Liraglutide Reduces Postprandial Hyperlipidemia by Increasing ApoB48 (Apolipoprotein B48) Catabolism and by Reducing ApoB48 Production in Patients With Type 2 Diabetes Mellitus

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Objective—Treatment with liraglutide, a GLP-1 (glucagon-like peptide-1) agonist, has been shown to reduce postprandial lipidemia, an important feature of diabetic dyslipidemia. However, the underlying mechanisms for this effect remain unknown. This prompted us to study the effect of liraglutide on the metabolism of ApoB48 (apolipoprotein B48).

Approach and Results—We performed an in vivo kinetic study with stable isotopes (D_8-valine) in the fed state in 10 patients with type 2 diabetes mellitus before treatment and 6 months after the initiation of treatment with liraglutide (1.2 mg/d). We also evaluated, in mice, the effect of a 1-week liraglutide treatment on postload triglycerides and analysed in vitro on jejunum, the direct effect of liraglutide on the expression of genes involved in the biosynthesis of chylomicron. In diabetic patients, liraglutide treatment induced a dramatic reduction of ApoB48 pool (65±38 versus 162±87 mg; P=0.005) because of a significant decrease in ApoB48 production rate (3.02±1.33 versus 6.14±4.27 mg kg^{-1} d^{-1}; P=0.009) and a significant increase in ApoB48 fractional catabolic rate (5.12±1.35 versus 3.69±0.75 pool d^{-1}; P=0.005). One-week treatment with liraglutide significantly reduced postload plasma triglycerides in mice and liraglutide, in vitro, reduced the expression of ApoB48, DGAT1 (diacylglycerol O-acyltransferase 1), and MTP (microsomal transfer protein) genes.

Conclusions—We show that treatment with liraglutide induces a significant reduction of the ApoB48 pool because of both a reduction of ApoB48 production and an increase in ApoB48 catabolism. In vitro, liraglutide reduces the expression of genes involved in chylomicron synthesis. These effects might benefit cardiovascular health.

Clinical Trial Registration—URL: https://www.clinicaltrials.gov. Unique identifier: NCT02721888.

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Key Words: apolipoprotein B-48 ■ diabetes mellitus ■ hyperlipidemias ■ kinetics ■ liraglutide ■ period, postprandial

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Postprandial hyperlipidemia is an important feature of diabetic dyslipidemia which includes elevated levels of chylomicrons and chylomicron remnants in plasma.[1] Patients with type 2 diabetes mellitus show an increase in chylomicron production[2] in conjunction with an increased rate of intestinal ApoB48 secretion[3] and an augmented expression of MTP (microsomal transfer protein; responsible for the addition of triglycerides to ApoB48 [apolipoprotein B48]) within the intestine.[4] In addition, the clearance of chylomicrons is impaired in type 2 diabetes mellitus.[5] Many data suggest that postprandial hyperlipidemia is atherogenic,[6] and epidemiological studies have identified postprandial triglyceride concentrations as a clinically significant independent risk factor for cardiovascular disease.[7–9] In addition, in patients with type 2 diabetes mellitus, the increase in postprandial triglycerides has been shown to be correlated with an increase in proinflammatory and proatherogenic factors (ie, TNFα [tumor necrosis factor-α], IL6 [interleukin 6], and vascular cell adhesion molecule 1[VCAM-1]) and associated with endothelial dysfunction assessed by reduction in flow-mediated dilatation.[10,11] For all these reasons, decreasing postprandial lipidemia can be considered key target for the reduction of cardiovascular risk in patients with type 2 diabetes mellitus.

Treatment with liraglutide, a GLP-1 (glucagon-like peptide-1) receptor agonist, has been shown to reduce postprandial triglycerides in patients with type 2 diabetes mellitus. In individuals with impaired glucose tolerance and recent-onset type 2 diabetes mellitus, a single subcutaneous injection of exenatide (10 µg) markedly reduced the postprandial elevation of triglycerides, ApoB48, and ApoC-III, as compared to placebo.[12] Three weeks

