals despite aggressive statin treatment.[5,6] It is therefore important to determine whether and to what extent statins dose-dependently increase circulating PCSK9 levels in such patients.

In contrast to statins, anti-PCSK9 monoclonal antibodies promote an unexplained 25% to 30% reduction in circulating lipoprotein (a) [Lp(a)] levels.[7] Lp(a) is a lipoprotein subfraction analogous to LDL, where a unique protein homologous to plasminogen, apolipoprotein (a) [apo(a)], is covalently tethered to apolipoprotein B100 by a unique disulfide bond.[8] Approximately 20% of the Caucasian population have high Lp(a) levels (above 50 mg/dL)[9] and a consequent increased risk of coronary heart disease, stroke, calcific aortic valve stenosis, and heart failure.[10–13] The molecular mechanisms of Lp(a) assembly that likely occurs at the surface of hepatocytes between a newly synthesized apo(a) and apoB100 containing lipoproteins (LpB) remain elusive.[14] Apo(a) is never found associated with triglyceride-rich lipoproteins but rather on cholesterol-rich LDL particles.[15,16] The impact of statin therapy on plasma Lp(a) levels is somewhat controversial with studies documenting slight decreases in Lp(a) while others suggest that statins actually increase Lp(a) levels as well as the amount of oxidized phospholipids carried by Lp(a).[17] Similar to PCSK9, whether statins dose-dependently influence Lp(a) plasma levels and whether this could be dependent on metabolic disturbances is unknown.

To shed light on the metabolic states favoring Lp(a) assembly, and thus elevated Lp(a) levels, in conjunction with the ongoing development of PCSK9 inhibitors, we aimed to determine the impact of different doses of statins, with and without metabolic disturbances of triglyceride-rich lipoproteins (eg, in type II diabetes [T2D]) and with and without modulation of their cholesterol content (eg, by inhibition of the cholesterol ester transfer protein [CETP]) on circulating PCSK9 and Lp(a) levels in patients at high cardiovascular risk.

Methods

Study design

The Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events (ILLUMINATE) trial included 15,067 men and women at high cardiovascular risk (Trial Registration Number: NCT00134264). The details of the study population have been previously described.[18] Briefly, men and women aged 45 to 75 years were eligible if they had a prior history of myocardial infarction, stroke, acute coronary syndrome, unstable angina, peripheral vascular disease, or cardiac revascularization within the period of 30 days to 5 years before screening. Patients with T2D who met American Diabetes Association criteria or who were currently on hypoglycemic agents were also eligible. During a run-in period of 4 to 10 weeks, patients received atorvastatin, which was titrated (if needed) at 2-week intervals to achieve LDL cholesterol levels <100 mg/dL with atorvastatin 10, 20, 40, or 80 mg/d. Patients whose LDL cholesterol level met the target were randomly assigned to receive either atorvastatin (at a dose established during the run-in period) plus 60 mg of torcetrapib or atorvastatin

plus placebo. Noteworthy, the use of torcetrapib in ILLUMINATE was found to have serious adverse off-target effects with an increase in cardiovascular events and related deaths.[18] We randomly selected 594 patients receiving torcetrapib and atorvastatin matched in a 1:2 ratio to 1151 patients receiving placebo and atorvastatin. This subset of patients was selected to ensure patients with T2D were overrepresented (48%) and that all atorvastatin doses (10, 20, 40, and 80 mg/d) were also equally represented.

Laboratory analyses

The level of high-density lipoprotein (HDL) cholesterol was determined through enzymatic analyses with dextran sulfate, polyethylene glycol–modified cholesterol esterase, and cholesterol oxidase, to generate a peroxide that was measured colorimetrically. Total cholesterol and triglyceride levels were determined by standard enzymatic techniques. LDL cholesterol was quantified by the Friedewald formula, except when the triglyceride level was above 400 mg/dL, in which case LDL cholesterol levels were measured by direct beta quantification. Lp(a) was measured by immunoturbidimetry (Randox, Parramata, Australia) and PCSK9 measured by enzyme-linked immunosorbent assay (Cyclex, Nagano, Japan). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: glucose (mg/dL) $\times$ insulin (pmol/L) $\div$ 7.175 $\times$ 405.

Statistical analyses

Comparisons of continuous variables were conducted using the 2-sided Wilcoxon rank-sum test. A chi-square test was used to compare categorical variables. Signed-rank tests were used to compare baseline and 3-month PCSK9 and Lp(a) levels.

