Effects of extended-release niacin on the postprandial metabolism of lipoprotein(a) and apolipoprotein B-100-containing lipoproteins in statin-treated men with type 2 diabetes

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Abstract

Objective: The effects of extended-release niacin (ERN; 1-2g/day) on the metabolism of lipoprotein(a) [Lp(a)] and apolipoprotein (apo) B-100-containing lipoproteins were investigated in 11 statin-treated Caucasian men with type 2 diabetes in a randomized, crossover trial of 12-weeks duration.

Approach and Results: The kinetics of Lp(a) and very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low-density lipoprotein (LDL) apoB-100 were determined following a standardized oral fat load (87% fat) using intravenous administration of D_3-leucine, gas chromatography-mass spectrometry and compartmental modeling. ERN significantly decreased fasting plasma total cholesterol, LDL cholesterol and triglyceride concentrations. These effects were achieved without significant changes in body weight or insulin resistance. ERN significantly decreased plasma Lp(a) concentration (-26.5%) and the production rates (PR) of apo(a) (-41.5%) and Lp(a)-apoB-100 (-32.1%); the effect was greater in individuals with elevated Lp(a) concentration. ERN significantly decreased VLDL (-58.7%), IDL (-33.6%) and LDL (-18.3%) apoB-100 concentrations and the corresponding PR (VLDL: -49.8%, IDL: -44.7%, LDL: -46.1%). The number of VLDL apoB-100 particles secreted increased in response to the oral fat load. Despite this, total VLDL apoB-100 production over the 10-hour postprandial period was significantly decreased with ERN (-21.9%).

Conclusions: In statin-treated men with type 2 diabetes, ERN decreased plasma Lp(a) concentrations by decreasing the production of apo(a) and Lp(a)-apoB-100. ERN also decreased the concentrations of apoB-100-containing lipoproteins by decreasing VLDL production and the transport of these particles down the VLDL to LDL cascade. Our study provides further mechanistic insights into the lipid-regulating effects of ERN.

Abbreviations: Lp(a): lipoprotein(a); VLDL: very low-density lipoprotein; IDL: intermediate density lipoprotein; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ERN: extended-release niacin; CVD: cardiovascular disease, FCR: fractional catabolic rate, PR: production rate; PCSK9: proprotein convertase subtilisin-like/kexin type 9; HOMA: homeostatic model assessment

Introduction

Type 2 diabetes is a powerful cardiovascular disease (CVD) risk factor[1,2]. This may in part be due to the presence of the atherogenic dylipidemia characterized by elevated plasma triglyceride and low high-density lipoprotein (HDL) cholesterol concentrations[3]. Statin therapy is the frontline treatment for dyslipidemia in type 2 diabetes[3]. Statins effectively lower low-density lipoprotein (LDL) cholesterol with modest triglyceride lowering and HDL cholesterol raising effects. Statins generally do not lower plasma concentrations of lipoprotein(a) [Lp(a)], an emerging CVD risk factor in the general population[4,5] and type 2 diabetes[6,7].

Nicotinic acid or niacin is an essential B-complex vitamin. At pharmacological doses, niacin has moderate LDL cholesterol lowering effects, however[8,9]. Niacin, nonetheless, effectively lowers plasma triglycerides and raises HDL cholesterol concentrations[8,9]. Niacin is also one of few agents that can significantly lower plasma Lp(a) concentrations[8-10]. Niacin is not widely used, however, owing to poor tolerability. The newer extended release formulation is better tolerated, however.

The role of niacin in CVD risk reduction remains unclear. In the Coronary Drug Project secondary prevention trial, niacin monotherapy showed a 26% reduction in risk of myocardial infarction[11]. However, recent clinical outcome trials including the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglyceride: Impact on Global Health Outcomes (AIM-HIGH) and Heart Protection Study 2-Treament of HDL to Reduce the Incidence of Vascular Events (HPS-2 THRIVE) where niacin is added to aggressive LDL cholesterol lowering statin therapy failed to demonstrate benefits[12,13]. However, the cardiovascular benefits of niacin may be restricted to patients with elevated plasma triglyceride and HDL cholesterol[14]. In addition, there is compelling data to support the use of niacin in combination with statins to treat the atherogenic dyslipidemia[8-10]. Studies to better understand the mechanism of action of niacin, therefore, are merited.

Few studies have examined the effect of niacin on lipoprotein metabolism. That the findings have been inconclusive may be, in part, related to small sample size and varying participant characteristics[15-17]. Furthermore, no studies have examined the effect of niacin in combination with optimal statin therapy, in the postprandial state or in type 2 diabetes. This study, therefore, aimed to investigate the effect of niacin on lipoprotein metabolism, with a focus on Lp(a), in men with type 2 diabetes on background optimal statin therapy. We also examined the effect of niacin on the metabolism of apolipoprotein (apo) B-100-containing lipoproteins. The effect of niacin on HDL metabolism in these individuals were reported previously[18]. We hypothesized that niacin would decrease Lp(a) concentrations by decreasing the production rates of apo(a) and Lp(a)-apoB-100, in men with type 2 diabetes. We also hypothesized that niacin would decrease the concentrations of apoB-100-containing lipoproteins by decreasing the production rates of these particles.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Table 1 shows the clinical and biochemical characteristics of the 11 men with type 2 diabetes at randomization. These individuals had well controlled plasma LDL cholesterol, low HDL

cholesterol and moderately elevated triglyceride concentrations. All participants took 100mg of aspirin daily for the duration of the trial to minimize flushing.

Table 2 shows the plasma lipid, lipoprotein, apolipoprotein, glucose and insulin concentrations at the end of each intervention period. Compared with rosuvastatin, the combination of rosuvastatin + ERN significantly decreased plasma total, LDL and non-HDL cholesterol, apoB-100, and triglyceride, and increased HDL cholesterol and apoA-I concentrations. There was a significant increase in plasma glucose with rosuvastatin + ERN, but insulin and the homeostatic model assessment (HOMA) score was not altered significantly. Plasma proprotein convertase subtilisin-like/kexin type 9 (PCSK9) concentration decreased with rosuvastatin + ERN, but this failed to reach statistical significance (*P*=0.06). Carry-over effects were tested and not found significant for the abovementioned parameters. No changes (rosuvastatin *vs.* rosuvastatin + ERN) in body weight (96.9 ± 4.67 *vs.* 96.5 ± 4.56 kg; *P*=0.21), systolic blood pressure (129 ± 4.63 *vs.* 123 ± 3.44 mmHg; *P* =0.20), diastolic blood pressure (74.8 ± 2.92 *vs.* 70.9 ± 3.57 mmHg; *P* =0.17), or dietary intake (7306 ± 523 *vs.* 7659 ± 508 kJ/day; *P* =0.43) between intervention periods were observed.

Tracer metabolic studies were performed in the non-steady state. Participants consumed a liquid formulation high-fat test meal (oral fat load; 87% fat), resulting in postprandial changes in plasma triglycerides (Supplemental Figure I), but not plasma Lp(a). Figure 1 shows the fit of the model to the tracer-to-tracee ratio (TTR) for Lp(a) protein components, apo(a) and Lp(a)-apoB-100 in a representative participant at the end of each intervention period. The corresponding apo(a) and Lp(a)-apoB-100 fractional catabolic rates (FCR) are 0.88 and 1.20, and 1.09 and 1.87 pools/day for rosuvastatin only and rosuvastatin + ERN, respectively. Figure 2 shows the fit of the model to the very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and LDL apoB-100 tracer data following administration of D_3-leucine, and to the VLDL apoB-100 concentrations in response to the oral fat load in a representative participant at the end of each intervention period. The corresponding VLDL, IDL and LDL apoB-100 FCR are 5.28 and 8.38, 2.33, 1.28, and 0.38 and 0.25 pools/day for rosuvastatin and rosuvastatin + ERN, respectively. Plasma leucine tracer curves did not differ significantly between interventions. Details of the multi-compartmental models for Lp(a) and apoB-100-containing lipoproteins are provided in the Supplemental Material.

Table 3 shows the concentrations and kinetic parameters for Lp(a) protein components, apo(a) and Lp(a)-apoB-100, as well as VLDL, IDL and LDL apoB-100 following interventions with rosuvastatin and rosuvastatin + ERN. Compared with rosuvastatin alone, rosuvastatin + ERN significantly decreased plasma Lp(a) concentration and the production rates (PR) of apo(a) and Lp(a)-apoB-100. No significant changes to apo(a) or Lp(a)-apoB-100 FCR were observed. The basal (synonymous with the fasted state) concentrations and PR of VLDL, IDL and LDL apoB-100 were significantly decreased with rosuvastatin + ERN compared with rosuvastatin alone. Basal IDL and LDL, but not VLDL, apoB-100 FCR were also significantly decreased with rosuvastatin + ERN. The number of VLDL apoB-100 particles secreted into plasma on top of basal VLDL apoB-100 secretion increased in response to the oral fat load. The increase was greater with rosuvastatin + ERN (3.31 ± 0.13 *vs* 4.05 ± 0.32 mg/kg; *P*=0.05; Supplementary Figure II). Despite this, total (basal + post-oral fat load) VLDL apoB-100 production over the 10-hour postprandial period was significantly decreased with rosuvastatin + ERN compared with rosuvastatin alone (Table 3; Supplemental Figure II). The total area under the curve (AUC) over the 10-hour postprandial period was decreased with rosuvastatin + ERN (689 ± 107 *vs* 450 ± 57.9, mg/L*h; *P*=0.03). No significant changes in the direct secretion of IDL (mean ± SEM: 0.69 ± 0.37 *vs* 0.58 ± 0.24, mg/kg/day; *P*=0.75) or LDL (0.53 ± 0.14 *vs* 0.42 ± 0.13, mg/kg/day; *P*=0.42) were observed with rosuvastatin + ERN compared with rosuvastatin alone. The

transport of VLDL to IDL (7.97 ± 0.88 *vs* 4.64 ± 0.71, mg/kg/day; *P*<0.01) and IDL to LDL (8.59 ± 0.88 *vs* 4.95 ± 0.60, mg/kg/day; *P*<0.01) were significantly decreased with rosuvastatin + ERN. The aforementioned kinetic parameters remained significantly different between rosuvastatin + ERN compared with rosuvastatin alone following adjustment for fasting glucose concentration.

