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ORIGINAL ARTICLE

WILEY

Effects of liraglutide on the metabolism of triglyceride-rich lipoproteins in type 2 diabetes

Marja-Riitta Taskinen PhD¹ | Elias Björnson MS² | Niina Matikainen PhD^{1,3}  |
 Sanni Söderlund PhD^{1,3} | Kirsi H. Pietiläinen PhD^{1,3} | Mari Ainola PhD¹ |
 Antti Hakkarainen MS^{4,5} | Nina Lundbom PhD⁴ | Johannes Fuchs PhD⁶ |
 Annika Thorsell PhD⁶ | Linda Andersson PhD² | Martin Adiels PhD² |
 Chris J. Packard PhD⁷ | Jan Borén PhD^{2,8} 

¹Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

²Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden

³Endocrinology, Abdominal Center, Helsinki University Hospital, Helsinki, Finland

⁴HUS Medical Imaging Center, Radiology, Helsinki University Hospital, University of Helsinki, Finland

⁵Department of Neuroscience and Biomedical Engineering, Aalto University School of Science, Espoo, Finland

⁶Proteomics Core Facility at Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden

⁷Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK

⁸Wallenberg Laboratory/Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden

Correspondence

Jan Borén, MD, Wallenberg Laboratory, Sahlgrenska University Hospital, SE-41345 Gothenburg, Sweden.
 Email: jan.boren@wlab.gu.se

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Abstract

Aim: To elucidate the impact of liraglutide on the kinetics of apolipoprotein (apo) B48- and apoB100-containing triglyceride-rich lipoproteins in subjects with type 2 diabetes (T2D) after a single fat-rich meal.

Materials and Methods: Subjects with T2D were included in a study to investigate postprandial apoB48 and apoB100 metabolism before and after 16 weeks on 1.8 mg/day liraglutide ($n = 14$) or placebo ($n = 4$). Stable isotope tracer and compartmental modelling techniques were used to determine the impact of liraglutide on chylomicron and very low-density lipoprotein (VLDL) production and clearance after a single fat-rich meal.

Results: Liraglutide reduced apoB48 synthesis in chylomicrons by 60% ($p < .0001$) and increased the triglyceride/apoB48 ratio (i.e. the size) of chylomicrons ($p < .001$). Direct clearance of chylomicrons, a quantitatively significant pathway pretreatment, decreased by 90% on liraglutide ($p < .001$). Liraglutide also reduced VLDL₁-triglyceride secretion ($p = .017$) in parallel with reduced liver fat. Chylomicron-apoB48 production and particle size were related to insulin sensitivity ($p = .015$ and $p < .001$, respectively), but these associations were perturbed by liraglutide.

Conclusions: In a physiologically relevant setting that mirrored regular feeding in subjects with T2D, liraglutide promoted potentially beneficial changes on postprandial apoB48 metabolism. Using our data in an integrated metabolic model, we describe how the action of liraglutide in T2D on chylomicron and VLDL kinetics could lead to decreased generation of remnant lipoproteins.

KEYWORDS

dyslipidaemia, GLP-1 analogue, type 2 diabetes

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1 | INTRODUCTION

Increasing evidence indicates that triglyceride-rich lipoproteins (TRLs) and their remnants are causally associated to cardiovascular disease (CVD).¹ Given that these lipoproteins are often elevated in type 2 diabetes (T2D), it is probable that they contribute to the high residual CVD risk seen in individuals with T2D even when on optimal LDL-lowering therapies.^{2–4}

TRLs comprise two major classes: apolipoprotein (apo) B48-containing chylomicrons, which are produced by the intestine during lipid absorption; and apoB100-containing very low-density lipoproteins (VLDL), which are synthesized continuously by the liver. Gaining insights into the metabolism of apoB48 is challenging because of its low plasma concentration and transient appearance.⁵ Recently, we developed techniques to overcome these issues and found that apoB48 is secreted into the VLDL as well as the chylomicron density ranges in both the fasting state and in response to a fat-rich meal.^{6,7} Further, we found that apoB48-containing TRLs and their remnants accumulate in the circulation of subjects with raised plasma triglycerides, have a long residence time, and can account for around 25% of particles in the VLDL density range.^{6,7} These observations have implications for understanding the increased CVD risk in subjects with high plasma triglycerides.⁸

Use of glucagon-like peptide-1 (GLP-1) receptor agonists to treat T2D increased rapidly following reports of their beneficial effects on CVD.^{9–11} They are known to influence triglyceride metabolism,¹² to decrease the production¹³ and increase the clearance rate of apoB48,¹⁴ and to increase the clearance of chylomicron-triglyceride.¹⁵ However, the physiological relevance of earlier studies may be questioned as food was given as either an infusion or micro-meals to induce a quasi-steady-state condition in apoB48 plasma concentration.⁷ The impact of GLP-1 receptor agonists on chylomicron and apoB48 metabolism in a more normal dietary setting remains unclear.

We have previously shown that liraglutide promotes reductions in postprandial plasma triglyceride responses, remnant-like particle (RLP)-cholesterol, TRL-cholesterol, and apoC-III in subjects with T2D given a standard fat-rich meal.^{16,17} The aim of the present study was to provide an integrated view of the actions of liraglutide on the kinetics of apoB48- and apoB100-containing TRLs in subjects with T2D using our newly developed protocol for non-steady-state kinetic studies.^{6,7}

2 | RESEARCH DESIGN AND METHODS

The study was approved by the ethics committee of Helsinki University and Helsinki University Hospital and the National Agency of Medicines, Helsinki, Finland (EudraCT 2013-005075-40) (Clinical Trials NCT 02765399), and performed in accordance with the Declaration of Helsinki and the European Medicines Agency Note for guidance on Good Clinical Practice. Written informed consent was obtained from all subjects.

