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Controlled study of the effect of proprotein convertase subtilisin-kexin type 9 inhibition with evolocumab on lipoprotein(a) particle kinetics

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Aims Lipoprotein(a) [Lp(a)], a low-density lipoprotein (LDL) particle covalently bound to apolipoprotein(a) [apo(a)], is a potentially potent heritable risk factor for cardiovascular disease. We investigated the mechanism whereby evolocumab, a monoclonal antibody against proprotein convertase subtilisin-kexin type 9 (PCSK9), lowers Lp(a).

Methods and results We studied the kinetics of Lp(a) particles in 63 healthy men, with plasma apo(a) concentration >5 nmol/L, participating in an 8-week factorial trial of the effects of evolocumab (420 mg every 2 weeks) and atorvastatin (80 mg daily) on lipoprotein metabolism. Lipoprotein(a)-apo(a) kinetics were studied using intravenous D3-leucine administration, mass spectrometry, and compartmental modelling; Lp(a)-apoB kinetics were also determined in 16 subjects randomly selected from the treatment groups. Evolocumab, but not atorvastatin, significantly decreased the plasma pool size of Lp(a)-apo(a) (-36%, $P < 0.001$ for main effect). As monotherapy, evolocumab significantly decreased the production of Lp(a)-apo(a) (-36%, $P < 0.001$). In contrast, in combination with atorvastatin, evolocumab significantly increased the fractional catabolism of Lp(a)-apo(a) (+59%, $P < 0.001$), but had no effect on the production of Lp(a)-apo(a). There was a highly significant association between the changes in the fractional catabolism of Lp(a)-apo(a) and Lp(a)-apoB in the substudy of 16 subjects ($r = 0.966$, $P < 0.001$).

Conclusions Evolocumab monotherapy lowered the plasma Lp(a) pool size by decreasing the production of Lp(a) particles. In combination with atorvastatin, evolocumab lowered the plasma Lp(a) pool size by accelerating the catabolism of Lp(a) particles. This dual mechanism may relate to an effect of PCSK9 inhibition on Lp(a)-apo(a) production and to marked up-regulation of LDL receptor activity on Lp(a) holoparticle clearance.

Clinical Trial Registration Information NCT02189837

Keywords Evolocumab • Lp(a) • apo(a) • Atorvastatin • PCSK9

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Introduction

Lipoprotein(a) [Lp(a)] consists of low-density lipoprotein (LDL), containing apolipoprotein B-100 (apoB), covalently bound to a plasminogen-like glycoprotein, apolipoprotein(a) (apo(a)).[1] Lipoprotein(a) has potentially potent atherogenic, inflammatory, and thrombotic properties.[2] Recent observational studies suggest that elevated Lp(a) is causally related to atherosclerotic cardiovascular disease (ASCVD) and calcific aortic stenosis.[2,3]

Lipoprotein(a) is a quantitative heritable trait regulated by the apo(a) (*LPA*) gene locus.[1] Lipoprotein(a) has a wide range of plasma concentrations that are kinetically determined by the rates of Lp(a) production and catabolism; copy number variation in the *LPA* gene leads to heterogeneity in apo(a) isoform size, which is inversely related to the production of Lp(a).[4] Apolipoprotein(a) and apoB are assembled intrahepatically, forming an Lp(a) particle which is subsequently secreted into plasma.[4,5] The Lp(a) particle may also be assembled in the circulation (e.g. on the hepatocyte surface) from its constituent proteins after these are independently secreted from the liver into plasma.[4] Details of the assembly process remain unclear.[4] There is also uncertainty concerning whether the kinetics in plasma of the two protein components of Lp(a) are coupled,[6–8] and specifically whether apo(a) is recycled or cleared with apoB as an Lp(a) holoparticle.[9] The contribution of receptor-mediated catabolism to the clearance of plasma Lp(a) is also debated, there being evidence for and against a role for LDL receptors (LDLRs).[10–12] Understanding the metabolism of Lp(a) and mode of action of therapies in humans requires investigation of Lp(a) particle kinetics. This entails stable isotope tracers and compartmental modelling methods, but data are scarce.[13]

Proprotein convertase subtilisin-kexin type 9 (PCSK9), a secretory protease expressed chiefly in liver, regulates hepatic cell surface receptors involved in lipoprotein metabolism.[14] The role of PCSK9 in Lp(a) metabolism may predominantly involve regulation of the LDLR,[10,14] but there is also evidence that PCSK9 may control the hepatic secretion of apo(a).[12] Proprotein convertase subtilisin-kexin type 9 inhibition with monoclonal antibodies (mAbs) enhances LDLR activity that profoundly lowers plasma LDL-cholesterol by increasing clearance of LDL-apoB via the LDLR pathway.[15–17] If sufficiently potent, this effect may also enhance clearance of Lp(a) particles, accounting for the lowering of plasma Lp(a) concentrations by PCSK9 mAbs.[15,16,18–21] Statins and ezetimibe enhance LDLR activity, but do not lower plasma Lp(a),[22,23] suggesting a specific effect of PCSK9 mAbs in reducing production of Lp(a) particles.

In a factorial trial, we recently reported that evolocumab decreased the plasma concentration of LDL possibly by increasing the catabolism of LDL particles and to a lesser extent by decreasing the production of apoB in intermediate-density lipoprotein (IDL) and LDL.[18] The primary aim of the present study was to investigate whether reduction in plasma Lp(a) particle concentration with evolocumab, as monotherapy and as dual therapy with atorvastatin, involved kinetic effects on the catabolism and production of Lp(a) particles. The secondary aim was to explore the effect of evolocumab on the kinetic coupling of apo(a) and apoB within Lp(a) particles.