Table 4 shows Lp(a) concentrations and the kinetic parameters of its protein components in individual participants, grouped according to low and high plasma Lp(a) concentrations, with low defined as below and high as above the median [42nmol/L] plasma Lp(a) concentration. In between-group analyses, the high Lp(a) group had significantly lower number of Kringle IV domains. This group also had higher plasma Lp(a) concentration and apo(a) and Lp(a)-apoB-100 PR (all *P*<0.01). The FCR of Lp(a)-apoB-100 was significantly slower in the high Lp(a) group, but apo(a) FCR was not different between groups. Within the low Lp(a) group, apo(a) FCR and PR were significantly lower compared with those of Lp(a)-apoB-100. By contrast, within the high Lp(a) group, apo(a) FCR and PR were significantly higher than those of Lp(a)-apoB-100. Changes in Lp(a) concentration (low *vs.* high: -9.5% *vs.* -28%) and apo(a) (-3.5% *vs.* -37.2%) and Lp(a)-apoB-100 (-6.4% *vs.* -30.6%) PR were significantly greater [all $P_{group\ effect}$<0.05; Supplemental Figure III] with rosuvastatin + ERN in the high Lp(a) group.

Discussion

We report on the effects of extended-release niacin (ERN), added to the background of statin therapy, on Lp(a) metabolism and apoB-100-containing lipoproteins in men with type 2 diabetes. We showed that ERN significantly decreased plasma Lp(a) concentration and the PR of apo(a) and Lp(a)-apoB-100; the effect being greater in individuals with higher Lp(a) concentrations. These changes were achieved without alterations to body weight, blood pressure or dietary intake, and independent of changes in plasma glucose concentration. In addition, ERN significantly decreased the concentrations of apoB-100-containing lipoproteins by decreasing the transport of these particles down the VLDL to LDL cascade. The number of VLDL apoB-100 particles secreted increased in response to the fat load. Despite this, total VLDL apoB-100 production over the 10-hour postprandial period was significantly decreased with ERN. The plasma triglyceride and LDL cholesterol lowering, and HDL cholesterol raising effects are consistent with previous reports[8,9].

Previous kinetic studies using radioisotope and stable isotope methods have examined the effect of niacin on apoB-containing lipoproteins in the steady state. Kushwaha *et al* reported that niacin (0.5g, thrice/day for 4 weeks) accelerated the catabolism of autologous ^{125}I-labelled VLDL in a male participant with hypertriglyceridemia[17]. By contrast, Fabbrini *et al* reported that ERN (2g/day for 16 weeks) significantly decreased VLDL apoB-100 and triglyceride PR in 27 obese and predominantly female participants with non-alcoholic fatty liver disease[15]. In 5 men with combine hyperlipidemia, Lamon-Fava *et al* reported that ERN (2g/day for 12 weeks) decreased VLDL apoB-100 concentration by increasing its FCR, with no changes to IDL and LDL concentrations or kinetics[16]. Discrepancies among studies may relate to differences in participant clinical characteristics, in study design and sample size, and in dose and duration of intervention. To date, only one study has examined the effect of niacin on the metabolism of Lp(a). In 8 non-diabetic, obese men with hypertriglyceridemia, Croyal *et al* reported that ERN (2g/day for 8 weeks) decreased plasma apo(a) concentration, PR and FCR measured using stable isotope, liquid chromatography–tandem mass spectrometry method and monocompartmental modeling in the fasted state[19]. No studies have examined the effects of niacin on the metabolism of Lp(a) and its protein components, apo(a) and Lp(a)-apoB-100, or that of apoB-100-containing lipoproteins following an oral fat load. We, therefore, extend

previous reports by examining the effect of ERN on the metabolism of Lp(a) and its protein components in men with type 2 diabetes on background optimal statin therapy using a randomized, crossover study design. This study also examined, in the same individuals, the effect of ERN on the metabolism of apoB-100-containing lipoproteins in the non-steady state.

Studies in transgenic mice have shown reductions in apo(a) concentration and *APOA* mRNA expression with niacin intervention[20]. Niacin has also been shown to down-regulate human *APOA* promoter activity and hence, *APOA* transcription in HepG2 cells[20]. Our finding that ERN decreased apo(a) PR is consistent with this notion. The previously reported effects of niacin on hepatic triglyceride synthesis may explain the reductions in Lp(a)-apoB-100 PR with ERN. In particular, niacin inhibits the activity of diacylglycerol acyltransferase (DGAT)-2, the rate-limiting enzyme that catalyzes the final reaction in triglyceride synthesis[21], and upregulates intrahepatic fatty acid (FA) oxidation[15] and hence, its availability for triglyceride synthesis. This results in a reduction in the lipid pool that protects apoB-100 from post-translational degradation[22] and hence, apoB-100 secretion and the pool of apoB available to bind with apo(a). Of interest, the effects of ERN on Lp(a) concentration and apo(a) and Lp(a)-apoB-100 kinetics were greater in individuals with the smaller apo(a) isoform size [elevated Lp(a) concentration], consistent with recent reports[23]. Whether niacin could confer cardiovascular benefits via Lp(a) reductions in these individuals is unclear, however. The plasma Lp(a) reduction of 19% with ERN in the AIM-HIGH study was not associated with reduction in CVD events[10] even though recent sub-analyses suggest potential cardiovascular benefits of ERN in individuals with mixed dyslipidemia[14]. It is also possible that niacin may confer CVD benefits in those who sustain acute coronary syndrome and/or myocardial infarction given its favorable effect on atherosclerotic plaque structure[24]. Whether lowering Lp (a) specifically confers, CVD benefits can only be addressed by therapeutic interventions that selectively lower Lp(a)[10].

Recent *in vitro* studies point to a role of the LDL receptor in the catabolism of Lp(a)[25]. These data, however, suggest that the catabolism of Lp(a) is primarily mediated via apoB-100 rather than apo(a) and chiefly at supraphysiological LDL receptor levels[25]. In contrast, studies in homozygous and heterozygous familial hypercholesterolemia patients with little or no LDL receptor activity showed that the absence of a functional LDL receptor was not associated with delayed Lp(a) catabolism[26]. While ERN decreased plasma PCSK9 concentration in our study and potentially increased LDL receptor recycling, this was not associated with any significant changes in apo(a) or Lp(a)-apoB-100 catabolism. Our finding differs to that reported by Croyal *et al* showing reductions in apo(a) FCR with ERN[19]. The authors concluded that the LDL receptor and PCSK9 did not play a central role in apo(a) catabolism, but conceded that this may be a consequence of not examining the kinetics of Lp(a)-apoB-100. It is noteworthy that these participants did not have type 2 diabetes, were hypertriglyceridemic and were not on statin treatment. By contrast, our participants were on optimal background statin treatment and it is possible that the magnitude of PCSK9 reduction and by implication, increase in LDL receptor activity was insufficient to alter Lp(a) catabolism. Future studies on the effect of PCSK9 inhibitors on Lp(a) metabolism are warranted.

That apo(a) secretion is higher in individuals with elevated Lp(a) supports the notion that apo(a) secretion and isoform size are key determinants of Lp(a) concentration[27,28]. In parallel, the higher Lp(a)-apoB-100 secretion supports the notion that apoB-100 is a major determinant of Lp(a) concentrations[29]. Moreover, it may provide a mechanism by which therapies targeting apoB-100 synthesis and secretion could lower Lp(a) concentrations[30,31], although this remains to be investigated. The uncoupling of apo(a) and Lp(a)-apoB-100 metabolism is consistent with the notion that apo(a) and apoB-100 may associate and dissociated in plasma[32]. It is also consistent with the concept that Lp(a) may be assembled intracellularly and extracellularly[32-36].

We demonstrated that ERN decreased VLDL, IDL and LDL apoB-100 PR in the basal state. The reductions in VLDL apoB-100 PR are consistent with the notion that niacin decreases hepatic apoB secretion[22] The reductions in IDL and LDL apoB-100 PR may relate to reductions in transport of these particles down the VLDL to LDL cascade, or to decreases in the direct hepatic secretion of these particles. Our results were more consistent with the former mechanism and are likely attributable to markedly reduced VLDL apoB-100 secretion. We also showed concurrent reductions in IDL and LDL apoB-100 FCR with ERN. The fall in PCSK9 concentration with ERN might translate to increased LDL receptor activity[37], and thus increased LDL catabolism. However, that IDL and LDL FCR fell with ERN suggests that changes to IDL and LDL concentration are primarily due to decreased apoB hepatic secretion and transport.