2.1 | Study subjects

Subjects were recruited to a study of the effects of liraglutide on glycaemia, ectopic fat deposits and cardiometabolic risk factors; findings on metabolic status and plasma lipid and lipoproteins have been published.¹⁶ In this current report, which explores the mechanistic basis for the effects of liraglutide, we included 14 of the 15 subjects randomized to receive liraglutide and four of the seven subjects randomized to receive placebo (the remaining four subjects were given a fat-rich mixed meal and included in the earlier report¹⁶ but did not perform the kinetic protocol with stable isotopes). The study had a single-blind design, and clinical characteristics of the participants have previously been presented.¹⁶

Inclusion criteria were T2D, body mass index (BMI) of 27–40 kg/m², waist measurements of more than 88 cm in women and more than 92 cm in men, age 30–75 years, HbA1c of 42–75 mmol/mol (6%–9%), plasma triglyceride 1.0–4.0 mmol/L, and LDL cholesterol of less than 4.5 mmol/L at the screening visit. For the exclusion criteria, refer to Appendix S1.

2.2 | Study treatment and visits

If participants were not on metformin (2.0 g/day) and/or statin therapy (simvastatin 20 mg/day), these treatments were initiated and continued for a 4-week run-in period. To avoid gastrointestinal side effects, the liraglutide dose was increased stepwise starting at 0.6 mg/day (administered subcutaneously) for 1 week, followed by 1.2 mg/day for 1 week and thereafter 1.8 mg/day for 16 weeks.

All subjects underwent, on separate occasions, a kinetic study, determination of liver and intra-abdominal fat, and measurement of heparin-releasable lipases. These investigations were repeated after 16 weeks on liraglutide or placebo injections. Participants were instructed to avoid alcohol and strenuous exercise within 72 h of each study visit. Because an earlier report indicated that subjects on liraglutide would experience a weight loss of about 3 kg,¹⁸ a weight-loss programme was implemented for subjects receiving placebo to match the weight loss in the liraglutide group.¹⁶

2.3 | Kinetic study

The kinetic study was performed as previously described.^{6,7} On the evening of the first visit, ²H₂O (2 g/kg) was given to assess de novo lipogenesis of fatty acids in VLDL-triglycerides. After a 12-h overnight fast (at 8:00 AM, 0-h time point), subjects received a bolus injection of 500 mg ²H₅-glycerol (1,1,2,3,3-D5, Euriso-Top) and deuterated leucine (5,5,5-D3 Euriso-Top; 7 mg/kg body weight) to determine the kinetics of triglycerides, apoB100 and apoB48 in VLDL, and apoB48 in chylomicrons. At the 2-h time point, subjects were given a fat-rich mixed meal (Appendix S1). Blood sampling was started at the 0-h time point and continued at frequent intervals until 10 h after tracer administration, when a dinner was served. The subjects returned the following morning to give blood at 24-h post-tracer administration.

2.4 | Quantification of apoB48, tracer enrichment in apolipoproteins and triglycerides, multicompartmental modelling and variable estimation

The protocols and the compartmental model structure have been described in detail.^{6,7} Please refer to Appendix S1.

2.5 | Determination of intra-abdominal fat depots

Proton magnetic resonance spectroscopy was performed with a 1.5-T whole-body device to quantify liver fat content.¹⁹ Magnetic resonance imaging was used to determine subcutaneous abdominal and intra-abdominal fat.²⁰ Subjects were advised to fast for 4 h before the imaging. All imaging results were analysed by AH.

2.6 | Lipoprotein isolation, biochemical analyses and measurement of postheparin lipoprotein and hepatic lipase activity

Chylomicrons, large VLDL₁ particles and smaller VLDL₂ particles were isolated by density gradient ultracentrifugation as described.²¹ TRL-cholesterol (TRL-C) (i.e. the cholesterol in the aggregate of chylomicron remnants, VLDL and IDL) and remnant-lipoprotein cholesterol (RLP-C) (i.e. cholesterol in lipoproteins not binding to anti-apoAI and anti-apoB100) were analysed using automated direct assays (Denka Seiken, Tokyo, Japan). Refer to Appendix S1 for further biochemical analyses and measurements of postheparin lipoprotein and hepatic lipase activity.

2.7 | Statistics

R (version 3.6.3) was used for all analyses. Data are presented as mean ± SE. Area under the curve (AUC) was calculated using the trapezoidal rule using the function *trapz* from the package *pracma*. Correlation coefficients were calculated using Spearman's rank test. Repeated measures ANOVA was performed using the function *lmer* from package *lme4*. The significance of associations between variables across both visits and a post hoc test of between-visit differences was calculated using ANCOVA using the *ancova* function in the package *jmv*. *p* values were calculated using the Wilcoxon signed-rank test; *p*-values of less than .05 were considered statistically significant.

3 | RESULTS

3.1 | Effect of liraglutide on cardiometabolic risk factors and fasting plasma lipoproteins

The effects of liraglutide on glycaemia, ectopic fat deposits, cardiometabolic risk factors¹⁶ and apoC-III¹⁷ in the subjects included in this current report have been reported. Liraglutide promoted an

improvement in glycaemic control, a decrease in liver fat content but no change in fasting plasma triglyceride levels (which were in the normal range before treatment), significant reductions in fasting plasma apoB, LDL-cholesterol and TRL-cholesterol, and a non-significant reduction in fasting RLP-cholesterol (Table 1). No change in liraglutide was seen in either lipoprotein lipase (LpL) activity, or LpL mass (note that heparin-released LpL may not be reflective of the activity of the enzyme at its site of action on capillary endothelium²²). ApoC-III was reduced whereas the plasma concentration of ANGPTL-3 was increased by 23% in liraglutide (Table 1).

3.2 | Impact of liraglutide on triglyceride and apoB48 kinetics in chylomicrons and VLDL

The effects of liraglutide on the triglyceride content in chylomicrons, VLDL₁ and VLDL₂ in response to a standard fat-rich meal in these subjects with T2D have been published,¹⁶ and are reported again here to facilitate interpretation of the apoB kinetic data. Liraglutide treatment reduced the AUC (postprandial response) for plasma triglycerides, chylomicron- and VLDL₁-triglycerides, but not for VLDL₂-triglycerides (Figure 1A-C, Table S1). Liraglutide also reduced the AUC for RLP-cholesterol and TRL-cholesterol (Table S1).