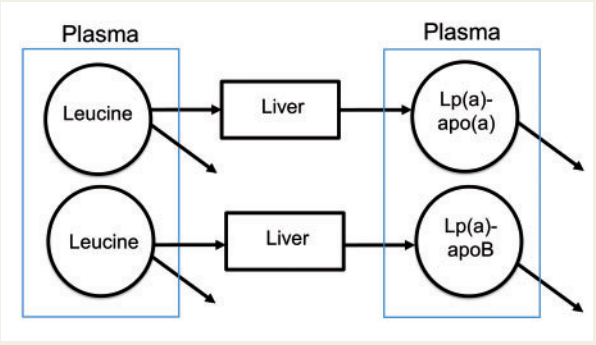


Figure 1 Compartmental model for the metabolism of lipoprotein(a)-apolipoprotein(a) and lipoprotein(a)-apolipoprotein B. See main text and Supplementary material online for details.

Methods

Subjects, study design, clinical protocol

Full details of study subjects, design, and protocols were published previously.[18] Briefly, the main study recruited healthy normolipidaemic, non-obese men aged 18–65 years with fasting plasma LDL-cholesterol of ≥2.5 mmol/L and <4.9 mmol/L and triglycerides of <1.7 mmol/L. Subjects were randomized into a double-blind, placebo-controlled, two-by-two factorial trial of the effects of oral atorvastatin 80 mg everyday (QD), subcutaneous injection (SC) evolocumab 420 mg Q2W, or SC evolocumab 420 mg Q2W, and oral atorvastatin 80 mg QD for 8 weeks. Subjects were studied after a 14-h fast, pre- and post-interventions, using a single bolus D-3 leucine (5 mg/kg body weight), with serial blood samples taken over 10 h and 24, 48, 72, and 96 h after the injection of isotope. For analytical reasons (see Supplementary material online), we only reported on the effect of interventions in 63 subjects with baseline plasma apo(a) concentrations >5 nmol/L. The kinetics of Lp(a)-apo(a) and Lp(a)-apoB were investigated in a subset of 16 subjects chosen randomly from the four interventions (four per group), in whom we also compared the kinetics of apo(a) measured from whole plasma and from the LDL–high-density lipoprotein (HDL) subfraction.

Laboratory methods

Full details of methods, including quantification of Lp(a) and apo(a), determination of apo(a) isoform size and isolation and measurement of isotopic enrichment of Lp(a)-apo(a) and Lp(a)-apoB are given in the Supplementary material online or have been published elsewhere.[18,24]

Lipoprotein(a) particle kinetic model and calculation of kinetic parameters

The Lp(a)-apo(a) and Lp(a)-apoB enrichment data were modelled using a single-pool model with the SAAM II programme (The Epsilon Group, Charlottesville, VA, USA).[19,25] Fractional catabolic rates (FCRs) were estimated by fitting leucine enrichment in Lp(a)-apo(a) and Lp(a)-apoB proteins with a single-pool model, precursor enrichment being set at the same level as in the apoB model described earlier.[18] More complex models for Lp(a)-apo(a) and Lp(a)-apoB were developed, with the best quality model selected on the basis of the lowest Akaike information criterion; refer also to Supplementary material online.[26] *Figure 1* shows the compartmental model for Lp(a) kinetics; the precision of the estimated kinetic parameters was derived by iteratively fitting the model to the enrichment

Table 1 Clinical and biochemical characteristics of the 63 subjects at baseline

Characteristic	Placebo ($n = 16$)	Atorvastatin ($n = 17$)	Evolocumab ($n = 14$)	Evolocumab/ Atorvastatin ($n = 16$)
Age (years)	32.4 (26.7–39.2)	31.7 (28.0–35.9)	34.3 (27.9–42.1)	30.0 (26.0–34.4)
BMI (kg/m^2)	24.4 (22.8–26.2)	25.2 (23.7–26.7)	24.8 (23.6–26.1)	25.6 (23.9–27.4)
Systolic BP (mmHg)	127 (123–131)	122 (119–128)	123 (118–129)	123 (116–130)
Diastolic BP (mmHg)	78.4 (74.3–82.7)	76.7 (71.9–81.8)	75.0 (66.0–85.2)	76.4 (71.9–81.2)
Glucose (mmol/L)	5.22 (5.02–5.42)	5.31 (5.12–5.50)	5.36 (5.03–5.72)	5.39 (5.21–5.58)
Total cholesterol (mmol/L)	4.60 (4.39–4.82)	4.74 (4.41–5.11)	4.58 (4.27–4.92)	4.62 (4.20–5.09)
Triglycerides (mmol/L)	0.86 (0.77–0.96)	0.92 (0.77–1.10)	0.75 (0.62–0.90)	0.93 (0.74–1.16)
HDL-cholesterol (mmol/L)	1.22 (1.09–1.36)	1.22 (1.10–1.36)	1.17 (1.01–1.36)	1.15 (1.00–1.32)
LDL-cholesterol (mmol/L)[a]	3.02 (2.83–3.23)	3.13 (2.89–3.39)	3.09 (2.90–3.30)	3.00 (2.71–3.33)
ApoB (g/L)	0.82 (0.77–0.88)	0.85 (0.79–0.91)	0.84 (0.78–0.91)	0.85 (0.77–0.93)
Lipoprotein(a) (nmol/L)	31.7 (17.2–58.3)	14.5 (9.72–21.7)	26.8 (13.8–52.0)	28.6 (15.6–52.4)
Apo(a) (nmol/L)	25.0 (15.2–41.0)	15.5 (10.8–22.2)	23.3 (13.2–41.1)	26.5 (16.4–42.9)
Predominant apo(a) isoform K_4	19.7 (17.5–22.2)	24.2 (21.4–27.4)	20.9 (18.3–23.0)	22.0 (19.1–25.4)