We found that the number of VLDL apoB-100 particles secreted into plasma increased in response to the oral fat load, the increase being marginally higher with ERN. Whether this relates to altered suppression of FA spillover, a consequence of lipoprotein lipase hydrolysis of meal triglyceride, a potential contributor for FA pool and substrate for hepatic triglyceride synthesis[38,39], is unclear. Despite this, total VLDL apoB-100 production over the 10-hour postprandial period was significantly decreased with ERN.

Our study found that fasting plasma glucose concentrations increased with ERN, consistent with earlier reports[8,9]. Plasma glucose concentration did not change significantly in response to the oral fat load (data not shown) with ERN, however. It is noteworthy that the lipid-regulating effects of ERN were independent of the changes in glucose concentrations.

Our study has limitations. First, we studied only Caucasian men. While this largely eliminates population admixture as a potential confounder, future studies in women and populations of different ethnicity and disease states are warranted. Second, our study focused on the metabolic fate of the predominant apo(a) isoform and in whole plasma. Future studies to examine the effect of ERN on the concentrations and kinetics of the secondary apo(a) isoform are warranted. Future studies that selectively recruit individuals with low and elevated [>95th percentile] plasma Lp(a) concentrations are also warranted. Third, while plasma Lp(a) concentrations remained constant following the oral fat load, we cannot exclude that the distribution of apo(a) between lipoprotein fractions may be altered. Studies on the metabolism of apo(a) within the triglyceride-rich lipoprotein fraction, following an oral fat load, are also of interest. Fourth, the kinetics of VLDL subspecies and triglycerides were not examined, but we anticipate that their production will also be decreased with ERN. Fifth, while we separated apoB-100 from apoB-48 within the d<1.006g/mL fraction, we cannot exclude a small degree of contamination by apoB-48-containing particles. In addition, the triglyceride-lowering effect of ERN might be in part due to reductions in apoB-48 containing particles. The effects of ERN on the metabolism of these particles merit investigation. Finally, apoB-100-containing lipoprotein particles may exhibit kinetic and structural heterogeneity based on the complement of regulatory apolipoproteins. Studies to examine the effect of ERN on the concentration and kinetics of these apolipoproteins may further corroborate our findings.

In conclusion, in statin treated men with type 2 diabetes, ERN decreased plasma Lp(a) concentrations by decreasing the production of apo(a) and Lp(a)-apoB-100. ERN also decreased the concentrations of apoB-100-containing lipoproteins by decreasing VLDL production and the transport of these particles down the VLDL to LDL cascade. Our study provides further mechanistic insights into the lipid-regulating effects of ERN.

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Significance

Statins are the frontline treatment for dyslipidemia in type 2 diabetes. While statins effectively lower LDL cholesterol, statins have little effect on plasma Lp(a), an increasingly recognized cardiovascular disease (CVD) risk factor, and modest triglyceride lowering and HDL cholesterol raising properties. Niacin, by contrast, is one of few agents to effectively lower plasma Lp(a). Niacin also lowers plasma triglycerides and raises HDL cholesterol. The mechanism by which niacin regulates Lp(a) metabolism is not known, however. Consequently, we sought to examine the effects of extended-release niacin (ERN) on Lp(a) metabolism in statin-treated men with type 2 diabetes. We also examined the effects of ERN on apoB-100-containing lipoprotein metabolism. ERN lowered plasma Lp(a) concentrations by decreasing the production of apo(a) and Lp(a)-apoB-100. ERN also lowered the concentrations of apoB-100-containing lipoproteins by decreasing the transport of these particles. Our study provides mechanistic insights into the lipid-regulating effects of ERN.

Figure 1: Fit of the model to the tracer to tracee ratio for apo(a) (A) and Lp(a)-apoB-100 (B) with D_3-leucine in a representative participant on rosuvastatin and rosuvastatin + extended release niacin

Figure 2: Fit of the model to the tracer to tracee ratio for VLDL (A), IDL (B) and LDL (C) apoB-100 with D_3-leucine, and VLDL apoB-100 concentration (D) in a representative participant on rosuvastatin and rosuvastatin + extended release niacin

Table 1: Clinical and biochemical characteristics of study participants at randomization

N=11	Mean	SD
Age (years)	63	6.20
Weight (kg)	96.7	15.2
Body mass index (kg/m^2)	32.2	4.60
Waist circumference (cm)	112	11.7
Systolic blood pressure (mm/Hg)	126	13.4
Diastolic blood pressure (mm/Hg)	73.3	11.8
Total cholesterol (mmol/L)	3.95	0.38
LDL cholesterol (mmol/L)	2.25	0.36
HDL cholesterol (mmol/L)	0.98	0.15
Triglycerides (mmol/L)	1.54	0.67
Glucose (mmol/L)	6.70	1.51
Insulin (mU/L)	13.2	6.48
HOMA score	3.90	1.90
Duration of diabetes (years)	6.27	3.13
On lipid-lowering medication (n)	11	
On anti-diabetic medication (n)	10	
On anti-hypertensive medication (n)	8	

To convert total, LDL and HDL cholesterol in mmol/L to mg/dl, divide by 0.0259; triglycerides in mmol/L to mg/dl, divide by 0.0113; SD: standard deviation

Table 2: Plasma lipid, lipoprotein, apolipoprotein and other biochemical measurements on rosuvastatin and rosuvastatin + extended release niacin

N=11	Treatment				*P*
	Rosuvastatin		Rosuvastatin + ER Niacin		
	Mean	**SEM**	**Mean**	**SEM**	
Total cholesterol (mmol/L)	3.55	0.15	3.32	0.14	0.02
LDL cholesterol (mmol/L)	2.28	0.12	1.93	0.09	<0.01
HDL cholesterol (mmol/L)	1.00	0.04	1.17	0.07	<0.01
Non-HDL cholesterol (mmol/L)	2.55	0.13	2.14	0.12	<0.01
Plasma triglyceride (mmol/L)	1.67	0.26	1.31	0.21	0.04
Plasma apoB (g/L)	0.70	0.05	0.58	0.04	<0.01
Plasma apoA-I (g/L)	1.31	0.05	1.40	0.06	0.03
Glucose (mmol/L)	7.61	0.76	8.32	0.73	0.03
Insulin (mmol/L)	14.20	2.77	17.39	3.95	0.10
HOMA score	4.71	0.86	6.03	1.05	0.06
HbA1c (%)	7.20	0.33	7.51	0.27	0.17
Plasma PCSK9 (ng/mL)	249	15.8	223	8.80	0.06

To convert total, LDL, HDL and non-HDL cholesterol in mmol/L to mg/dl, divide by 0.0259; triglycerides in mmol/L to mg/dl, divide by 0.0113; PCSK9: proprotein convertase subtilisin-like/kexin type 9.

Table 3: Kinetic estimates of the metabolism of apoB-100-containing lipoproteins and Lp(a) after treatment with rosuvastatin and rosuvastatin + extended release niacin

	Treatment				*P*
	Rosuvastatin		Rosuvastatin + ER Niacin		
	Mean	**SEM**	**Mean**	**SEM**	
N=10					
Plasma concentration (nmol/L)					
Lp(a)†	95.8	29.7	70.4	20.7	<0.01
Fractional catabolic rate (pools/day)					
apo(a)	0.95	0.11	0.99	0.16	0.81
Lp(a)-apoB-100	0.97	0.13	1.00	0.17	0.70
Production rate (nmol/kg/day)					
apo(a)	4.27	1.56	2.50	0.78	0.04
Lp(a)-apoB-100	2.99	0.86	2.03	0.48	0.03
N=11					
Basal concentration (mg/L)					
VLDL	51.8	10.3	21.4	2.52	<0.01
IDL	86.6	13.0	58.8	6.65	0.01
LDL	569	37.2	465	24.4	<0.01
Basal fractional catabolic rate (pools/day)					
VLDL	7.38	0.74	8.26	0.84	0.50
IDL	2.74	0.34	2.01	0.24	0.01
LDL	0.39	0.03	0.26	0.03	<0.01
Basal production rate (mg/kg/day)					
VLDL	15.0	1.65	7.53	0.94	<0.01
IDL	9.29	0.95	5.23	0.61	<0.01
LDL	9.80	0.97	5.36	0.63	<0.01
Conversion (%)					
VLDL to IDL	61.0	7.43	63.1	6.50	0.77
IDL to LDL	99.8	0.11	95.5	3.49	0.18
Total VLDL production (mg/kg/10h)*	9.54	0.64	7.19	0.39	<0.01
Postprandial VLDL secreted (mg/kg)	3.31	0.13	4.05	0.32	0.05

**Total VLDL production: basal + post-prandial VLDL production over 10 hour postprandial period; † Measured by the monoclonal antibody-based ELISA method*

Table 4: Plasma Lp(a) concentrations and the kinetic parameters of apo(a) and Lp(a)-apoB-100, grouped by low or high plasma Lp(a) concentration. Low Lp(a) concentration was defined as below and high as above the median plasma Lp(a) concentration (42nmol/L), respectively. Data is from rosuvastatin only treatment period