Liraglutide treatment lowered the total plasma apoB48 AUC by 17% (*p* = .042), which was attributable to the 54% reduction in AUC for chylomicron-apoB48 (*p* < .001); the postprandial changes in VLDL₁- and VLDL₂-apoB48 were not altered by therapy (Figure 1D-F, Table S1). Of note, liraglutide induced a disproportionately greater reduction in the chylomicron-apoB48 AUC (Figure 1D) than in the chylomicron-triglyceride AUC (Figure 1A). In addition, the chylomicron-triglyceride/apoB48 molar ratio (a surrogate measure of the triglyceride content per chylomicron as each particle has one apoB48 moiety) was significantly higher across the postprandial period following liraglutide treatment (Figure S1A). On average, chylomicrons contained about 30,000 more triglyceride molecules per particle, and were therefore larger, after liraglutide treatment. The placebo group achieved an average weight loss similar to that in the liraglutide group (mean ± SD of 3.5 ± 3.0 vs. 2.5 ± 2.0 kg, respectively; *p* = .43), but showed no change in the chylomicron triglyceride/apoB48 ratio (mean ± SD of 55,500 ± 27,400 vs. 64,300 ± 26,400 triglyceride molecules per apoB48 'before' vs. 'on placebo', respectively; *p* = 1.0).

Liraglutide treatment reduced the total production of apoB48-containing lipoproteins by 33% (*p* = .0023), which was explained by a 60% reduction in postprandial chylomicron-apoB48 secretion (*p* < .001); the postprandial VLDL₁- and VLDL₂-apoB48 production rates were not altered by therapy (Table 2). The apoB48 tracer enrichment curves in the chylomicron, VLDL₁ and VLDL₂ fractions were virtually identical before and on liraglutide (Figure 1G-I), indicating little change in catabolic processes in response to liraglutide. Indeed, the overall fractional catabolic rates (FCRs) for apoB48 in chylomicrons, VLDL₁ and VLDL₂ were unaltered by therapy (Table 2). However, liraglutide reduced the fractional rate of direct

TABLE 1 Baseline characteristics at fasting and their corresponding changes during liraglutide treatment in subjects with type 2 diabetes

	Before liraglutide (n = 14)	On liraglutide (n = 14)	Change	p-value
BMI (kg/m ²)	32 ± 3.4	31.4 ± 3.4	-0.6 ± 0.5 (-2%)	.0023
Body weight (kg)	99.1 ± 11.2	97.1 ± 10.9	-1.9 ± 1.6 (-2%)	.0039
HbA1c (%)	7.1 ± 1.0	6.5 ± 0.7	-0.6 ± 0.7 (-7%)	.0096
Glucose (mmol/L)	8.4 ± 2.5	6.5 ± 1.2	-2.0 ± 1.4 (-22%)	.0017
Insulin (μU/mL)	14.1 ± 4.9	15.2 ± 4.4	1.0 ± 3.9 (+13%)	.30
Matsuda index	2.6 ± 1.2	3.1 ± 1.1	0.7 ± 0.9 (+36%)	.031
BOHB (mg/dL)	1.1 ± 0.7	0.9 ± 0.5	-0.2 ± 0.5 (-13%)	.24
Liver fat (%)	15.3 ± 7.3	11.4 ± 5.9	-3.9 ± 2.9 (-27%)	<.001
VAT area (cm ²)	3460 ± 949	3300 ± 940	-157 ± 326 (-4%)	.09
SAT area (cm ²)	4160 ± 1070	3950 ± 1060	-213 ± 196 (-5%)	.004
Total chol. (mmol/L)	3.9 ± 0.7	3.8 ± 0.7	-0.2 ± 0.4 (-4%)	.12
LDL-chol. (mmol/L)	2.0 ± 0.7	1.8 ± 0.6	-0.2 ± 0.3 (-9%)	.01
HDL-chol. (mmol/L)	1.3 ± 0.4	1.3 ± 0.4	-0.01 ± 0.2 (-0.3%)	.6
RLP-chol. (mg/dL)	7.6 ± 4.8	6.3 ± 4.7	-1.3 ± 3.1 (-12%)	.19
TRL-chol. (mg/dL)	21.2 ± 9.4	18.2 ± 7.7	-3.0 ± 5.8 (-8%)	.049
TG (mmol/L)	1.7 ± 0.6	1.4 ± 0.6	-0.2 ± 0.4 (-11%)	.10
TG VLDL ₁ (mmol/L)	0.7 ± 0.4	0.6 ± 0.4	-0.1 ± 0.3 (-10%)	.33
TG VLDL ₂ (mmol/L)	0.3 ± 0.1	0.3 ± 0.1	-0.01 ± 0.06 (+1%)	.67
ApoB (mmol/dL)	68.7 ± 15.3	64.5 ± 12.8	-4.2 ± 6.2 (-6%)	.024
ApoB100 in VLDL ₁ (mg/L)	23.5 ± 13.4	21.3 ± 14.2	-2.2 ± 13 (+2%)	.67
ApoB100 in VLDL ₂ (mg/L)	39 ± 15	39.8 ± 16.5	0.8 ± 11 (+6%)	.72
ApoE (mg/dL)	3.6 ± 1.1	3.3 ± 1.2	-0.3 ± 0.7 (-7%)	.10
ApoC-III (mg/dL)	11.7 ± 4.3	10 ± 3.8	-3 ± 3 (-8%)	.035
ANGPTL-3 (ng/mL)	231 ± 53.4	270 ± 89.1	51 ± 71 (+23%)	.033
DNL (μmol/L)	15.1 ± 7.6	19.1 ± 13	4.4 ± 9.9 (+36%)	.17
LPL-activity (mU/mL)	160 ± 49.6	158 ± 55.6	-2 ± 26 (-1%)	.97
HL-activity (mU/mL)	292 ± 168	270 ± 137	-22 ± 53 (-2%)	.30
LPL mass (ng/mL)	413 ± 100	426 ± 144	13 ± 76 (+3%)	.50

Abbreviations: ANGPTL-3, angiopoietin-like protein 3; Apo, apolipoprotein; BMI, body mass index; BOHB, beta-hydroxybutyrate; DNL, de novo lipogenesis; HL, hepatic lipase; LPL, lipoprotein lipase; RLP, remnant-like particle; SAT, subcutaneous adipose tissue; TG, triglyceride; TRL, triglyceride-rich lipoprotein; VAT, visceral adipose tissue.