Values expressed as geometric mean (95% CI).
Apo, apolipoprotein; BMI, body mass index; BP, blood pressure; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]LDL-cholesterol directly measured after ultracentrifugation.

data. The concentration of apo(a) measured by liquid chromatography-mass spectrometry (LCMS) was used to estimate plasma Lp(a) particle concentration and pool size. Lipoprotein(a)-apo(a) pool size was calculated as apo(a) concentration (nmol/L) × plasma volume (L); plasma volume was calculated as 0.045 × body weight in kg. Absolute production rate (nmol/kg/day; equivalent to absolute catabolic rate) was estimated as plasma pool size × FCR of Lp(a)-apo(a). The average coefficient of variation (CV) of the estimated FCR, delay time, and plasma leucine to delay were 9.3%, 8.7%, and 3.9%, respectively.

Statistical analyses

Statistical methods were detailed previously[18] and included log-transformation of Lp(a) data, random-effects regression models, and adjustments for baseline co-variates and multiple comparisons (see Supplementary material online).

Results

Subject characteristics

The selection and disposition of subjects were summarized previously.[18] The 63 subjects had similar characteristics at baseline [with the exception of Lp(a)] to the 18 subjects excluded and parent cohort (Supplementary material online, *Table S1*).[18] Subjects were on average 31 years old, lean, normotensive, non-diabetic and had normal plasma lipid and lipoprotein profiles (*Table 1*). Baseline plasma Lp(a) and apo(a) concentrations among the treatment groups were higher than in the main cohort; mean Lp(a) and apo(a) concentrations were lower in the atorvastatin than other groups, but the difference was not statistically significant. Apolipoprotein(a) isoform size was higher with atorvastatin, but group differences were not statistically significant. The plasma concentrations of Lp(a) and apo(a) were highly correlated ($r = 0.945$, $P < 0.001$). Adherence to randomized treatments was 100%. Adverse events were reported previously.[18]

Lipoprotein(a)-apolipoprotein(a) and lipoprotein(a)-apolipoprotein B-100 enrichment curves and kinetic parameters

The characteristics of the 16 subjects selected for the Lp(a)-apo(a) and Lp(a)-apoB kinetic substudy were comparable to the remaining groups (Supplementary material online, *Table S2*). *Figure 2* shows the fit of the model to the leucine tracer/tracee ratio of Lp(a)-apo(a) and Lp(a)-apoB over time (pre- and post-intervention) in a representative subject from the placebo, atorvastatin, evolocumab, and evolocumab/atorvastatin groups. The isotopic enrichment curves for both protein components of Lp(a) were superimposable in all subjects pre- and post-interventions. The FCRs of Lp(a)-apo(a) and Lp(a)-apoB were highly correlated pre-intervention ($r = 0.982$) and post-intervention ($r = 0.995$; *Figure 3*), confirming tight coupling of the metabolism of both protein components of the Lp(a) particle. The FCR of apo(a) derived from whole plasma and from the LDL–HDL fraction were closely correlated pre-intervention ($r = 0.961$, $P < 0.001$) and post-intervention ($r = 0.976$, $P < 0.001$). The precision (CV) of the FCRs was <10%.

Treatment effects on plasma pool size and kinetics of apolipoprotein(a)

Treatment effects on plasma lipid, lipoprotein, and apolipoprotein concentration and apoB-100 kinetics were comparable to our earlier study[18] (see Supplementary material online, *Tables S3 and S4*). At baseline, there was an inverse association ($r = -0.373$, $P < 0.01$) between apo(a) isoform size and production of Lp(a)-apo(a).