Results

Plasma PCSK9 and Lp(a) levels were measured at baseline (at the end of the run-in period) and 3 months after randomization in a subset of 594 patients receiving torcetrapib and atorvastatin and in 1151 patients receiving placebo and atorvastatin. The characteristics of the study participants at baseline are presented in Table 1.

At baseline, that is, after the run-in period, both plasma PCSK9 (Fig. 1) and Lp(a) levels (Fig. 2) were dose-dependently elevated with increasing atorvastatin doses. Compared with patients without T2D, those with T2D at baseline had higher PCSK9 (357 $\pm$ 123 vs 338 $\pm$ 115 ng/mL, P = .0012) and lower Lp(a) levels (28 $\pm$ 32 vs 32 $\pm$ 33 mg/dL, P = .0005). Regardless of T2D status, higher atorvastatin doses were associated with higher PCSK9 levels (Fig. 1). Similar to PCSK9 levels, higher atorvastatin doses were associated with higher Lp(a) levels (Fig. 2). Spearman correlation coefficients between PCSK9 and Lp(a) levels with parameters of lipid metabolism and glucose homeostasis are presented in Table 2. Despite statin therapy, which increases PCSK9[3,4] and reduces total and LDL cholesterol levels, circulating PCSK9 levels were positively associated with total cholesterol, LDL cholesterol, triglycerides, and apoB as well as with HDL cholesterol and apoA-I levels, albeit to a lower extent. Plasma PCSK9 levels were also positively associated with fasting blood glucose, insulin, and HOMA-IR. Circulating Lp(a) levels were positively

Table 1 Baseline characteristics of study participants classified on the basis of treatment group

	Atorvastatin and placebo, N = 1151	Atorvastatin and torcetrapib, N = 594	*P* value
Male gender, %	83	85	.24
Diabetes mellitus, %	48	50	.42
Age, y	62 $\pm$ 7.5	62 $\pm$ 7.6	.75
Total cholesterol, mg/dL	153 $\pm$ 26	153 $\pm$ 27	.99
LDL cholesterol, mg/dL	77 $\pm$ 20	77 $\pm$ 21	.79
HDL cholesterol, mg/dL	46 $\pm$ 11	47 $\pm$ 12	.49
Triglycerides, mg/dL	151 $\pm$ 75	146 $\pm$ 78	.02
Apolipoprotein B, mg/dL	72 $\pm$ 15	71 $\pm$ 16	.06
Apolipoprotein A-I, mg/dL	123 $\pm$ 22	122 $\pm$ 22	.06
Glucose, mg/dL	116 $\pm$ 36	118 $\pm$ 41	.95
Insulin, pmol/L	118 $\pm$ 135	117 $\pm$ 109	.88
HOMA-IR	5.02 $\pm$ 8.09	5.04 $\pm$ 5.80	.78
Lipoprotein(a), mg/dL	30 $\pm$ 33	31 $\pm$ 33	.99
PCSK9, ng/mL	350 $\pm$ 120	340 $\pm$ 117	.11

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

Data are presented as percentage or mean $\pm$ standard deviation.

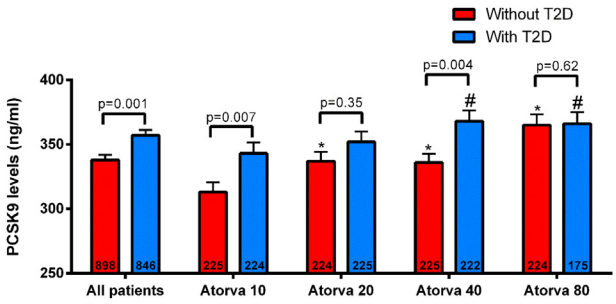

Figure 1 Plasma PCSK9 levels at baseline in patients with or without type II diabetes in the entire study sample and in patients separated on the basis of atorvastatin dose. *Significantly different from atorvastatin 10 mg group in patients without type II diabetes. #Significantly different from atorvastatin 10 mg group in patients with type II diabetes.

associated with LDL cholesterol, and to a much lower extent with total cholesterol, apoB, and HDL cholesterol. There was a weak but significant negative association between Lp(a) and triglycerides, fasting blood glucose, insulin, and HOMA-IR (Table 2). There was no significant correlation between PCSK9 and Lp(a) plasma levels.