Note: Data in this table have been reported earlier (in table S1 in Adiels et al.¹⁷) but some values differ because of a 2-h difference in sampling time. Data are shown as mean ± SD; p-values have been calculated using the Wilcoxon signed-rank test.

clearance of apoB48 in chylomicrons by more than 90% ($p < .001$) (Table 2), and there was a compensating increase in the fractional transfer rate (FTR) of apoB48 from chylomicrons to VLDL₁ ($p < .001$) (Table 2). This metabolic perturbation resulted in a substantial decrease in the amount of chylomicron-apoB48 cleared directly from the circulation (Table 2). Because of the large reduction in the chylomicron-apoB48 pool in response to liraglutide, the amount of apoB48 transferred from chylomicrons into the VLDL₁ density range was reduced ($p = .017$), despite the increased FTR from chylomicrons to VLDL₁ (Table 2).

By applying our previously developed metabolic model^{6,7} to the triglyceride and apoB48 postprandial data shown in Figures 1 and S2, we estimated that the FCR for chylomicron-triglyceride increased by 40% on liraglutide ($p = .011$) (Table 2). Given that the

chylomicron-apoB48 FCR was not altered by liraglutide (Table 2), this change was probably attributable to an increased lipolytic rate for chylomicrons.

3.3 | Impact of liraglutide on triglyceride and apoB100 kinetics in VLDL

In contrast to the substantial effects of liraglutide on apoB48 metabolism, there was only a modest impact of liraglutide on VLDL₁- and VLDL₂-apoB100 kinetics over the postprandial period (Figure S2). We observed a small but non-significant reduction in the AUC for VLDL₁-apoB100 (11%, $p = .33$), which was similar to the fall in the AUC for VLDL₁-triglycerides (Table S1). Liraglutide did not affect the VLDL₂-

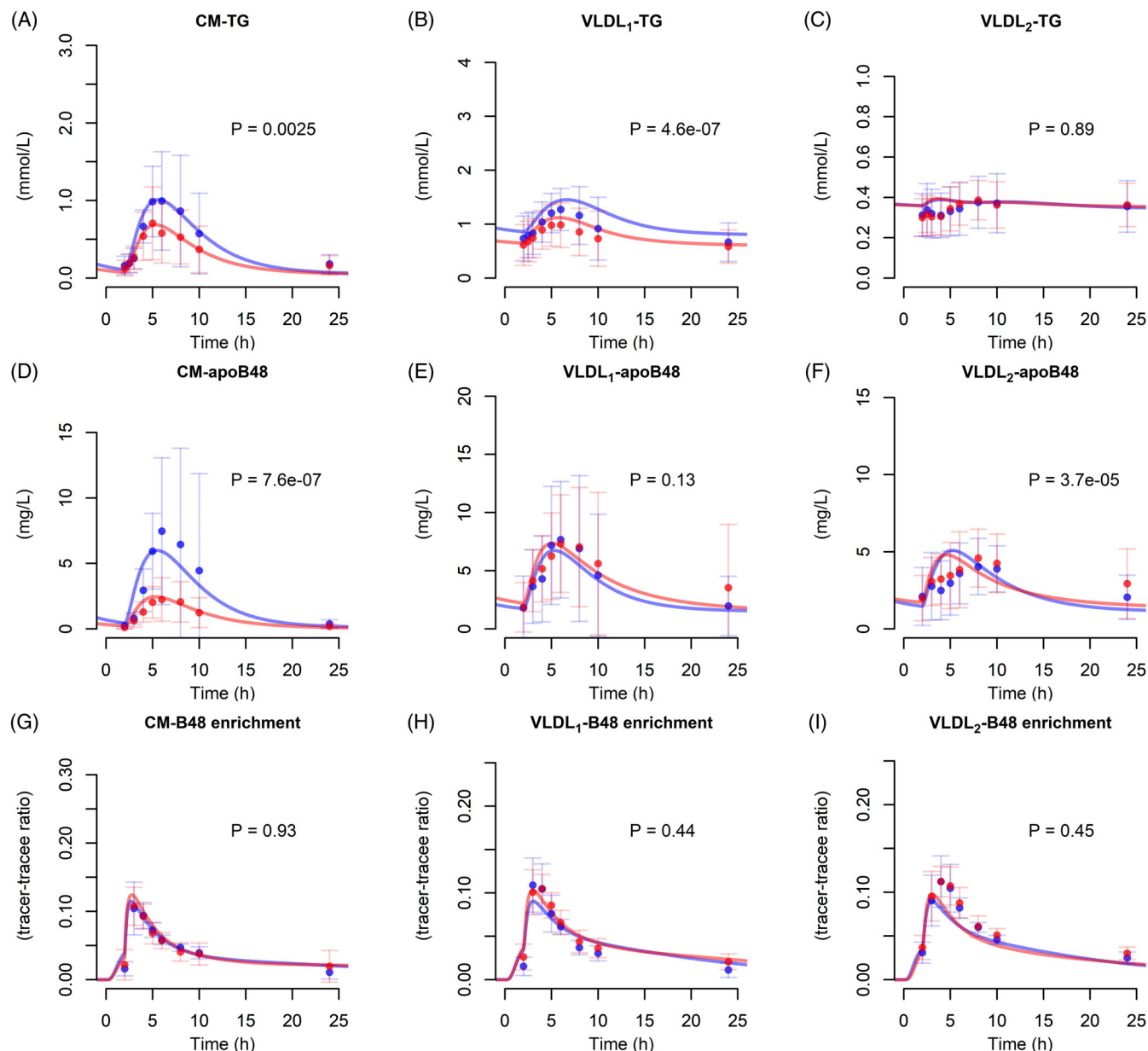


FIGURE 1 Chylomicron (CM) and very low-density lipoprotein (VLDL) metabolism in subjects with type 2 diabetes ($n = 14$) before and on liraglutide treatment. A-C, CM-, VLDL₁- and VLDL₂-triglyceride (TG) levels, D-F, CM-, VLDL₁- and VLDL₂-apoB48 levels, and G-I, CM-, VLDL₁- and VLDL₂-apoB48 tracer enrichment (following administration of deuterated leucine at time 0) in response to a standard fat-rich mixed meal before (blue) and after 16 weeks on liraglutide (red). The fat meal was administered 2 h after the injection of the tracer as described.^{6,7} Data are shown as mean with 1 SD error bars, and the lines show the model fits to the data; p -values refer to repeated measures ANOVA with visit as independent variable

apoB100 AUC (Table S1) or the rates of production and clearance of apoB100 in VLDL₁ and VLDL₂ (Table 2).