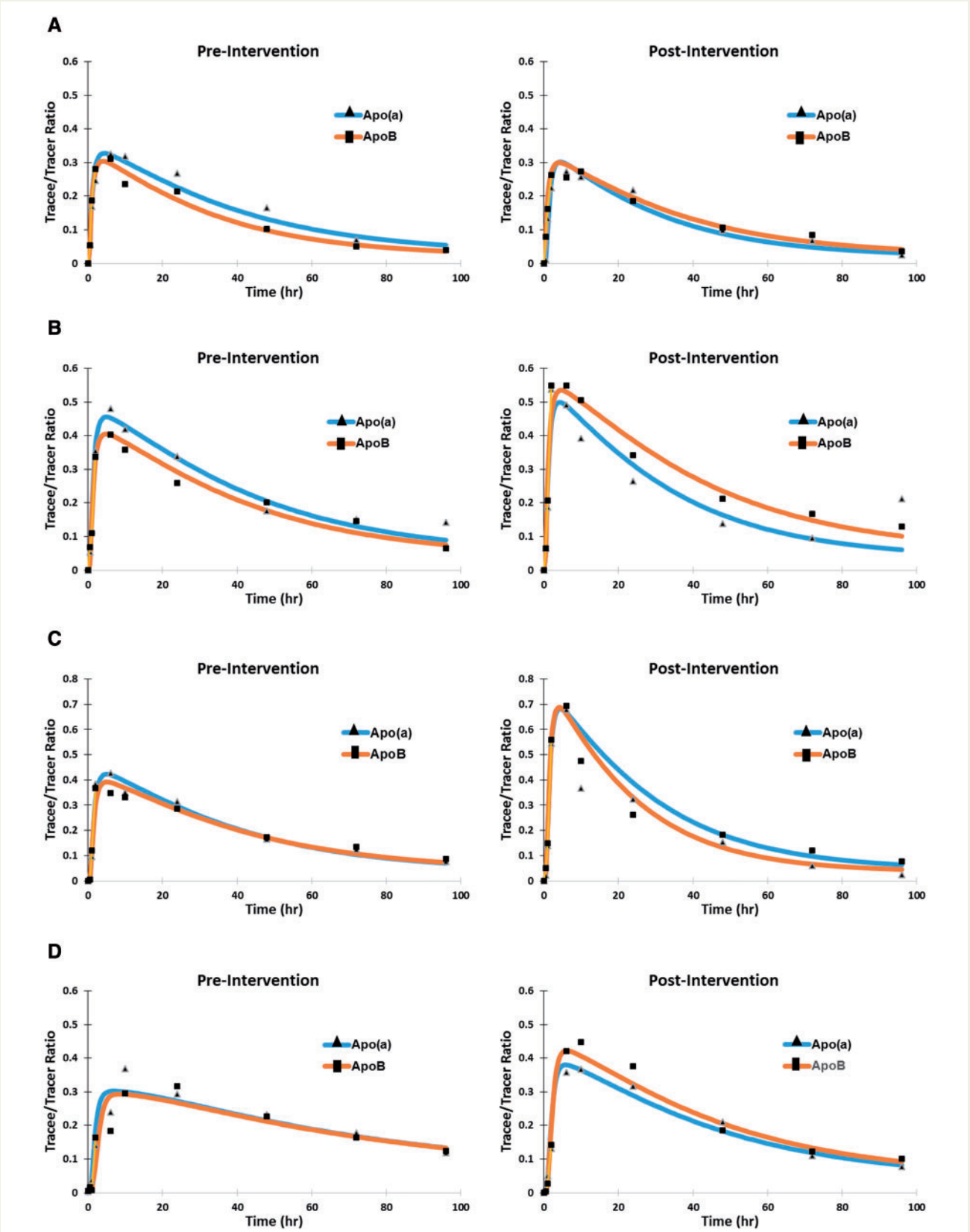


Figure 2 Fit of the model to tracer/tracee ratio of leucine in lipoprotein(a)-apolipoprotein(a) and lipoprotein(a)-apolipoprotein B over time (pre-intervention and post-intervention) in a representative subject from the placebo (*A*), atorvastatin (*B*), evolocumab (*C*), and evolocumab/atorvastatin (*D*) Groups. The precision of the estimated fractional catabolic rates was <10%.

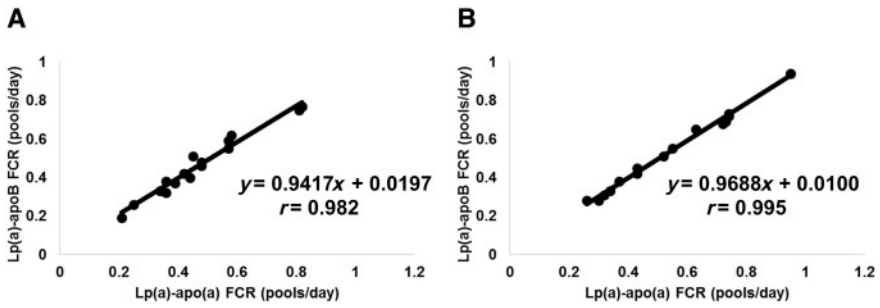


Figure 3 Association between fractional catabolic rates of lipoprotein(a)-apolipoprotein(a) and lipoprotein(a)-apolipoprotein B in a subset of 16 subjects at pre-intervention (*A*) and post-intervention (*B*).

Table 2 Effect of the interventions on kinetic indices of lipoprotein(a)-apolipoprotein(a) metabolism in the subjects

		Placebo ($n = 16$)	Atorvastatin ($n = 17$)	Evolocumab ($n = 14$)	Evolocumab/ atorvastatin ($n = 16$)
Pool size (nmol)	Baseline	87.6 (53.1–144)	55.1 (38.2–79.6)	82.7 (48.4–141)	95.6 (59.8–153)
	Week 8	81.1 (47.2–140)	48.1 (31.7–73.0)	55.0 (29.2–104)	59.3 (34.1–103)
	Ratio	0.93 (0.83–1.03)	0.87 (0.76–1.00)	0.67* (0.58–0.77)	0.62** (0.52–0.74)
	% change	-7% (-13% to +3%)	-13% (-24% to 0%)	-33%* (-42% to -23%)	-38%** (-48% to -26%)
FCR (pools/day)	Baseline	0.36 (0.31–0.41)	0.48 (0.38–0.59)	0.38 (0.29–0.50)	0.39 (0.30–0.51)
	Week 8	0.37 (0.30–0.45)	0.57 (0.44–0.75)	0.37 (0.26–0.52)	0.62 (0.47–0.82)
	Ratio	1.03 (0.89–1.20)	1.20 (1.03–1.40)	0.97 (0.76–1.25)	1.59† (1.40–1.81)
	% change	+3% (-11% to +20%)	+20% (+3% to +40%)	-3% (-24% to +25%)	+59%† (+40% to +81%)
PR (nmol/kg/day)	Baseline	0.40 (0.24–0.68)	0.33 (0.24–0.46)	0.40 (0.24–0.67)	0.47 (0.30–0.72)
	Week 8	0.38 (0.21–0.68)	0.34 (0.34–0.49)	0.26 (0.15–0.43)	0.46 (0.30–0.71)
	Ratio	0.95 (0.79–1.14)	1.04 (0.91–1.18)	0.64‡ (0.51–0.81)	0.98 (0.81–1.19)
	% change	+5% (-21% to +14%)	+4% (-9% to +18%)	-36%‡ (-49% to -19%)	-2% (-19% to +19%)