We also measured PCSK9 and Lp(a) levels after 3 months of treatment either with placebo or with torcetrapib on top of atorvastatin doses established during the run-in period. Plasma PCSK9 levels significantly increased in patients treated with torcetrapib (+13.1 ± 125.3 ng/mL [ie, +3.7%], $P = .005$), but not in patients treated with placebo (+2.6 ± 127.9 ng/mL [ie, +0.7%], $P = .39$). Increases in PCSK9 levels with torcetrapib were statistically significant in patients with T2D only (Fig. 3). In contrast, plasma Lp(a) levels significantly decreased in patients treated with torcetrapib (−3.4 ± 10.7 mg/dL [−11.1%], $P < .0001$), but not in patients treated with placebo (+0.3 ± 9.4 mg/dL [+1%], $P = .92$). Decreases in Lp(a) were observed in patients with and without T2D (Fig. 4). Changes in PCSK9 were not associated with changes in Lp(a) levels in patients treated with torcetrapib (Spearman correlation coefficient r = −0.03, $P = .21$).

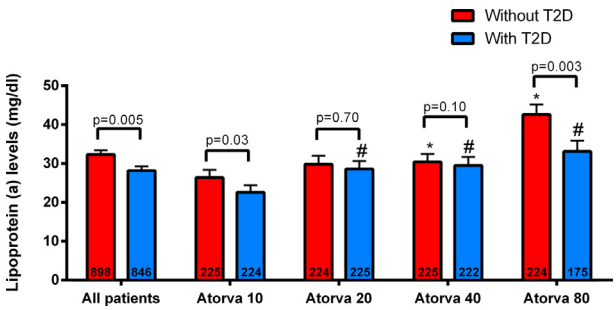

Figure 2 Plasma Lp(a) levels at baseline in patients with or without type II diabetes in the entire study sample and in patients separated on the basis of atorvastatin dose. *Significantly different from atorvastatin 10 mg group in patients without type II diabetes. #Significantly different from atorvastatin 10 mg group in patients with type II diabetes.

Table 2 Spearman correlation coefficients between PCSK9 and lipoprotein(a) levels and parameters of lipid metabolism or of glucose homeostasis

	PCSK9	Lipoprotein(a)
Total cholesterol	0.25 ($P < .0001$)	0.11 ($P < .0001$)
LDL cholesterol	0.15 ($P < .0001$)	0.21 ($P < .0001$)
HDL cholesterol	0.08 ($P = .0007$)	0.05 ($P = .03$)
Triglycerides	0.16 ($P < .0001$)	−0.11 ($P < .0001$)
Apolipoprotein B	0.22 ($P < .0001$)	0.06 ($P = .01$)
Apolipoprotein A-I	0.15 ($P < .0001$)	0.01 ($P = .63$)
Lipoprotein(a)	0.03 ($P = .38$)	–
PCSK9	–	0.03 ($P = .38$)
Glucose	0.14 ($P < .0001$)	−0.05 ($P = .04$)
Insulin	0.17 ($P < .0001$)	−0.05 ($P = .05$)
HOMA-IR	0.18 ($P < .0001$)	−0.06 ($P = .01$)

HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

Discussion

In this sub-study of ILLUMINATE, a randomized clinical trial that documented the impact of CETP inhibition with torcetrapib on cardiovascular outcomes, we found that atorvastatin therapy was dose-dependently associated with higher PCSK9 and Lp(a) levels. The presence of T2D was also associated with higher PCSK9, but lower Lp(a) levels. We also showed that CETP inhibition with torcetrapib for 3 months slightly increased PCSK9 levels and decreased Lp(a) levels.

We found that atorvastatin therapy was dose-dependently associated with higher PCSK9 levels. It is well established that all statins significantly increase circulating PCSK9 levels compared either with placebo or pretreatment values in humans (as reviewed by Sahebkar

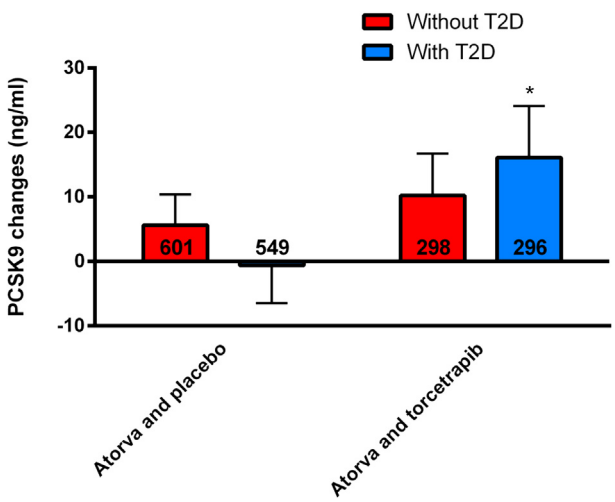

Figure 3 Changes in plasma PCSK9 levels after 3 months on placebo or torcetrapib in patients with and without type II diabetes. *Significantly different from baseline.