Liraglutide reduced VLDL₁-triglyceride production by 32% ($p = .017$) (Figures 1 and S1), but did not change direct VLDL₂-triglyceride production or the FCR for VLDL₁- or VLDL₂-triglyceride (Tables 2 and S2).

Results from the placebo group indicated that there was no discernible impact of modest weight loss per se on the kinetics of apoB48, apoB100 or triglyceride over the postprandial period (Figure S3 and Table S3).

3.4 | Liraglutide reduces the number of chylomicron particles produced postprandially

To account for the difference in molecular weight between apoB48 and apoB100, we also calculated the production, transfer and clearance rates for apoB48- and apoB100-containing particles in the chylomicron, VLDL₁ and VLDL₂ density ranges in nmol/day (Figure 2). Before liraglutide treatment, 1802 nmol apoB48-containing particles were produced postprandially per day across the chylomicron-VLDL₁-VLDL₂ spectrum, similar to the number of apoB100 particles released

TABLE 2 Effects of liraglutide on apolipoprotein (apo)B48 and apoB100 metabolism in triglyceride (TG)-rich lipoproteins in subjects with type 2 diabetes

	Before liraglutide (n = 14)	On liraglutide (n = 14)	p-value
APOB48 LIPOPROTEINS			
Production rates			
Basal production (mg/day)	49 ± 18	40 ± 17	.12
Total production (mg/day)	490 ± 190	329 ± 140	.002
Postprandial CM (mg/day)	284 ± 130	113 ± 53	<.001
Postprandial VLDL ₁ (mg/day)	75 ± 34	79 ± 54	.95
Postprandial VLDL ₂ (mg/day)	82 ± 38	98 ± 83	.9
Fractional catabolic rates			
CM apoB48 (pools/day)	22 ± 8	27 ± 13	.27
CM to VLDL ₁ FTR (pools/day)	12 ± 4	26 ± 13	<.001
CM FDC (pools/day)	9 ± 5	0.8 ± 0.4	<.001
VLDL ₁ apoB48 (pools/day)	25 ± 18	19 ± 15	.39
VLDL ₂ apoB48 (pools/day)	36 ± 23	31 ± 17	.81
CM-TG (pools/day)	33 ± 13	46 ± 20	.011
VLDL ₁ -TG (pools/day)	25 ± 18	19 ± 15	.39
VLDL ₂ -TG (pools/day)	36 ± 23	31 ± 17	.81
Clearance rates			
Direct CM-apoB48 clearance (mg/day)	106 ± 63	3.8 ± 2.5	<.001
CM-apoB48 to VLDL ₁ transfer (mg/day)	127 ± 120	110 ± 57	.017
APOB100 LIPOPROTEINS			
Production rates			
VLDL ₁ -apoB100 (mg/day)	871 ± 140	799 ± 160	.1
VLDL ₂ -apoB100 direct (mg/day)	251 ± 79	246 ± 68	.58
VLDL ₁ to VLDL ₂ transfer (mg/day)	582 ± 260	453 ± 250	.1
VLDL ₁ -TG (g/day)	51 ± 21	35 ± 9.8	.017
VLDL ₂ -TG direct (g/day)	3.5 ± 1.3	3.5 ± 1.1	1
Fractional catabolic rate			
VLDL ₁ (pools/day)	16 ± 11	16 ± 10	.86
VLDL ₂ (pools/day)	6.7 ± 2.6	5.6 ± 2.2	.068
VLDL ₁ -TG (pools/day)	29 ± 20	27 ± 16	1

Abbreviations: CM, chylomicron; FDC, fractional direct clearance; FTR, fractional transfer rate; VLDL, very low-density lipoprotein. Data are shown as mean ± SD; p-values are calculated using the Wilcoxon signed-rank test.

by the liver (2191 nmol/day). After liraglutide treatment, we observed a notable reduction in the number of chylomicron particles produced postprandially and a profound decrease in the direct removal pathway (Figure 2).

3.5 | Metabolic determinants of apoB48, apoB100 and triglyceride kinetics

The chylomicron-apoB48 production rate was inversely correlated with the Matsuda insulin sensitivity index ($p = .015$) (Figure S4A). However, the nature of the association was altered by treatment

(between-visit difference in association, $p < .001$); on liraglutide, chylomicron-apoB48 production rates appeared to be lower for a given value of the Matsuda index, especially when the index was less than 3. Looking at the two components of the Matsuda index, it appeared that insulin had a stronger effect on CM-apoB48 production than glucose at baseline. On liraglutide, the slope of the association between plasma insulin levels and CM-apoB48 production was diminished (Figure S4B,C). The association between the chylomicron-triglyceride/apoB48 ratio (a surrogate of particle size) and the Matsuda index was also altered by treatment (between-visit difference in association, $p < .001$) (Figure S4D and Table S4). Chylomicrons from subjects who were comparatively insulin resistant (low Matsuda index)