Values expressed as geometric mean (95% CI); ratio: refers to ratio of geometric means of post-/pre-values for treatment effect and equates to an average proportional (or percentage) change from baseline values; e.g. ratio of 0.67 refers to a 33% reduction and 1.59 refers to a 59% increase in the corresponding outcome variable.
CI, confidence interval; FCR, fractional catabolic rate (equivalent to fractional production rate); PR, production rate (equivalent to absolute catabolic rate).
*$P < 0.005$ (adjusted $P < 0.02$) compared with placebo; $P < 0.02$ (adjusted $P < 0.05$) compared with atorvastatin.
**$P < 0.001$ (adjusted $P < 0.01$) compared with placebo; $P < 0.01$ (adjusted $P < 0.05$) compared with atorvastatin.
†$P < 0.001$ (adjusted $P < 0.01$) compared with placebo and evolocumab; $P < 0.01$ (adjusted $P < 0.05$) compared with atorvastatin.
‡$P < 0.001$ compared with placebo, atorvastatin, and evolocumab/atorvastatin (adjusted $P < 0.01$).

Evolocumab significantly lowered the plasma pool size of Lp(a)-apo(a) (-36%, $P < 0.001$ for main effect); the reductions with monotherapy (-33%, $P < 0.001$) and dual therapy with atorvastatin (-38%, $P = 0.004$) were significantly greater than with atorvastatin and placebo alone (*Table 2*, Supplementary material online, *Figure S1*). Atorvastatin alone did not significantly alter the plasma pool size of

Lp(a)-apo(a). Changes in plasma apo(a), measured by LCMS, and Lp(a), measured by immunoassay, were highly correlated ($r = 0.845$, $P < 0.001$).

There was a statistically significant interaction ($P = 0.028$) between atorvastatin and evolocumab on the FCR of Lp(a)-apo(a), with the increase with combination therapy (+59%, $P < 0.01$) being significantly greater compared with placebo and each monotherapy (*Table 2*). Neither atorvastatin nor evolocumab alone significantly increased the FCR of Lp(a)-apo(a). There was a statistical significant interaction ($P = 0.044$) between atorvastatin and evolocumab on the production rate of Lp(a)-apo(a), the decrease with evolocumab alone (-36%, $P < 0.001$) being significantly greater than with placebo, atorvastatin, and combination therapy. Neither atorvastatin nor combination therapy significantly decreased the production rate of Lp(a)-apo(a). *Figure 4* shows the individual percentage changes in kinetic parameters following treatment with the interventions, with corresponding absolute changes shown in Supplementary material online, *Figure S2*. There was a highly statistically significant association ($r = 0.996$, $P < 0.001$) between the interventional changes in FCR of Lp(a)-apo(a) and Lp(a)-apoB in the 16 subjects selected for this substudy (Supplementary material online, *Figure S3*). The precision (CV) of the FCRs was <10%.

In evolocumab/atorvastatin group, the change in Lp(a)-apo(a) pool size was significantly correlated with change in Lp(a)-apo(a) production ($r = 0.745$, $P = 0.001$), but not with change in Lp(a)-apo(a) catabolism; this association was diminished by including apo(a) isoform size in the model ($r = 0.574$, $P = 0.011$). In this group, the increase in the FCR of Lp(a)-apo(a) was directly correlated with the increase in LDL-apoB FCR ($r = 0.522$, $P = 0.038$), but not with changes in other kinetic indices of apoB metabolism in very low-density lipoprotein (VLDL) and IDL. Apolipoprotein(a) isoform size was inversely correlated ($r = -0.572$, $P = 0.021$) with the change in FCR of Lp(a)-apo(a) in the combination therapy group. After adjusting for apo(a) isoform size, change in Lp(a)-apo(a) pool size in the evolocumab alone group was significantly associated with change in Lp(a)-apo(a) production ($r = 0.273$, $P = 0.048$), but not with Lp(a)-apo(a) catabolism ($r = 0.167$, $P = 0.208$). The decrease in Lp(a)-apo(a) production in this group was not significantly correlated with any kinetic changes in apoB in VLDL, IDL, and LDL.

Discussion

In the most comprehensive kinetic investigation to date, we demonstrated that evolocumab lowered plasma Lp(a) concentration by decreasing the production of Lp(a) particles as monotherapy, and by increasing the catabolism of Lp(a) particles in combination with atorvastatin. Another novel finding was that the fractional turnover rate of the apo(a) and apoB proteins within Lp(a) particles were also tightly coupled before and after interventions.