ID	Lp(a) group	Apo(a) isoforms (Number of Kringle 4 domains)						
		Predominant	Secondary	Lp(a) (nmol/L)	Apo(a) FCR (pools/day)	Lp(a)-apoB-100 FCR (pools/day)	Apo(a) PR (nmol/kg/day)	Lp(a)-apoB-100 PR (nmol/kg/day)
1	Low	28	34	21.30	0.88	1.20	0.83	1.14
2	Low	19	28	21.34	0.76	0.91	0.73	0.87
3	Low	19	20	15.50	0.81	1.32	0.57	0.92
4	Low	21	27	18.10	1.14	1.64	0.93	1.33
5	Low	28	34	12.58	0.75	1.25	0.42	0.71
Mean				***17.76***	***0.87***	***1.27****	***0.69***	***1.00****
SEM				***1.52***	***0.06***	***0.10***	***0.08***	***0.10***
6	High	21	19	62.55	1.31	1.05	3.68	2.97
7	High	17	24	203.31	1.64	0.89	14.98	8.12
8	High	17	26	201.70	1.10	0.50	9.98	4.59
9	High	17	25	239.10	0.65	0.66	6.95	7.05
10	High	16	26	162.88	0.49	0.29	3.59	2.16
Mean				***173.91†***	***1.04***	***0.68*†***	***7.84†***	***4.98*†***
SEM				***27.14***	***0.19***	***0.12***	***1.92***	***1.03***

*FCR: fractional catabolic rate, PR: production rates; *P<0.05 for within-group analysis by paired student t-test; †P<0.01 for between-group analysis by independent student t-test*

SUPPLEMENTAL MATERIAL

Detailed Methods

Participants

Twelve non-smoking men with type 2 diabetes (T2DM) aged between 18-75 years with a body mass index (BMI) of less than 40kg/m^2 (mean ± SD: age 63.0±5.7 years, BMI 31.7±4.4kg/m^2) were recruited [1]. Type 2 diabetes was defined as a fasting plasma glucose concentration of ≥7.1mmol/L. Participants with fasting total plasma cholesterol >6.0mmol/L or triglyceride >4.5mmol/L, genetic hyperlipidemia, proteinuria, hypothyroidism, cholelithiasis, alcohol consumption >30 g alcohol/day, HbA1c >8.5%, daytime insulin treatment, uncontrolled hypertension (>150/90mmHg), creatinemia (>150µmol/L), hepatic dysfunction (AST or ALT>3x ULN), abnormal thyroid function, muscle disorders or creatinine kinase >3xULN, major systemic illness, used steroids or agents that may influence lipid metabolism including fish oils, cardiovascular event within the last 6 months, lactose intolerance or intolerance to cream and eggs (ingredients in the test meal) were excluded. Three participants were already on rosuvastatin at recruitment, the other nine subjects were on atorvastatin (n=6), pravastatin (n=2) and simvastatin (n=1). None were on fibrates or niacin at recruitment. Eleven participants were on anti-diabetic medication (biguanides (n=11), sulfonylureas (n=6)) and eight were on anti-hypertensive medication (calcium channel blockers (n=3), angiotensin-converting-enzyme inhibitors (n=5), angiotensin receptor blockers (n=3), beta-blocker (n=1); three participants were on multiple anti-hypertensive medication). Eleven participants were of Caucasian descent and one, of Indian descent. Given that plasma Lp(a) concentrations and isoform distribution, and by inference Lp(a) metabolism, may differ according to ethnicity [2], the participant of Indian descent was excluded from the study. One participant had Lp(a) concentration that was below detection limit. Therefore, the impact of niacin on Lp(a) metabolism could not be explored in this individual.

All participants provided informed written consent and the study was approved by the Royal Perth Hospital Human Research Ethics Committee (EC2009/018). The study was registered on the Australian New Zealand Clinical Trials Registry (ACTRN12609000448246).

Study Design and Clinical Protocol

This was a randomized, crossover design study. Participants were treated with rosuvastatin (Crestor, AstraZeneca) at a stable dose for a ≥6-week run-in period and attained a target LDL-cholesterol of <2.5mmol/L, fasting triglycerides <4.5mmol/L and HDL-cholesterol ≤1.0mmol/L to be eligible for the study. Rosuvastatin dose varied across the participants (5 x 10mg/day, 4 x 20mg/day and 2 x 5mg/day). Given that statins are the frontline treatment for dyslipidemia in populations with high cardiovascular disease risk, including type 2 diabetes, all participants remained on statin treatment for the study duration, to be consistent with current guidelines. Participants were advised to continue their habitual isocaloric diet and maintain physical activity constant throughout the study. Dietary intake was assessed using four 3-day food records (FoodWorks 2007 Version 5, Xyris Software, Brisbane, Australia). At the end of the run-in period, participants were randomized to either rosuvastatin or rosuvastatin plus extended-release (ER) niacin (Niaspan, Abbott Laboratories) (10 titrated to 2g, 1 to 1.5g and 1 to 1g) for 12 weeks, then crossed over to the alternate therapy with a 3-week washout period. Niacin dose titration was based on participant tolerance to ER niacin. During the washout period, only niacin was discontinued. Rosuvastatin and all concomitant medications remained unaltered. Participants took 100mg of aspirin once daily in the evening, prior to taking niacin to help reduce

flushing. To minimize confounding due to aspirin intake, all participants took aspirin for the duration of the study. Compliance with study medications were determined by tablet count at the end of each treatment period.

Metabolic studies were performed at the end of each treatment period. All participants were admitted to the metabolic ward in the morning after a 12-hour fast. Body weight and height were measured and arterial blood pressure recorded using a Dinamap1846 SX/P monitor (Critikon, Tampa, USA). Fasting baseline blood samples were collected for biochemical measurement.

After the collection of fasting baseline blood samples, participants consumed a liquid formulation high-fat test meal (oral fat load) and two 50,000U vitamin A capsules over a 5-minute period. The test meal consisted of 100mL full cream milk (3.4% fat), 150mL of pure cream (35% fat), 70mL of corn oil, 90g of whole egg, 10g of sugar and 3.5g flavouring. This fat load yielded a total of 4800kJ with an energy distribution of 87% fat [130g], 7% carbohydrates [21g] and 6% protein [17g]. Immediately following the test meal, a single bolus of D_3-leucine (5mg/kg) was administered intravenously within a 2-minute period into an antecubital vein via a Teflon cannula. Blood samples were collected at 5, 10, 20, 30, and 40 minutes, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 hours post-injection. During this 10-hour period, participants were asked to rest quietly in a semi-recumbent posture and were allowed to drink water only. Additional fasting blood samples were collected in the morning on the following 4 days (24, 48, 72 and 96 hours).

Isolation and measurement of isotopic enrichment of lipoproteins

Lp(a): Lp(a) was isolated by an immunoprecipitation method, which uses the antigen-antibody reaction principle to identify a protein that reacts specifically with an antibody from mixtures of protein. Briefly, 250μL human Lp(a) antibody (Lp(a) Antibody HA R2; Wako Pure Chemical Industries) were immobilized onto 80μL magnetic beads G-proteins (MBGP) by incubation for 1.5h at room temperature. The Lp(a) antibody-MBGP complexes were then incubated overnight with 80μL plasma at 4°C to form plasma Lp(a)-Lp(a) antibody-MBGP complexes. Lp(a) was eluted from the support and Lp(a) was reduced by dithiothreitol in sample buffer to apo(a) and Lp(a)-apoB-100. The proteins were separated by SDS gel electrophoresis and transferred onto PVDF membrane by Western Blotting. The apo(a) and Lp(a)-apoB-100 bands were excised and hydrolyzed with 200μl 6M HCl at 110^{o}C overnight. After evaporation of the acid, the amino acids were derivatized using the oxazolinone method. Isotopic enrichment was determined using gas chromatography-mass spectrometry (GCMS) with selected ion monitoring of samples at a mass to charge ratio (m/z) of 212 and 209 and NCI.

ApoB-containing lipoproteins: The triglyceride-rich lipoprotein (TRL; density 1.006g/mL) fraction was isolated from 3.5mL plasma using ultracentrifugation and subsequent extraction using diethyl ether. TRL samples were then prepared for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a Tris-glycine buffer system and a 5% acrylamide gel. The gel was then electro-blotted onto polyvinylidene fluoride (PVDF) membrane using a 1 x Tris-glycine (10% methanol) buffer. The PVDF membrane was stained with amido black protein stain and destained with 100% methanol. Two bands, apoB-100 (550kDa) and apoB-48 (240kDa), were clearly visible after de-staining. The apoB-100 band was excised from the membrane, hydrolyzed with 200μL 6M HCl at 110°C for 16 hours. Derivatization of leucine to the oxazolinone derivative was described previously [3]. Isotopic enrichment was determined using gas chromatography-mass spectrometry (GCMS) with selected ion monitoring of samples at a mass to charge ratio (m/z) of 212 and 209 and negative ion chemical ionization. Intermediate density lipoprotein (IDL) and LDL were isolated from 3.5mL plasma using sequential ultracentrifugation at densities of 1.019 and 1.063g/mL respectively. IDL and LDL apoB-100

were precipitated by the isopropanol method and isotopic enrichment determined using GCMS, as described above. The apoB concentration in each lipoprotein fraction was determined using precipitation methods and GCMS, with norleucine as the internal standard. The concentration of VLDL apoB-100 was derived following subtraction of TRL apoB-48 concentration from total TRL apoB concentration.

Plasma leucine: Plasma free leucine were isolated by cation-exchange chromatography using AG 50 W-X8 resin (BioRad, Richmond, CA) after removing plasma proteins with 60% perchloric acid. Isotopic enrichment was determined using GCMS as described above.