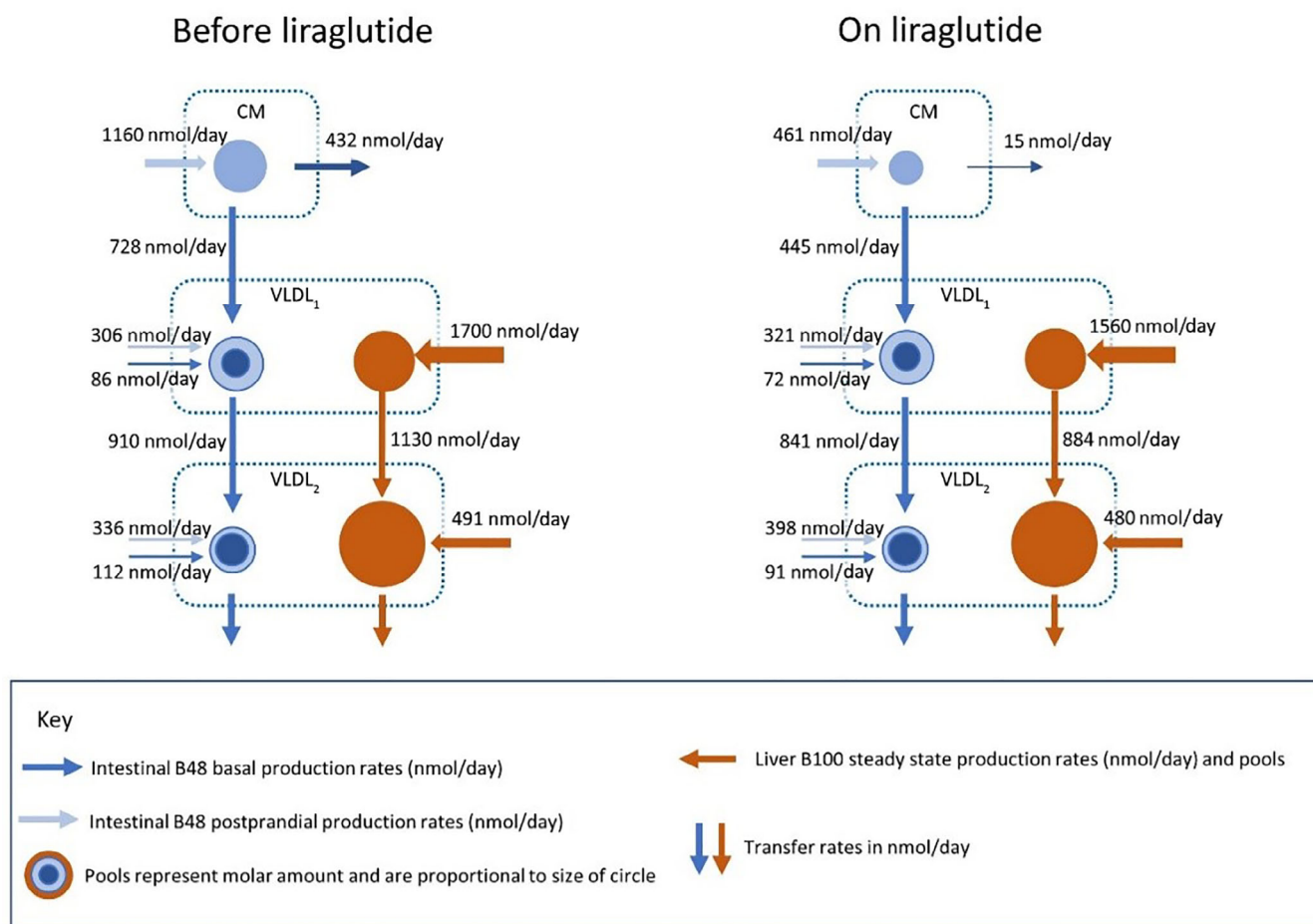


FIGURE 2 Flux rates for apolipoprotein (apo)B48 (shown in blue) in chylomicron (CM), VLDL₁ and VLDL₂, and apoB100 (shown in red) in VLDL₁ and VLDL₂ in subjects with type 2 diabetes ($n = 14$) before and on liraglutide treatment. Production rates and transfer rates for apoB48 and apoB100 are given in nmol/day to allow for the difference in molecular weight between the two forms of apoB (we assumed here 512 and 245 kDa for apoB100 and apoB48, respectively). These are equivalent to particle production rates (as there is one apoB molecule per particle). Relative particle numbers are indicated by the diameter of the circles. For apoB48-containing very low-density lipoprotein (VLDL), the inner (darker) circle is the fasting concentration and the outer (lighter) circle the postprandial increment averaged across the day. Rightward arrows indicate influx from the intestine, leftward arrows indicate influx from the liver, and downward arrows indicate lipolysis products

before treatment had a low triglyceride/apoB48 ratio, close to that of the triglyceride/apoB100 ratio in VLDL₁ (Figure S4D). On liraglutide, the chylomicron-triglyceride/apoB48 ratio increased markedly whereas the VLDL₁-triglyceride/apoB100 ratio was not altered (-Figure S4D). Thus, the improvement of insulin sensitivity on liraglutide (Table 1) does not appear to be the main reason for the liraglutide-induced reduction in chylomicron-apoB48 production.

ApoC-III, a key regulator of lipolysis, was positively associated with VLDL₁-triglyceride levels ($r = 0.70$, $p = .007$) and negatively correlated with the FCRs for VLDL₁-apoB100 ($p = .0034$) (Figure S4E) and VLDL₁-triglycerides ($r = -0.83$, $p = .0003$). These associations were not altered by liraglutide. Liver fat content was positively related to the VLDL₁-triglyceride production rate ($p = .0024$) (Figure S4E) and again this association was not altered by liraglutide ($p = .054$). It appeared that reduction in liver fat (Table 1) was accompanied by a proportionate decrease in VLDL₁-triglyceride production (Table 2 and Figure S4F).

4 | DISCUSSION

Our objective was to develop an integrated view of the effects of liraglutide on triglyceride transport in subjects with T2D. We showed that liraglutide reduced the postprandial production rate of chylomicron-apoB48 by 60%, increased the size of chylomicrons appearing in the circulation during fat absorption, and increased the lipolysis rate of chylomicrons. A pathway responsible for direct clearance of chylomicrons was virtually eliminated by liraglutide treatment, and less chylomicron-apoB48 was transferred into the VLDL density range. Liraglutide reduced the production rate of VLDL₁-triglyceride but there was no significant effect on VLDL₁-apoB100 synthesis or overall VLDL-apoB100 clearance rates. The impact of liraglutide on the postprandial response provides an explanation for the reduction in TRL- and RLP-cholesterol reported previously in these subjects.¹⁶ Finally, we observed that although the rate of chylomicron-apoB48 (over)production in these subjects with T2D was related to the degree

of insulin resistance, the liraglutide-induced reduction in apoB48 synthesis and increase in chylomicron particle size were not explained by the observed improvement in insulin sensitivity. Instead, a further effect of liraglutide on intestinal chylomicron assembly and transport not dependent on improved glycaemic control may be implicated.