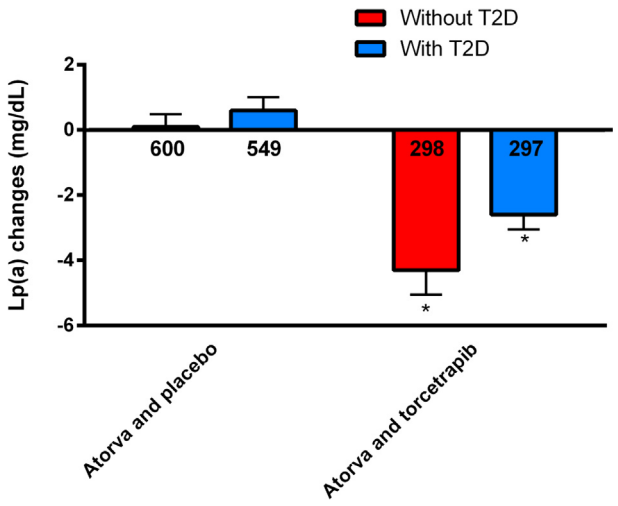

Figure 4 Changes in plasma Lp(a) levels after 3 months on placebo or torcetrapib in patients with and without type II diabetes. *Significantly different from baseline.

et al.[19]). Our study is the largest ever conducted on this topic and extends the results of a previous study on rosuvastatin[4] by comprehensively documenting for the first time a dose response association between statin and PCSK9 levels. Compared with patients on 10 mg atorvastatin, those on the maximal 80 mg atorvastatin treatment dose had 37 ng/mL higher plasma PCSK9 levels. This increase in PCSK9 levels is of the same magnitude to that reported in patients on 10 mg atorvastatin vs placebo or pretreatment values (ie, +40 ng/mL).[19] This observation underlines that the effects of atorvastatin on circulating PCSK9 levels are not linearly proportional to the treatment dose, similar to statin-mediated LDL cholesterol–lowering dose effects.[20]

We also observed in ILLUMINATE that higher atorvastatin doses were associated with elevated Lp(a) levels. The impact of statin therapy on Lp(a) levels is somewhat controversial. For instance, in the Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin trial, 40 mg rosuvastatin promoted a 20% increase in circulating Lp(a) levels.[17] In the Treating to New Targets trial, we have shown that uptitration of atorvastatin doses from 10 to 80 mg/d was associated with a small but significant increase in Lp(a).[21] In the Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin trial however, 20 mg rosuvastatin did not significantly increase Lp(a) levels compared with pretreatment values.[22] In contrast, both atorvastatin and simvastatin reduced Lp(a) in a smaller cohort of heterozygous familial hypercholesterolemic patients.[23] We therefore cannot rule out that ILLUMINATE patients requiring higher doses of atorvastatin to achieve LDL cholesterol <100 mg/dL were those with higher untreated baseline Lp(a) levels because assays that measure LDL cholesterol also include the contribution of Lp(a) cholesterol.[24] In that respect, the single nucleotide polymorphism rs10455872, that is a strong predictor of plasma Lp(a) levels, is also the second most important genetic determinant of LDL cholesterol lowering with statins.[25]

Next, we showed that patients with T2D had higher PCSK9 levels than patients without T2D, as recently reported in a smaller cohort.[26] However, PCSK9 levels were not different between patients with vs without T2D in another smaller study.[27] Important questions still remain regarding the directionality and causality of this association. In that respect, insulin has been shown to enhance PCSK9 expression, which may argue in favor of T2D causing elevations in PCSK9 levels.[28,29] This observation is also supported by the recent publication of 2 large-scale genetic associations studies that have shown that loss-of-function variants at the PCSK9 loci associated with impaired PCSK9 function may be associated with an increased risk of T2D.[30,31]