Biochemical analyses

Laboratory methods for measurements of lipids, lipoproteins and other biochemical measurements were detailed previously [1]. Total plasma proprotein convertase subtilisin-like/kexin type 9 (PCSK9) concentration was measured by enzyme-linked immunosorbent assay (ELISA) using the CircuLex PCSK9 ELISA (CircuLex ELISA, CycLex Co, Nagano, Japan), according to the manufacturer's recommendations. Analysis of plasma Lp(a) concentration was performed by a monoclonal antibody-based ELISA method and considered “the gold standard” method for measuring Lp(a). Inter- and intra-assay coefficients of variation were 3.2% and 3.5%, respectively [4]. Given the contemporary view that there is one apo(a) to apoB-100 molecule per Lp(a) particle [5], the molar concentration of apoB-100 within the Lp(a) fraction was deemed to be identical to that of apo(a). Apo(a) isoforms were measured with a high sensitive agarose gel electrophoresis method as previously described [4]. The apo(a) isoform size in the samples are reported in terms of the relative number of K4 domains. These measurements were carried out at the Northwest Lipid Metabolism and Diabetes Research Laboratories (NWRL), University of Washington, Seattle, WA.

Kinetic analyses

Lp(a): The analyses of the data in this study started with the assumption that this was a postprandial study and as such, the concentrations of lipids and apolipoproteins may change over the course of the study. While this was true for apoB-100 containing lipoproteins, no significant change to plasma Lp(a) concentration was observed over the course of the study (Supplemental Figure IV). The data was therefore modelled assuming constant Lp(a) concentrations (Supplemental Figure V). The leucine compartment model consists of a 4-compartment subsystem (compartments 1-4) that describes plasma leucine kinetics. This subsystem is connected to two intrahepatic delay compartments (compartments 5 and 8) that account for the time required for leucine tracer to be incorporated into Lp(a)-apoB-100 and apo(a) associated with Lp(a), and secreted into plasma. The kinetics of Lp(a)-apoB-100 and apo(a) are described by two compartments (Lp(a)-apoB-100: compartments 6 and 7, apo(a): compartments 9 and 10), where one represents a slowly turning-over pool of the lipoproteins. The Lp(a)-apoB-100 and apo(a) concentration compartment model consists of compartments 11 and 14, delay compartments that represent 4 compartments in series, and compartments 12 and 13 represent Lp(a)-apoB-100, and compartments 15 and 16 represent Lp(a)-apo(a).

The fractional catabolic rate (FCR; pools/day) of apoB-100 and apo(a) associated with Lp(a) were derived from the model parameters giving the best fit. The corresponding basal production rates (PR; nmol/kg/day) were calculated as the product of FCR and pool size, which equals the plasma concentration multiplied by plasma volume; plasma volume was estimated as 4.5% of body weight.

ApoB-100 containing lipoproteins: A non-steady state compartment model, based on previously published models [6], was developed using the SAAM II program (The Epsilon Group, VA) to account for changes in plasma concentrations of apoB-100 containing lipoproteins following consumption of the test meal (Supplemental Figure VI). The tracer to tracee ratio (TTR) of D_3-leucine was used for the kinetic analyses. Two separate, but linked, models were developed, one to account for the leucine tracer data including plasma leucine and VLDL, IDL and LDL apoB-100 leucine enrichment, and the other model for VLDL, IDL and LDL apoB-100 concentration data. The leucine compartment model consists of a 4-compartment subsystem (compartments 1-4) that describes plasma leucine kinetics. This subsystem is connected to an intrahepatic delay compartment (compartment 5) that accounts for the time required for leucine tracer to be incorporated into apoB-100 and secreted into plasma. The model provides direct secretion of apoB-100 into VLDL, IDL and LDL fractions. The kinetics of each fraction are described by one or two compartments (VLDL: compartments 6, IDL: compartments 8 and 9; LDL: compartments 10 and 11), where one represents a slowly turning-over pool of the particles. For each fraction, the lipoproteins may be converted [VLDL to IDL; IDL to LDL] stepwise down the delipidation cascade or removed directly from plasma. The VLDL, IDL and LDL apoB-100 concentration compartment model consists of compartment 12, a delay compartment, compartments 13 and 14 representing plasma VLDL, compartments 15 and 16 representing plasma IDL, and compartments 17 and 18 representing plasma LDL particles.

In this model, the initial conditions reflect basal apoB-100 concentrations i.e. in the fasting state. The concentration of apoB-100 in VLDL (compartments 13), IDL (compartments 15 and 16) and LDL (compartments 17 and 18) can be estimated assuming steady-state conditions before consumption of the test meal. The test meal is represented as an impulse input into compartment 12, which acts as a delay prior to observing increased concentrations of the apoB-100 in plasma. This single input into compartment 12 was an adjustable parameter, which reflects the number of new apoB-100 particles secreted into plasma on top of basal apoB-100 secretion rate. During model development, it was observed that the change in IDL and LDL apoB-100 concentration in response to the test meal was small to negligible. It is possible that the response to the test meal in these fractions is significantly diminished owing to maximally upregulated LDL receptor expression with rosuvastatin treatment. Consequently, the input parameter represents the number of new VLDL particles secreted into plasma on top of basal VLDL apoB-100 secretion rate. Whether the test meal increased the corresponding input into the leucine tracer model, [i.e. *de novo* synthesis of isotopically labelled apoB-100] was also explored. The present data does not support the presence of such an increased input. That there was no transient increase in the incorporation of tracer into apoB-100 remains consistent with current understanding of the apoB-100 synthesis and secretion pathways. First, it remains possible that apoB-100 synthesis remains constant irrespective of the oral fat load. This may relate to these pathways being highly regulated. Second, the apparent increase in apoB-100 secretion may be a function of decreased post-translational degradation of apoB-100, presumably “rescued” by lipids from the fat load. Therefore, there was no need for the system to invoke an additional mechanism whereby a fat load results in increased incorporation of tracer into apoB-100. It is, therefore, possible that the transient increase in postprandial VLDL apoB-100 concentration reflects the appearance of *de novo* secretion of isotopically labelled apoB-100. We however cannot exclude that mechanisms in addition to that described above could account for the *de novo* secretion of isotopically labelled apoB-100, particularly with ERN treatment.

The catabolism of apoB-100 particles from plasma was assumed to be constant over the period of the kinetic study. During model development, the potential for the catabolism of apoB-100 particles to change as a function of plasma triglyceride concentrations was explored. In

particular, it was hypothesized that as triglyceride (TG) concentrations increased, lipoprotein lipase mediated lipolysis of VLDL particles may be reduced and hence, lower VLDL particle catabolism. The rate constant k(0, 6) [and k(0, 13)] was a function of triglyceride concentration such that k(0, 6) = Vmax/(S + TG), where Vmax and S are maximal rate velocity and sensitivity to triglyceride concentrations, respectively. Assuming a constant rate of VLDL apoB-100 production, this model failed to describe the VLDL apoB-100 tracer and concentration data. That VLDL apoB-100 FCR did not change as a function of triglyceride concentration is consistent with the rate of increase in VLDL apoB-100 exceeding that of plasma triglyceride. The measurement and use of VLDL triglyceride concentration may better define the association between VLDL particle catabolism and triglyceride concentration.

The fit of the model to the tracer-to-tracee ratio (TTR) for VLDL, IDL and LDL following administration of D_3-leucine, and to the corresponding apoB-100 concentrations in response to the oral fat load over the 10-hour postprandial period averaged over all participants (mean ± SEM) at the end of each intervention period is provided in Supplementary Figures VII and VIII, respectively.

The VLDL-, IDL- and LDL- apoB-100 fractional catabolic rates (FCR; pools/day) were derived from the model parameters giving the best fit. The corresponding basal production rates (PR; mg/kg/day) were calculated as the product of FCR and pool size, which equals the plasma concentration multiplied by plasma volume; plasma volume was estimated as 4.5% of body weight. The number of VLDL apoB-100 secreted in response to the test meal on top of the basal VLDL apoB-100 secretion (mg/kg) was calculated as the product of the input (increase in VLDL apoB-100 concentration) and plasma volume. Total VLDL production (mg/kg/10h) was defined as the sum of basal and postprandial VLDL production over the 10-hour postprandial period.

Statistics

Data are presented as mean and SEM unless stated otherwise. Data at the end of the treatment periods were compared using a mixed-effect model (SAS Proc Mixed; SAS Institute, USA), which also tested for carry-over, treatment sequence and time-dependent effects. There were no significant carry-over, treatment sequence or time-dependent effects. Data between individuals with low [<42nmol/L, n=5] and high [≥42nmol/L, n=5] plasma Lp(a), with low defined as below and high defined as above the median [42nmol/L], were compared using independent Student's *t*-test. Data within individuals with low [<42nmol/L, n=5] and high [≥42nmol/L, n=5] plasma Lp(a), with low defined as below and high defined as above the median [42nmol/L], were compared using paired Student's *t*-test. The p-values are reported, with statistical significance set at the 5% level.

Sample size calculation

The sample size calculations were based on the randomized, placebo-controlled crossover design. ***Primary endpoint:*** Previous studies support that statin + extended release niacin compared with statin treatment lowers Lp(a) concentrations by 20% [7,8]. We hypothesize that decreased Lp(a) concentrations are due to decreased Lp(a) PR. Given an SD of PR of 20%, based on the many kinetic studies performed by this group, together with a minimal difference of Lp(a) PR of 19%, a sample size of 10 will have 80% power to detect a difference between rosuvastatin + extended release niacin and rosuvastatin treatments at two-sided α=0.05. ***Secondary endpoint:*** Previous studies reported that niacin lowers VLDL apoB-100 concentration by 44% [9,10]. We hypothesize that decreased VLDL apoB-100 concentrations are due to decreased VLDL apoB-100 PR. Given an SD of PR of 20%, based on the many kinetic

studies performed by this group, together with a minimal difference of VLDL apoB-100 PR of 44%, a sample size of 11 will have >80% power to detect a difference between rosuvastatin + extended release niacin and rosuvastatin treatments at two-sided α=0.05.