Previous lipoprotein(a) kinetic studies

Tracer studies suggest that the turnover of apo(a) is slower than apoB within the Lp(a) particle.[9,24] These were, however, smaller and analytically less detailed studies. They were also carried out under postprandial conditions, which increase exchange of apo(a) with triglyceride-rich lipoproteins (TRLs).[9] We concur with reports showing similar turnover rates of apo(a) and apoB within Lp(a),[6,7,27] as well as with lack of exchange of apo(a) with other lipoproteins in the post-absorptive state.[8] However, that our fractional catabolism of Lp(a) particles was comparable to LDL-apoB suggests a role of LDLRs in the metabolism of Lp(a).[10] We also confirmed the inverse association between apo(a) isoform and production of Lp(a)-apo(a),[28] but did not find that apo(a) isoform influenced the reduction in Lp(a) particle production with evolocumab monotherapy. A less rigorously designed and powered study has suggested that PCSK9 inhibition with alirocumab could increase the fractional catabolism of Lp(a),[19] but the sample was of mixed race and gender and the findings not statistically significant; apo(a) and apoB kinetics within Lp(a) were also not reported. Despite similar modelling,[19] we further present the basis for the goodness-of-fit of our model and the precision of the fractional catabolism of Lp(a). That atorvastatin alone did not alter Lp(a) kinetics is consistent with previous reports that statins do not specifically change Lp(a) concentrations.[22]

Decreased lipoprotein(a) production with evolocumab monotherapy

While *in vitro* experiments suggest that coupling of newly secreted apo(a) and LDL-derived apoB occurs at the plasma membrane of hepatocytes,[4] there is evidence from human and experimental studies for an intracellular assembly process.[5,6] Other data suggest that Lp(a) is reassembled after disassembly in the circulation.[9,24] Since atorvastatin and evolocumab decreased the plasma concentrations of LDL-apoB and triglycerides to a comparable extent, we cannot conclude that reduction in Lp(a) formation involves decreased availability of apoB for apo(a) in the circulation or a role for TRLs. Changes in VLDL, IDL, and LDL productions were also not significantly correlated with the fall in production of Lp(a) particles. Interventions that decrease intracellular formation of apoB can lower Lp(a),[22] but the effects are less potent than evolocumab.

Proprotein convertase subtilisin-kexin type 9 mAbs may decrease transcriptional regulation, intracellular availability, and/or extracellular release of the apo(a) protein,[4,12] but the molecular mechanisms are unclear. Reduction in the intracellular availability of apo(a) with evolocumab appears a more likely mechanism.[12] Consistent with our results, a recent tracer study in non-human primates concluded that alirocumab lowered Lp(a)-apo(a) production rate (Croyal *et al. Atherosclerosis* 2017;263:e27/abstract). Our finding that the effect of evolocumab on Lp(a)-apo(a) secretion was not dependent on apo(a) isoform size suggests decreased transport of apo(a) to the Golgi.[4] Our postulated effect of evolocumab monotherapy on apo(a) secretion was apparently ablated when combined with atorvastatin. In this setting, supraphysiological up-regulation of LDLRs enhances catabolism of Lp(a) particles and via a balancing, feed-forward mechanism may overcome the primary effect of evolocumab alone on the formation of Lp(a); at the molecular level this may entail to increased intracellular availability of apo(a) secondary to enhanced synthesis of PCSK9 due to atorvastatin and/or increased recycling of apo(a) following receptor-mediated uptake by hepatocytes.[10,29] Balancing feedback is a feature of complex metabolic systems following a perturbation, as demonstrated with other interventions.[13,30] The potential ablation of the rate limiting effect of PCSK9 inhibition on Lp(a) production in the presence of supraphysiological up-regulation of LDLR activity warrants further study.