These findings are in broad agreement with earlier publications.^{13,14} Xiao et al. reported that the GLP-1 receptor agonist exenatide decreased apoB48 production by 38%, resulting in a drop in circulating apoB48 levels when given to healthy subjects fed by continuous infusion into the duodenum, indicating a lack of effect on gastric emptying.¹³ No change was seen in the apoB48 FCR, as confirmed in the present investigation, nor were overall VLDL-apoB100 kinetics altered, again reflecting what we observed in VLDL₁ and VLDL₂. Xiao et al. were cautious in extrapolating their observations to the physiological setting in which regular meals are consumed, but the close correlation with our more physiologically relevant results indicates that the intestine responds to GLP-1 receptor agonists with an acute and continued suppression of apoB48 synthesis that affects the regular postprandial response. Verges et al., who used a micro-meal

feeding approach in subjects with T2D to provide a quasi-steady-state condition, showed that liraglutide not only lowered synthesis but also increased the FCR of apoB48.¹⁴ The discordancy with our study (and that of Xiao et al.¹³) may be attributable to the lipid status of the subjects. The individuals in Verges et al. had poorer glycaemic control than our group and were hypertriglyceridaemic (a mean plasma triglyceride at baseline of 2.48 mmol/L).¹⁴ Chylomicrons and VLDL compete for available lipoprotein lipase, and therefore raised triglyceride levels result in impaired chylomicron clearance^{23,24} and reduced apoB48 FCR.⁶ Thus, it is probable that the 29% decrease in fasting triglycerides (i.e. VLDL) in response to liraglutide in the study by Verges et al.¹⁴ would increase lipolytic capacity and lead to more efficient clearance of chylomicrons and apoB48 than observed in our study. Alternatively, background statin therapy in our study may have stimulated apoB48 clearance and the addition of liraglutide resulted in no further action²⁵; statin use was an exclusion criterion in the investigation by Verges et al.¹⁴

GLP-1 receptor agonists have a range of effects, including reduction in body weight and food intake, inhibition of gastric emptying, and alteration in small bowel motility and lymph flow.^{26,27} In our

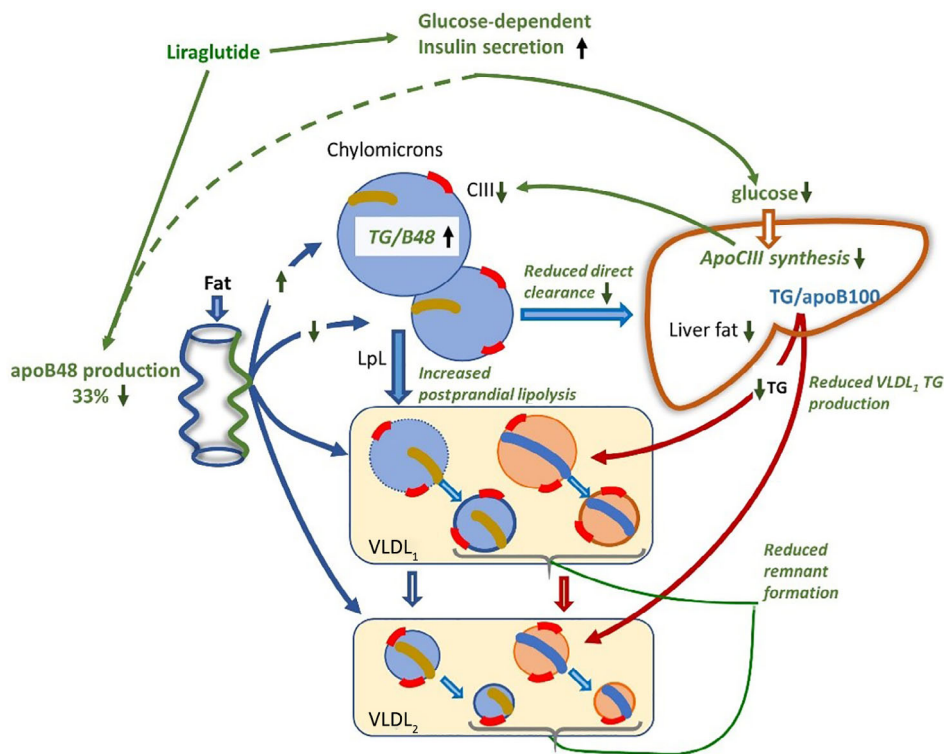


FIGURE 3 Proposed mechanism of action of liraglutide on chylomicron and very low-density lipoprotein (VLDL) metabolism in type 2 diabetes. In this schematic, we postulate that liraglutide has a direct suppressive action on apolipoprotein (apo)B48 synthesis in the gut, but also include the possibility that improved glycaemic control impacts on chylomicron secretion.²⁸ As a consequence of the reduced apoB48 availability, in response to a standard dietary fat load the intestine assembles larger chylomicron particles that are lipolysed more rapidly than their smaller counterparts. Before treatment, there appears to be substantial clearance of apoB48-containing particles directly from the chylomicron density range (possibly because their smaller size allows the particles to penetrate the liver sinusoidal space and be subjected to lipoprotein uptake pathways). On liraglutide, generation of larger chylomicrons limits clearance by this route, and in doing so possibly decreases the accumulation of liver fat. The fall in liver fat leads to less secretion of triglyceride (TG) in VLDL₁. The overall effect of liraglutide therapy is to diminish the generation of remnant lipoproteins. The impact of liraglutide on apoC-III metabolism is also included¹⁷; a fall in apoC-III production may contribute to the increased lipolytic capacity¹⁷