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Nonstandard Abbreviations and Acronyms	
Apo	apolipoprotein
ApoB48	apolipoprotein B48
DGAT1	diacylglycerol O-acyltransferase 1
FCR	fractional catabolic rate
GLP-1	glucagon-like peptide-1
HDL	high-density lipoprotein
LDL	low-density lipoprotein
MTP	microsomal transfer protein
PR	production rate

of treatment with liraglutide (1.8 mg/d) in patients with type 2 diabetes mellitus significantly reduced postprandial excursions of triglycerides and ApoB48, as compared to placebo, after a high-fat meal and independently of gastric emptying.[13]

To date, the mechanisms responsible for the reduction in postprandial lipidemia induced by liraglutide remain unknown. This prompted us to perform an in vivo kinetic study of ApoB48, the major apolipoprotein of chylomicrons with D_8-valine in patients with type 2 diabetes mellitus, to assess the effect of a 6-month treatment with liraglutide (1.2 mg/d) on the production and the catabolism of ApoB48. We also performed animal and in vitro studies to analyze the direct effect of liraglutide on the production of chylomicrons.

Materials and Methods

This prospective single-center study was approved by our regional ethics committee, and written informed consent was obtained from all patients before study inclusion (https://www.clinicaltrials.gov. Unique identifier: NCT02721888). The data that support the findings of this study are available from the corresponding author on reasonable request.

Subjects

For this study, we recruited 10 patients with type 2 diabetes mellitus and typical diabetic dyslipidaemia (defined by triglycerides >1.70 mmol/L or HDL (high-density lipoprotein)-cholesterol <1.03 mmol/L in men and <1.29 mmol/L in women) for whom treatment with liraglutide was indicated because of poorly controlled diabetes mellitus (glycohemoglobin A1c >7%). These patients were treated with oral glucose-lowering agents (metformin alone in 5 patients, metformin+sulfonylureas in 4 patients, metformin+acarbose in 1 patient) for at least 6 months and had stable glycohemoglobin A1c during the previous 6 months. Patients with LDL (low-density lipoprotein)-cholesterol >4.90 mmol/L, kidney failure (creatinine clearance <30 mL/min), liver failure (AST [aspartate aminotransferase] or ALT [alanine aminotransferase] >3× the upper limit of normal range), hyper or hypothyroidism, use of drugs known to affect lipid metabolism (corticosteroids, retinoids, antiproteases, estrogens, cyclosporin, glitazones, statins, fibrates, cholestyramine, ezetimibe, nicotinic acid, omega 3, or phytosterols), treatment with dipeptidyl peptidase-4 inhibitors during the 3 previous months, or previous treatment with thiazolidinediones or any GLP-1 agonists were excluded.

Study Design

Two kinetic studies were performed in each type 2 diabetic patient: the first one before initiation of the treatment with liraglutide and the second one after 6 months of treatment with liraglutide. The day after the baseline kinetic study, treatment with liraglutide was started at 08:00 AM at an initial dose of 0.6 mg/d which was uptitrated to 1.2 mg/d after 1 week. The 1.2 mg/d dose was maintained throughout the remainder of the study.

The day before the kinetic study, after a 12-hour fast, each patient was admitted to the diabetes mellitus ward in the morning to undergo a physical examination and blood sampling. The following day, a kinetic study was performed in the fed state. Food intake (1700 kcal/d, 55% carbohydrate, 39% fat, and 7% protein) was fractionated into small portions which were provided every 2 hours starting 6 hours before the tracer infusion up to the end of the study, to avoid variations in apolipoprotein plasma concentration, similar to protocol previously implemented by our group[14,15] and others.[16] The endogenous labeling of ApoB48 was performed by administration of L-D_8-Valine (99 atom %; Eurisotop, Saint Aubin, France) dissolved in 0.9% NaCl solution. At 08:00 AM, each subject received a primed infusion of 0.7 mg/kg of tracer intravenously, immediately followed by a 16-hour constant infusion of 0.7 mg/kg per hour. Blood samples were collected before then, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 16 hours after the primed infusion. Serum was separated by centrifugation for 10 minutes at 4°C and 3000*g*. To avoid the influence of acute exercise on lipid metabolism, all subjects were instructed to refrain from strenuous exercise 3 days before the kinetic study.