Supplemental References

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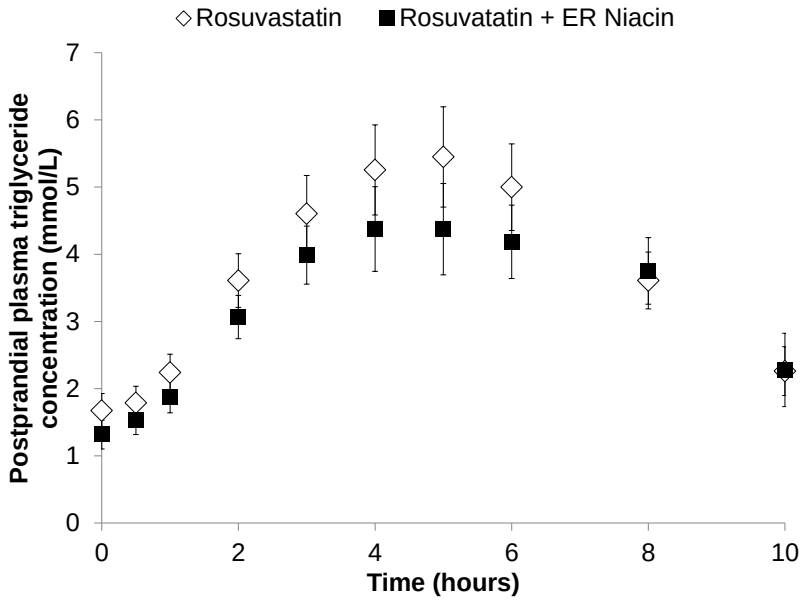
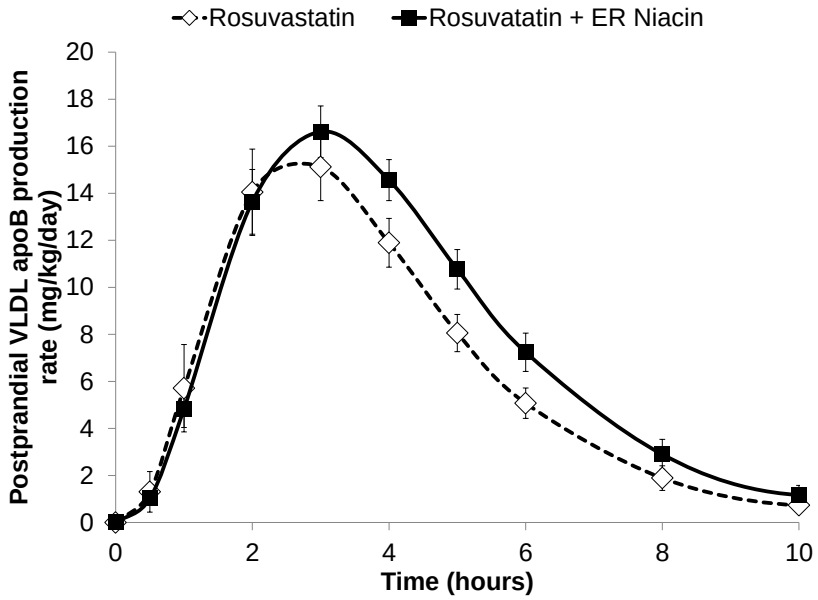
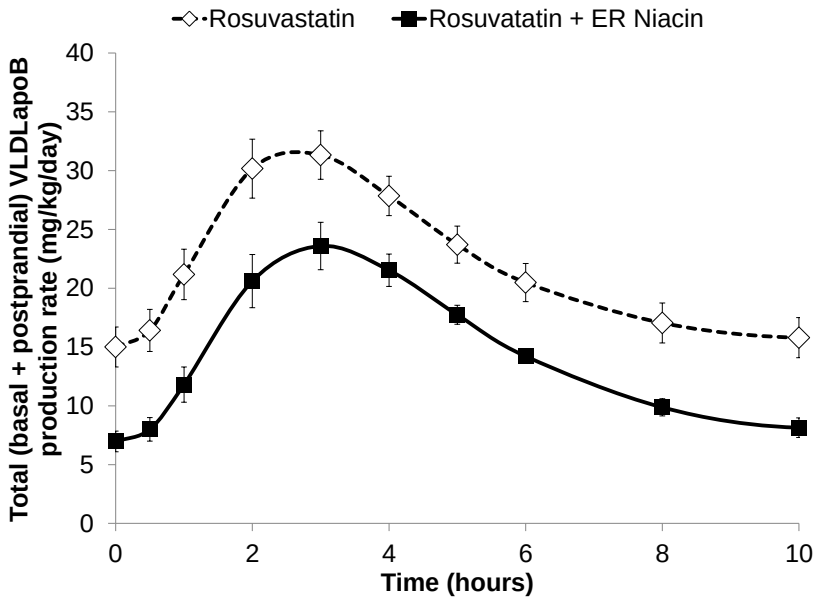
Supplemental Figure I: Postprandial plasma concentrations of plasma triglyceride from 0 to 10 hours in statin-treated men with type 2 diabetes on rosuvastatin and rosuvastatin + extended release niacin. Mean and SEM shown

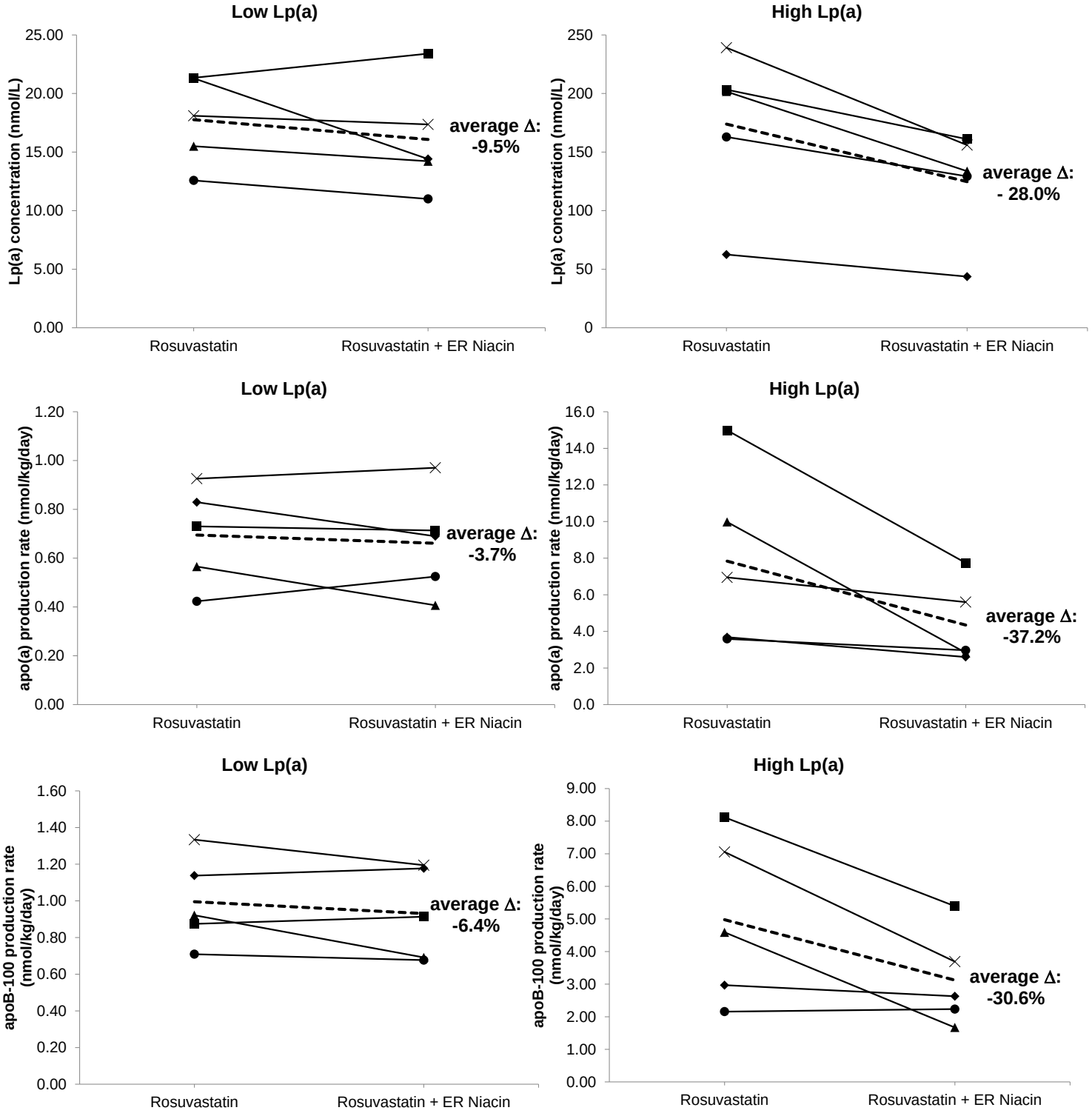


Supplemental Figure II: Postprandial and total (basal + postprandial) VLDL apoB-100 production rates from 0 to 10 hours in statin-treated men with type 2 diabetes on rosuvastatin and rosuvastatin + extended release niacin. Mean and SEM shown