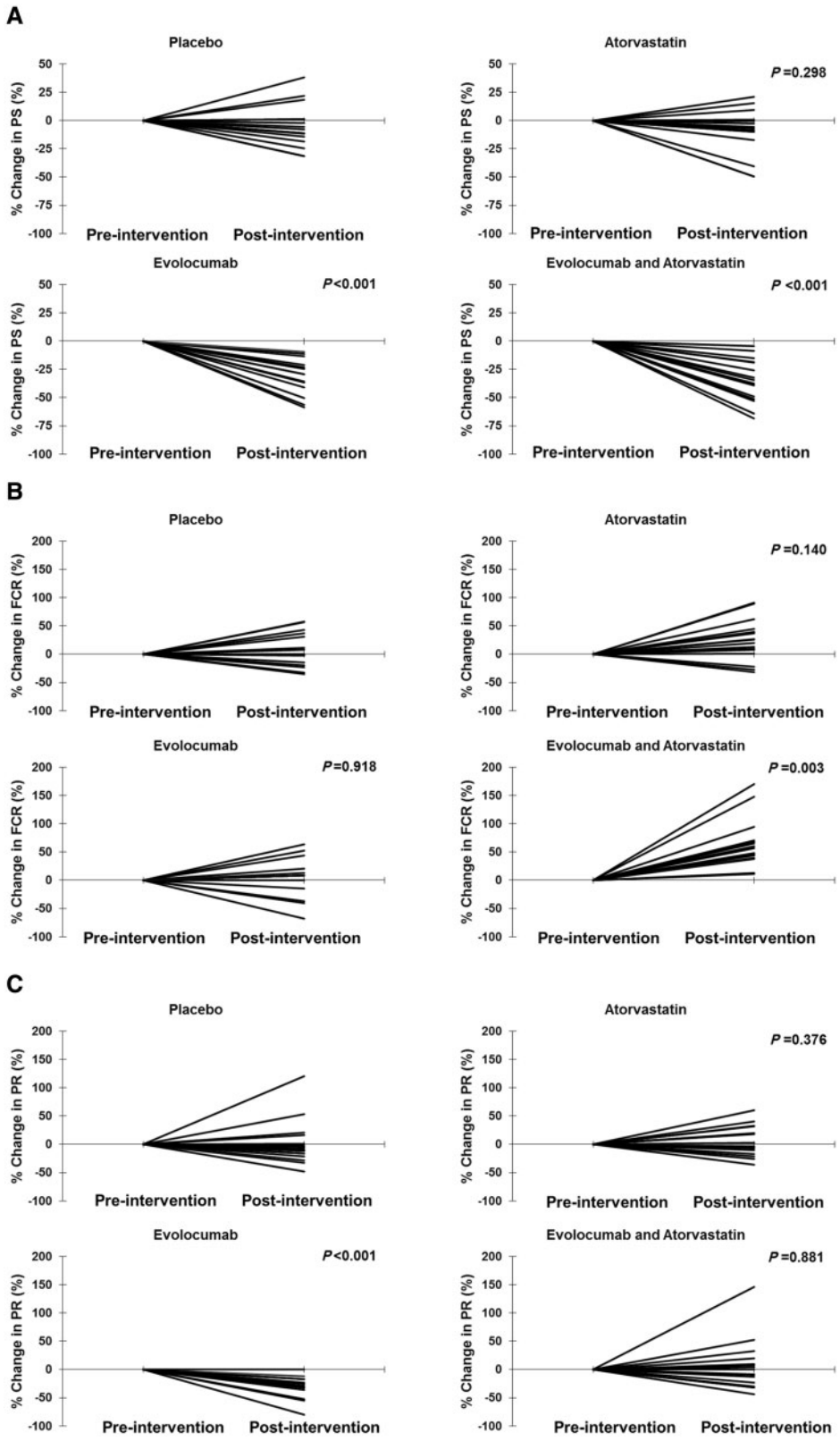
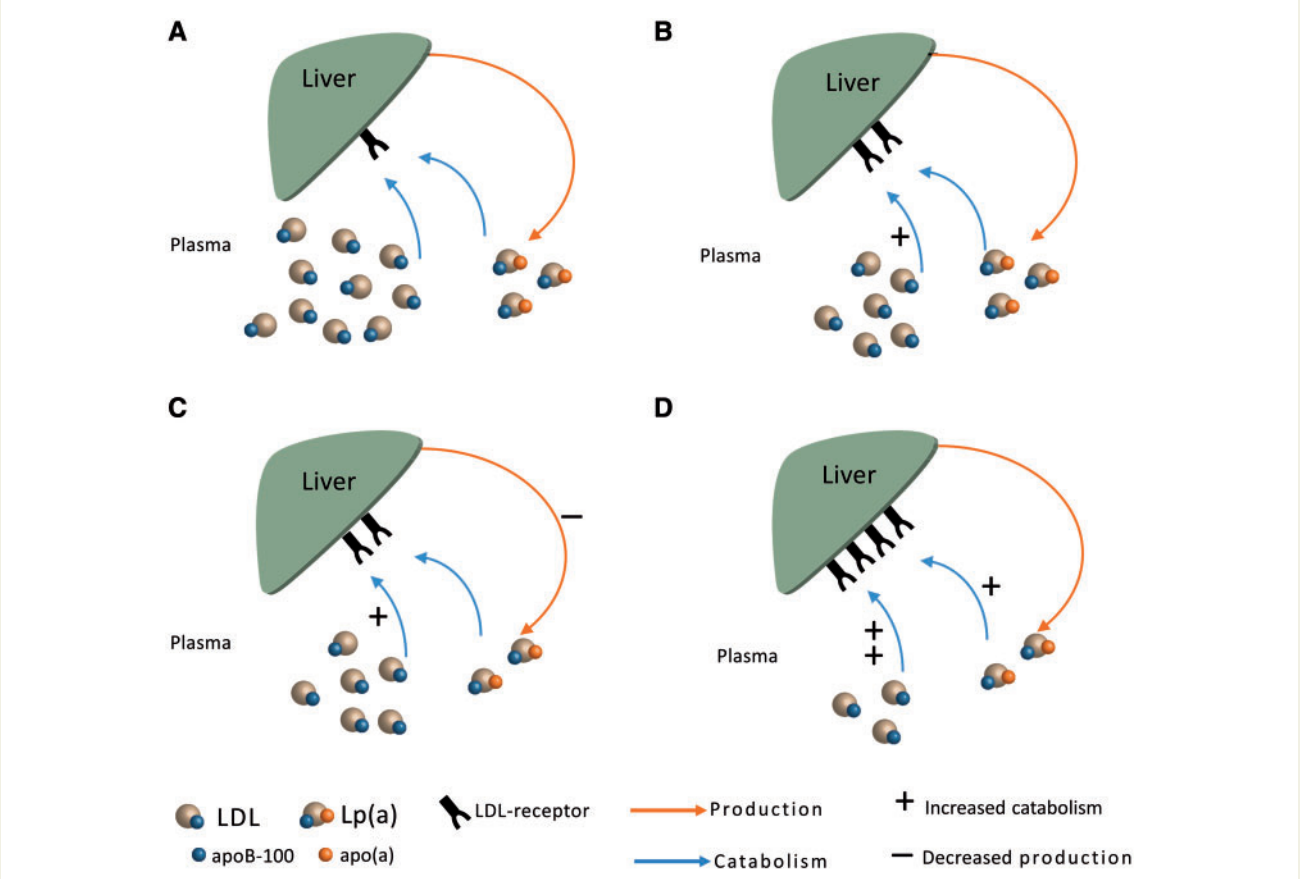


Figure 4 Individual percentage changes in the (*A*) pool size, (*B*) fractional catabolic rate and (*C*) production rate of lipoprotein(a)-apolipoprotein(a) following treatment with placebo, atorvastatin, evolocumab, and evolocumab/atorvastatin. *P*-values refer to changes compared with placebo group.