Isolation of ApoB48

Triglyceride-rich lipoproteins were isolated at each time point, at d=1.006 for 16 hours, 39 000 rpm at 12°C, then delipidated 1 hour at −20°C using 10 volumes of diethylether-ethanol 3:1. Delipidated proteins were separated by preparative SDS-PAGE. After staining with Coomassie Blue R-250, ApoB48 was cut from the gel and hydrolyzed in HCl 6 N for 16 hours at 110°C under nitrogen vacuum. Samples were then centrifuged to remove polyacrylamide. Supernatants were lyophilized in a Speed Vac (Savant Instrument, Farmingdale, NY). Lyophilised samples were dissolved in 50% acetic acid, applied to an AG-50 W-X8 200 to 400 mesh cation exchange column (Bio-Rad, Richmond, CA), and amino acids were recovered by elution with NH_4OH 4 N and lyophilized.

Determination of D8-Valine Enrichment by Gas Chromatograph/Mass Spectrometer

Derivatized samples, as di-tert-butyl-dimethylsilyl-ethers, were analyzed by gas chromatograph/mass spectrometer with a 7890A Gas chromatograph connected to a 5975C TAD Mass Selective Detector (Agilent Technologies). Separation was achieved on an HP-5MS 30 m×250 µm column (Agilent Technologies) using helium as a carrier gas. The gas-liquid chromatography operating conditions were as follows: sample injection 1 µL at 250°C using the pulsed split mode (ratio 5:1), oven temperature program: initial temperature at 110°C for 1 minute, up to 210°C at a rate of 10°C/min, up to 300°C at a rate of 20°C/min followed by 2 additional minutes at 300°C.

The mass selective detector was set up as follows: positive electronic impact ionization at 70 eV mode, source temperature at 230°C. Data were acquired in selected ion monitoring mode. The masses (m/z) used for quantification, corresponding to the [M-159]+ ions, were 186.2 and 194.2 for valine and D8-valine, respectively. The m/z used for qualification, corresponding to the [M-85]+ ions, were 260.2 and 268,2 for valine and D8-valine, respectively. D8-valine enrichment ratios were calculated for each sample as the response of ion m/z=194.2 divided by the response of ion m/z=186.2.

Modeling

Stable isotope enrichment curves for ApoB48 were fitted in a 3 compartment model using SAAM II computer software (SAAM II Institute, WA) as described in previous research.[17,18] Compartment 1 represented the plasma amino acids. Compartment 2 was an intracellular delay compartment, accounting for the synthesis, assembly and secretion of apolipoproteins, and compartment 3 represented circulating plasma lipoproteins. Because the experiment was performed in the steady state, the fractional synthetic rate equaled the fractional catabolic rate (FCR). Production rates (PRs) of ApoB48 were normalized to body weight and calculated as follows: PR=ApoB FCR×ApoB48 pool size/body weight, where ApoB48 pool size was

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calculated by multiplying the ApoB48 concentration by the estimated plasma volume (4.5% of body weight).[19] In obese subjects (body mass index ≥30), plasma volume was corrected as previously reported by several authors.[20,21]

Biochemical Analysis

Glycaemia, total cholesterol, HDL-cholesterol, triglycerides, ApoA-I, and ApoB were quantitated on a Vista analyzer with dedicated reagents (Siemens Healthcare Diagnostics, Deerfield, IL). LDL-cholesterol was calculated using the Friedewald formula because serum triglyceride levels were <3.8 mmol/L. Glycohemoglobin A1c was measured by high-pressure liquid chromatography with a Tosoh G8 analyzer (Tosoh Bioscience, Tokyo, Japan). ApoC-III concentrations were measured by commercial immunoturbidimetric assays following the procedure provided by the manufacturer (Diasys, Bouffemont, France).

ApoB48 was quantified in the triglyceride-rich lipoprotein fraction by analytic SDS-PAGE as previously reported.[22,23] Insulin was quantified with a chemiluminescent method on an Immulite 2000 XPi analyser (Siemens) with dedicated reagents. The degree of insulin resistance was estimated by the homeostasis model assessment according to the method described by Matthews et al.[24] The insulin resistance score (homeostasis model assessment–insulin resistance) was calculated with the following formula: fasting serum glucose (mmol/L)×fasting serum insulin (µU/mL)/22.5.