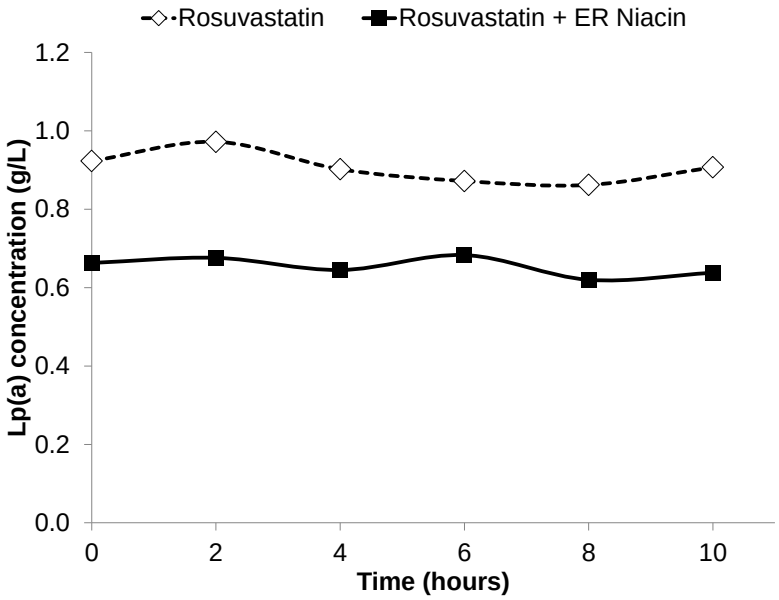




Supplemental Figure III: Changes in plasma molar concentration of Lp(a), and the production rates of its protein components, apo(a) and Lp(a)-apoB-100, with rosuvastatin and rosuvastatin + extended-release niacin, grouped by low [<42nmol/L] or high [≥42nmol/L] plasma Lp(a)

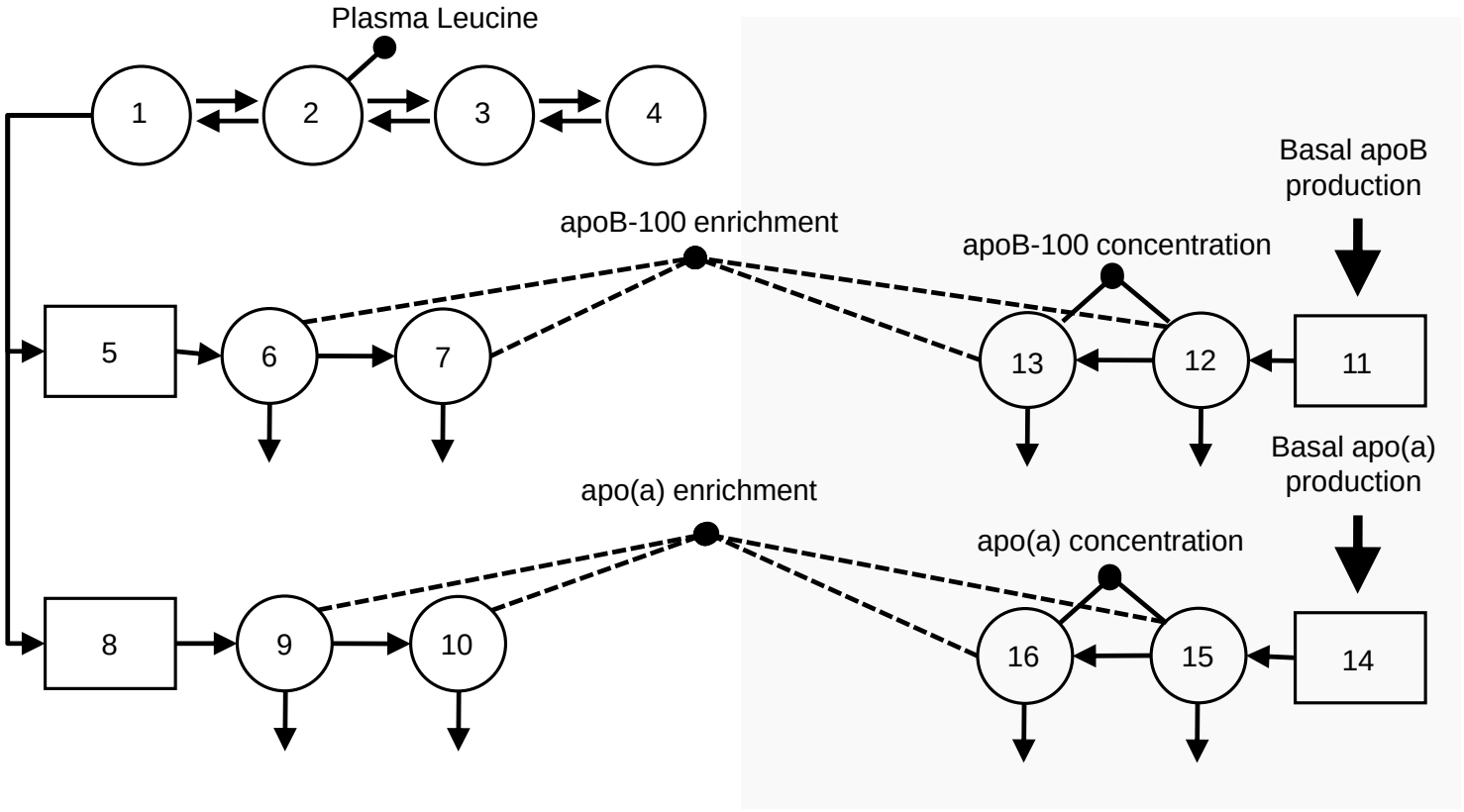


Supplemental Figure IV: Plasma Lp(a) concentration* over the study duration in a representative participant on rosuvastatin and rosuvastatin + extended release niacin



*Plasma Lp(a) concentration was measured in plasma samples by an automated latex enhanced immunoassay (QUANTIA Lp(a) assay and standard) using the Abbott Architect ci8200 platform (Abbott Laboratories, Abbott Park, IL).

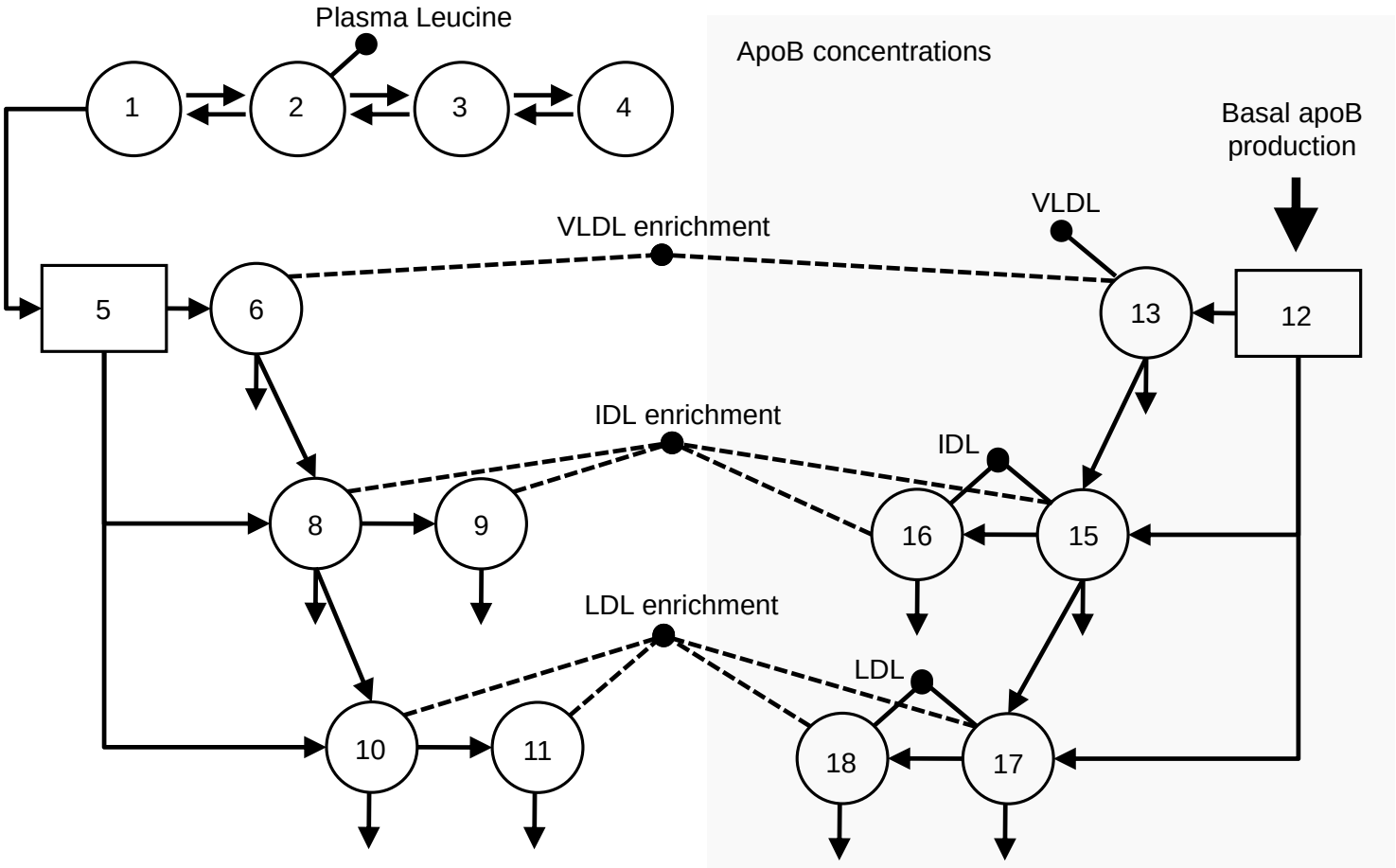
Supplemental Figure V: Multi-compartmental model describing Lp(a) kinetics