study, a similar mean weight loss was achieved in the placebo and liraglutide groups.¹⁶ With the caveat that the placebo group was small, we concluded that the modest weight loss did not explain the increased size of chylomicron particles, or altered the kinetics of apoB48, apoB100 or triglyceride observed on liraglutide. Rather, we propose that liraglutide acts to suppress apoB48 synthesis in the intestine (see Figure 3, which integrates features of liraglutide action seen in our study and is in line with the conclusions of others^{12,15}). The precise mechanism responsible for this effect is unclear. The glucose-lowering effect of GLP-1 receptor agonists may contribute, given that there is evidence that chylomicron synthesis is influenced by glucose levels.²⁸ Alternatively, liraglutide may have a further regulatory effect on apoB48 expression, as suggested by *in vitro* and animal studies.^{14,29,30} With this in mind it should be noted that while intact GLP-1 receptor signalling is needed for apoB48 expression, GLP-1 receptors are localized to intraepithelial lymphocytes and are not found on enterocytes (which synthesize apoB48).^{26,31} Therefore, effects mediated through GLP-1 receptors may involve complex intercellular lipid handling and/or postassembly pathways of intestinal lipid transport.

According to our model, following a decrease in apoB48 synthesis, enterocytes faced with handling the same amount of dietary triglyceride absorbed from the fat-rich meal would produce larger, more triglyceride-rich chylomicrons (Figure 3). Chylomicron assembly is known to be a flexible process with the diameter of the secreted particles ranging from 75 to 600 nm.³² In cell culture models in which protein translation (and thereby apoB48 synthesis) is inhibited, lipoprotein secretion continues, but the chylomicrons formed are larger.³³ Lipoprotein lipase hydrolyses larger chylomicrons more efficiently than smaller ones,^{34,35} which is consistent with the liraglutide-induced increase in the lipolysis rate we observed for chylomicron-triglycerides. As we previously reported, plasma apoC-III levels were reduced on liraglutide,¹⁷ which may also have contributed to the improved lipolytic capacity. This change may have been the result of glucose lowering by liraglutide as the apoC-III gene is regulated by carbohydrate.³⁶ Of note, although the rate of chylomicron-apoB48 overproduction and the chylomicron-triglyceride/apoB48 ratio were related to the degree of insulin resistance, these associations were altered by liraglutide; thus, it appeared that the liraglutide-induced reduction in apoB48 synthesis and increase in chylomicron particle size were not explained by the observed improvement in insulin sensitivity.

Liraglutide induced a pronounced reduction (90%) in the direct clearance of chylomicron-apoB48. The pathway responsible for direct clearance of chylomicrons in subjects with T2D is unknown. Given that small chylomicrons could potentially pass through the pores in the hepatic fenestrated endothelial layer (<150 nm),³⁷ we speculate that chylomicrons released from the intestine in subjects with T2D might be sufficiently small to be directly taken up by the liver (Figure 3). This pathway would increase the delivery of dietary triglyceride to hepatocytes and may contribute to fat deposition. We have earlier shown that high levels of liver fat are associated with increased production of VLDL₁.^{38,39} Liraglutide did not appear to alter the

association between liver fat and VLDL₁-triglyceride production, and therefore the decreased content of stored triglyceride in the liver may be sufficient to account for the decrease in VLDL₁-triglyceride production on liraglutide. Another potential route for chylomicron fluxes is by transport into the lymphatic system.^{40,41} Interestingly, lymphatic vessel dysfunction has been discovered in genetically modified mice.⁴²

Subjects with T2D are at a high risk of atherosclerotic CVD, partly because of their atherogenic lipoprotein profile, and in particular to increased circulating levels of remnant particles. We (and others) have shown that apoB48-containing lipoproteins are overproduced in T2D and our findings in relation to the response to regular food intake are especially relevant when the cumulative impact of consecutive large meals throughout the day is considered.^{43–45} The current data highlight how liraglutide may act to correct the abnormal intestinal lipid and lipoprotein metabolism in T2D and reduce considerably the generation of remnant particles as a result of suppressing apoB48 production. These observations may also advance understanding of the cardioprotective effects of GLP-1 receptor agonists reported in outcome trials.

The present study has design and technical limitations. The clinical trial was single-blind with a fixed sequence (pretreatment in the first phase and liraglutide or placebo in the second phase). All subjects were on background metformin and statin therapy and results should be interpreted in light of this clinical setting. There were constraints on overall subject numbers because of the complexity of the metabolic protocol. The number of subjects in the placebo arm was limited given that these individuals would gain no benefit from the intervention, but was judged adequate to discern if the qualitative effects of liraglutide on lipoprotein kinetics were attributable to change in weight. In addition, there was a limit on the volume of blood that could be drawn, and hence the sample times and dataset upon which to base the multicompartmental model.

In conclusion, our kinetic studies performed in a physiologically relevant setting in subjects with T2D showed that liraglutide has specific effects on postprandial chylomicron metabolism. Liraglutide markedly decreased apoB48 overproduction in the intestine, increased the size of the chylomicrons that appeared postprandially in the circulation, and dramatically reduced the direct clearance of chylomicrons. The changes in chylomicron metabolism were accompanied by decreased secretion of triglyceride from the liver in the VLDL₁ density range. These drug-induced metabolic changes in triglyceride transport would diminish the potential to generate remnant particles in subjects with T2D on liraglutide.

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CONFLICT OF INTEREST

The authors report no duality of interest.

AUTHOR CONTRIBUTIONS

The authors contributed to the present work as follows: MRT, NM, KHP and JB contributed to conception and design, EB, NM, SS, KHP, MA, AH, NL, AT, MA and LA to the acquisition of data or analysis, and MRT, EB, MA, CJP and JB to the interpretation of data. MRT, EB, CJP and JB drafted the original and revised manuscripts and all authors approved the final version to be published.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data that support the findings of this study but are not included in the article or in the on-line supplementary files are available from the corresponding author upon reasonable request. We are not able, however, to share detailed individual data that can be used to identify the study subjects.

ORCID

Niina Matikainen  <https://orcid.org/0000-0003-0041-1240>

Jan Borén  <https://orcid.org/0000-0003-0786-8091>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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