Take home figure Illustration summarizing the postulated mechanisms of Evolocumab on lipoprotein(a) particle kinetics. (*A*) Placebo: low-density lipoprotein is preferentially cleared by the low-density lipoprotein-receptor; circulating concentration of lipoprotein(a) determined by lipoprotein(a) production[27]; (*B*) Atorvastatin: low-density lipoprotein-receptor activity approximately two-fold elevated with halving of circulating low-density lipoprotein concentration,[18] but no effect on the clearance of lipoprotein(a); (*C*) Evolocumab: low-density lipoprotein-receptor activity also approximately two-fold elevated,[18] with similar effect to atorvastatin on the clearance of low-density lipoprotein and lipoprotein(a); evolocumab reduces hepatic production of lipoprotein(a) by decreasing intrahepatic synthesis of apo(a) and/or by decreasing availability of apolipoprotein B for coupling with apolipoprotein(a) in Space of Disse[4,12]; (*D*) Evolocumab/atorvastatin: low-density lipoprotein-receptor activity approximately four-fold elevated with reduction in concentrations of both low-density lipoprotein and lipoprotein(a); no reduction in hepatic production of lipoprotein(a).

Increased lipoprotein(a) catabolism with evolocumab and atorvastatin

The direct correlation between the fractional catabolism of Lp(a) and LDL-apoB particles implies a role of increased uptake of Lp(a) by the LDLR pathway. This could entail an impact of evolocumab and atorvastatin on LDLR activity and/or decrease competition of Lp(a) particles for clearance by this receptor.[4,10,16,17] Other hepatic receptors could mediate the catabolism of Lp(a), including VLDL and plasminogen receptors,[10,31,32] but their exact roles remain to be investigated. Since the circulating numbers of LDL particles exceed Lp(a) particles, the former are preferentially taken up by LDLRs. With combination therapy, Lp(a) particles compete better with very low plasma concentrations of LDL particles for uptake by LDLRs.[14,16] Reduced binding of Lp(a) particles to LDLRs may be due to camouflaging by the apo(a) moiety of the ligand-binding domain of apoB.[1,14] That apo(a) isoform size was negatively correlated with the catabolism of Lp(a) particles is compatible with this notion. Since PCSK9 can associate with both LDL and Lp(a) particles in plasma via a specific binding domain,[33] we cannot fully exclude a role for non-receptor mediated clearance in reducing the concentration of these two lipoproteins, but consider the magnitude of this effect would be incrementally smaller compared with receptor-mediated pathways.

Limitations

We only studied Caucasian men with predominantly normal plasma concentrations of Lp(a). We excluded those with extremely low plasma Lp(a) concentrations, because in this range our gel separation method was not sufficiently sensitive for isolating apo(a) and precisely measuring isotopic enrichment. We measured the turnover of apo(a) isolated from whole plasma, but demonstrated this was equivalent to that of apo(a) isolated from the LDL–HDL fractions. We did not measure LDLR activity directly, but inferred it from the fractional catabolism of LDL-apoB.[34] We anticipate that the kinetic changes in Lp(a) apply to other PCSK9 mAbs,[17] provided the dosing regimen is sufficient to substantially inhibit the concentration of

PCSK9 and its interaction with hepatic receptors. Our evolocumab regimen was designed to achieve maximal inhibition of PCSK9 and together with atorvastatin to maximally up-regulate LDLRs and the catabolism of LDL. Less intensive dose regimens may have comparable kinetic effects on LDL,[18,19] but whether this strictly extends to Lp(a) kinetics requires verification. Although the pool sizes of Lp(a) and apo(a) were numerically lower and FCR of Lp(a)-apo(a) higher in the atorvastatin alone group compared with the other groups, these differences did not significantly alter our findings. The per-group comparison clearly demonstrated that relative to placebo evolocumab alone decreased the production and combined with atorvastatin increased the catabolism of Lp(a) particles. Moreover, our random-effect regression models were adjusted for baseline Lp(a) as a covariate. Diet and apoE genotype can affect the metabolism of Lp(a),[35,36] but their impact is smaller than evolocumab. Finally, our overall findings may not apply to the postprandial state, when there could be recycling of apo(a) from TRLs.[9]

Conclusions

Using tracer kinetics we have shown under physiological conditions that evolocumab has a dual mechanism of action that lowers plasma Lp(a) concentration by decreasing production (as monotherapy) and increasing clearance (in combination with atorvastatin) of Lp(a) particles. Increased clearance of Lp(a) with PCSK9 inhibition may relate to supraphysiological up-regulation of hepatic LDLRs and/or decreased competition of Lp(a) with LDL particles for LDLR uptake.[14] Our postulated mechanisms of action of evolocumab are depicted in *Take home figure*. The sustainability of our findings is supported by longer term interventional and genetic studies.[15,20] Evolocumab may decrease cardiovascular events by increasing the clearance of LDL and Lp(a) particles against background statin therapy. The contribution that kinetic changes in Lp(a) makes to reduction in cardiovascular events and to future lipid guidelines requires further investigation.[37–39] Future studies should examine the mechanism of action of new therapies that lower Lp(a) in diverse patients with elevated Lp(a).[22]

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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