Constraining equations, using data averaged across all participants

Tracer	Tracee	Rate Constant (pools/hour)	95% Confidence Interval	
k(0,6)	k(0,12)	0.0630	0.0474	0.0786
k(0,7)	k(0,13)	0.0052	0.0009	0.0095
k(0,9)	k(0,15)	0.2907	0.1514	0.4300
k(0,10)	k(0,16)	0.0158	0.0032	0.0284
k(7,6)	k(13,12)	0.0083	0.0061	0.0105
k(10,9)	k(16,15)	0.0436	0.0116	0.0755

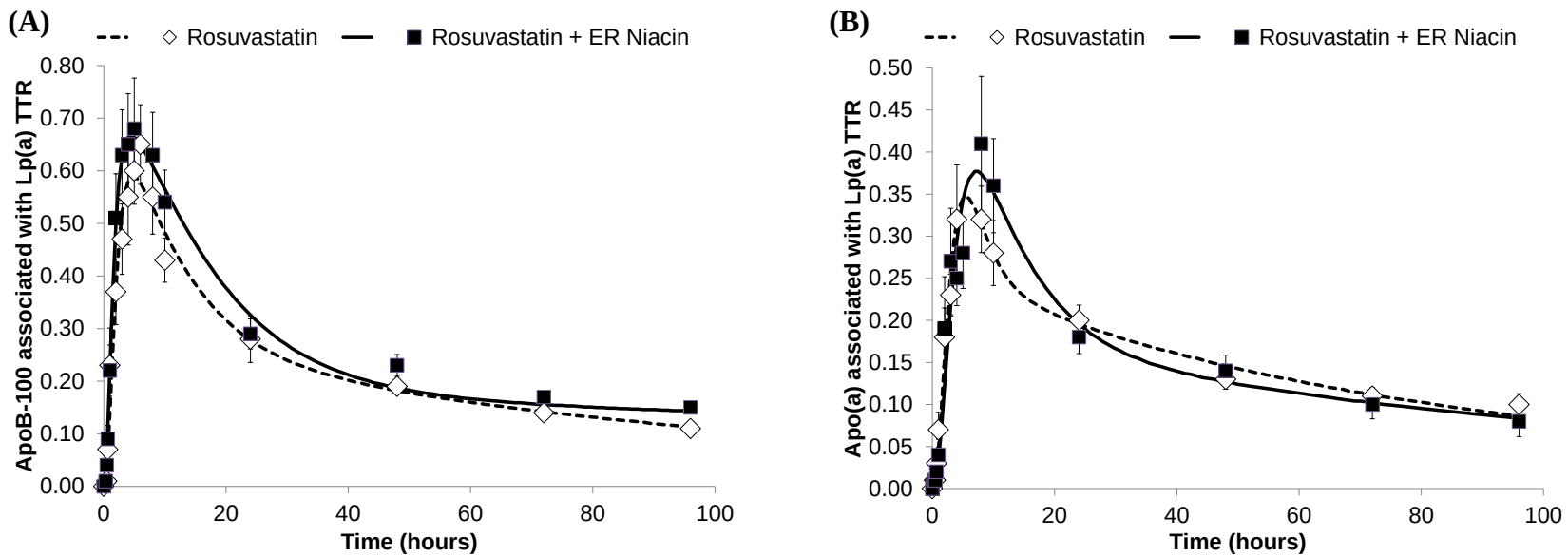
Supplemental Figure VI: Multi-compartmental model describing apoB-100-containing lipoprotein kinetics



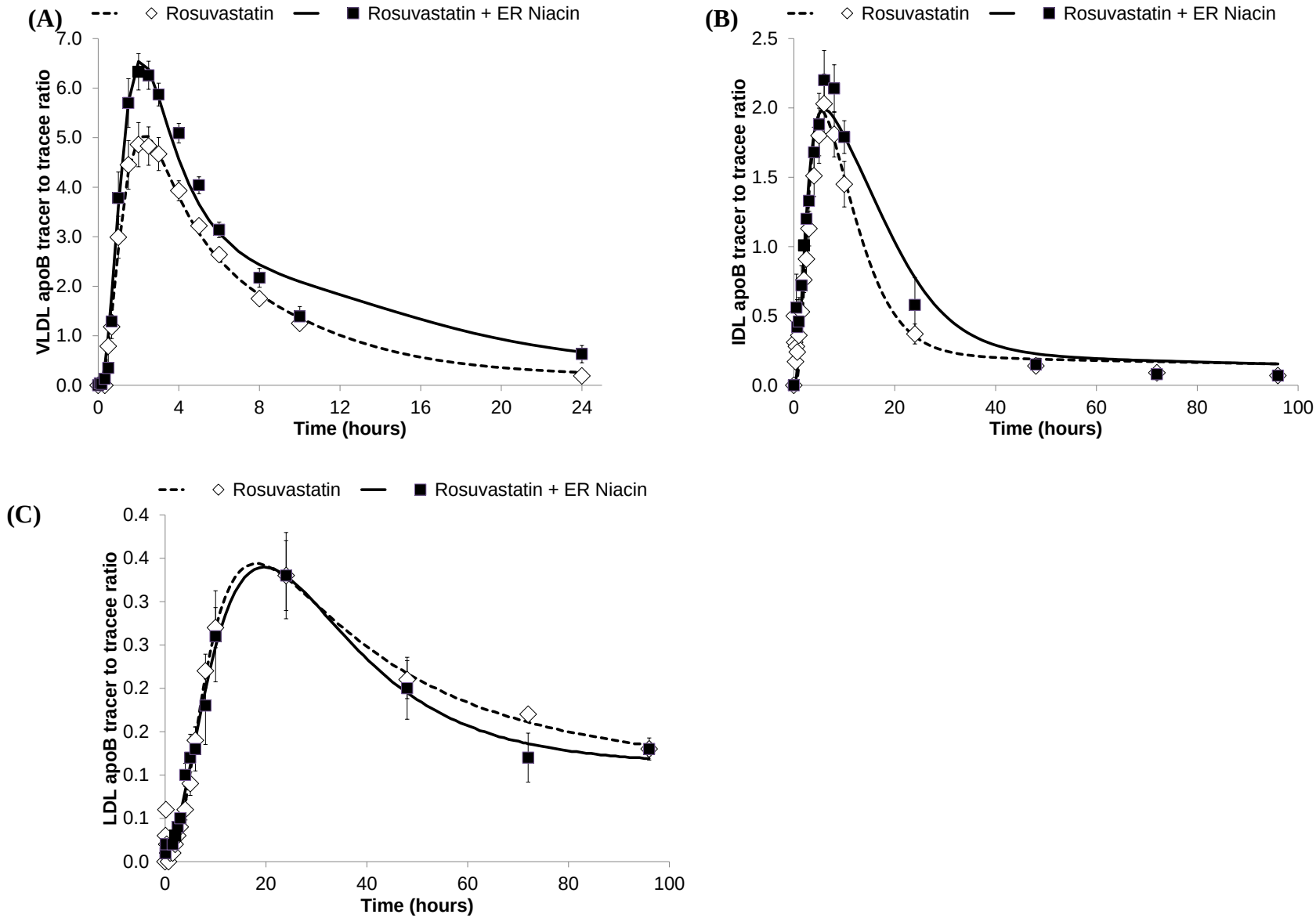
Constraining equations, using data averaged across all participants

Tracer	Tracee	Rate Constant (pools/hour)	95% Confidence Interval	
k(0,6)	k(0,13)	0.1051	0.0388	0.1712
k(0,8)	k(0,15)			
k(0,9)	k(0,16)	0.0474	0.0014	0.0081
k(0,10)	k(0,17)	0.0678	0.0514	0.0841
k(0,11)	k(0,18)	0.0010	0.0007	0.0013
k(9,8)	k(16,15)	0.0034	0.0005	0.0063
k(11,10)	k(18,17)	0.0070	0.0041	0.0098
k(8,6)	k(15,13)	0.1510	0.1290	0.1730
k(10,8)	k(17,15)	0.1234	0.1018	0.1449

Supplementary Figure VII: Fit of the model to the tracer to tracee ratio for Lp(a)-apoB-100 (A) and apo(a) (B) with D_3-leucine averaged across all participants on rosuvastatin and rosuvastatin + extended release niacin



Supplementary Figure VIII: Fit of the model to the tracer to tracee ratio for VLDL (A), IDL (B) and LDL (C) apoB-100 with D_3-leucine averaged across all participants on rosuvastatin and rosuvastatin + extended release niacin



Supplementary Figure IX: Fit of the model to the VLDL (A), IDL (B) and LDL (C) apoB-100 concentrations averaged across all participants on rosuvastatin and rosuvastatin + extended release niacin