In contrast, we found that Lp(a) levels were reduced in patients with T2D compared with patients without T2D. This result confirms previous studies showing lower Lp(a) levels in patients with T2D vs patients without T2D and extends the observations of 3 large prospective studies demonstrating a strong inverse association of Lp(a) levels with the risk of incident T2D (as reviewed by Tsimikas[8]). However, a genetic study challenged this association by showing that 4 single nucleotide polymorphism strongly correlated with the number of apo(a) kringle IV_2 repeats and Lp(a) levels did not predict T2D risk.[32] A potential explanation for reduced Lp(a) levels in patients with T2D is that insulin reduces apo(a) synthesis in primary hepatocytes.[33] More likely, because T2D is characterized by increased levels of triglyceride-rich apoB-containing lipoprotein, which is (unlike LDL) a poor acceptor for apo(a), there is probably a reverse causality between Lp(a) levels and T2D. In line with this hypothesis, Lp(a) was significantly and negatively correlated with plasma triglycerides in the present study.

We also found that torcetrapib reduced Lp(a) by 11%. Noteworthy, more potent CETP inhibitors such as anacetrapib, evacetrapib, and TA-8995 provide 25% to 40% reductions in Lp(a) levels.[34–36] Because CETP inhibition limits the net flux of triglycerides from triglyceride-rich lipoproteins to HDL and the net flux of cholesterol esters from HDL to triglyceride-rich lipoproteins particles, we speculate that fewer LDL-like particles might serve as substrate for Lp(a) assembly. In that respect, a recent genetic association study showed that genetic variants at the *LPA* locus linked with high Lp(a) levels were causally related with lower levels of circulating and triglyceride-rich lipoproteins particles. The directionality of this association clearly needs to be clarified.[37]

Finally, we found that CETP inhibition with torcetrapib provides a small but significant increase in PCSK9 levels, in patients with T2D, but not in patients without T2D. The fact that torcetrapib was found to have serious off-target effects[18] raises the possibility that the effects of torcetrapib observed in our study may have been unrelated to CETP inhibition. In contrast to our findings, other CETP inhibitors

were shown to reduce PCSK9 expression in vitro, in mice and monkeys.[38–41] Additional research will be needed to determine whether changes in PCSK9 levels result from a modulation of the canonical sterol regulatory element-binding protein 2 transcription factor pathway or from off-target effects of torcetrapib.

In humans, the impact of CETP inhibition on PCSK9 appears to rely on background lipid-lowering therapy.[42] In a study that included 39 mildly hypercholesterolemic patients, anacetrapib (100 mg/d) decreased PCSK9 levels by 18% in monotherapy but had no significant influence on PCSK9 levels (+3.4%) on top of atorvastatin 20 mg/d. Interestingly, plasma PCSK9 levels were weakly but positively associated with CETP activity in a cross-sectional study of 450 participants in a high-risk primary prevention setting.[43] Carriers of the PCSK9-R46L loss-of-function variant (linked with lower LDL cholesterol levels) also presented with lower CETP activity. This association has not been replicated in another study.[44] Thus, targeting CETP appears to influence PCSK9. Whether targeting PCSK9 also influences CETP activity will require confirmation.

We conclude that (1) both PCSK9 and Lp(a) levels are positively and dose-dependently increased by atorvastatin. The mechanisms by which statins increase PCSK9 levels are well known,[3,4] but those by which higher atorvastatin doses may increase Lp(a) levels remain elusive. (2) The presence of T2D is associated with higher PCSK9, but lower Lp(a) levels. Whereas impaired apoB-containing lipoprotein remodeling in T2D and on CETP inhibition appears to be a plausible causative mechanisms for reduced Lp(a) in those patients, it is not clear as to why patients with T2D have higher PCSK9 levels and why CETP inhibition with torcetrapib increased PCSK9 levels in these patients. The physiological link(s) between glycemic parameters, PCSK9, LDL receptor function, and Lp(a) metabolism clearly needs to be addressed in future studies.

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Authors' contributions: B.J.A. co-designed the statistical analysis plan and wrote the article. F.P. and F.T. conducted experiments. W.B. performed statistical analyses. G.K.H., S.M.B., S.R.-M., O.M., and K.-A.R. reviewed the article. D.D., D.D.W., J.J.P.K., and P.B. participated to the study design and reviewed the article. G.L. participated in study design, co-designed the statistical analysis plan, conducted experiments, and reviewed the article. All authors have approved the final article.

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