Oral Lipid Tolerance Test in Mice

For oral lipid tolerance tests, Balb/c mice (8–10 week-males) were fasted overnight for 14 to 16 hours in standard cages with ad libitum access to water. Mice were gavaged with olive oil (400 µL per 30 g of body weight), and blood was collected for measurement of triglycerides and ApoB at the indicated time points. In Triton WR1339 experiments, Triton was administrated by intraperitoneal route 1-hour prior gavage. Mice were injected with 500 mg of Triton WR1339 per Kg of body weight. Mice triglycerides were assessed by FS colorimetric assays (DiaSys, Condom, France), and mice ApoB concentrations were determined using a mouse ApoB ELISA diagnostic kit from Elabscience Biotechnology, TX, according to manufacturer instructions. We decided to use male mice in our animal study to avoid possible hormonal changes that may occur in females as previously reported.[25,26] We are confident that the use of only male mice for this mechanistic study was sufficient. This animal study was approved by our University Animal Care and Use Committee.

Measurement of Adipose Tissue Lipoprotein Lipase Activity in Mice

Periepididymal adipose tissue samples were collected from control and liraglutide-treated mice and were immediately homogenized in 20 mmol/L Tris (pH 7.5) and 150 mmol/L Nacl then centrifuged at 2000g for 10 minutes at 4°C to separate fat. Assays were performed immediately using a Fluorimetric LPL (lipoprotein lipase) Activity Assay Kit from Cell Biolabs, Inc, CA, according to manufacturer instructions.

Preparation and Processing of Jejunum Explant Samples

Protocol for preparation of precision-cut jejunum slices was adapted from de Graaf et al.[27] In brief, Balb/c mice were fasted for 16 hours with free access to water and were then euthanized by cervical dislocation. The jejunum was quickly collected, adhering fat tissue was detached, and remaining fecal constituents were gently removed from the lumen using a curved spatula. Finally, the lumen was rinsed once with ice-cold saline and stored in ice-cold Hank medium, pH 7.4.

The jejunum was cut into 3 cm long segments, and each segment was closed at 1 end using surgical thread. The intestine segments were then filled with a 3% agarose solution at 37°C using a syringe equipped with a drencher. The opposite end was then tied shut with another thread and maintained in ice-cold Hank medium. When the agarose had solidified, the segments were transferred into a cylindrical mould-plunger assembly of the tissue-embedding unit, and the mould was filled with a 3% agarose solution at 37°C and maintained on ice for 2 minutes to allow the agarose to solidify.

The plunger was removed, and the jejunum embedded in agarose was placed in a Brendon/Vitron slicer (Vitron, Inc, Tucson, AZ) containing Hank medium at 4°C to obtain ≈200 µm-precision-cut slices. After carefully removing the agarose, jejunum slices were used within the half hour after the preparation for incubation with different doses of liraglutide.

Jejunum slices were randomly distributed in 6-well plates (3–4 slices per well) containing 3 mL of oxygenated William medium E, supplemented with heat-inactivated nondelipidated FBS (10%), 5.5 mmol glucose, 4 mmol/L glutamine, 1 mmol/L pyruvate, and an antibiotic-antifungal cocktail (1%) and were treated with saline (control), 10 or 20 µM of commercial solution of liraglutide (Victoza, Novo Nordisk, France). Plates were incubated on a rocking shaker for 4 hours in a 5% atmosphere at 37°C. At the end of the incubation period, slices were collected and snap frozen until total RNA extraction.

Gene Expression

Total mRNAs were extracted from jejunum slices with Tri-Reagent (Euromedex, Souffelweyersheim, France) and were reverse-transcripted using the Iscript cDNA kit (Bio-Rad, Hercules, CA). Real-time reverse transcriptase polymerase chain reaction was performed, as previously described,[28] in a 96-well plate using a Bio-Rad iCycler iQ. The sequences of forward and reverse primers used for amplification are represented in Table I in the online-only Data Supplement. For each gene, a standard curve was established from 4 cDNA dilutions (1/10 to 1/10,000) and was used to determine relative gene-expression variation after normalization, with a geometric average of 18S and TATA box–binding protein expression.

Statistical Analysis

Data are reported as mean±SD. The area under the curve (AUC) for triglycerides was calculated by the trapezoidal method then the incremental AUC was calculated after correction for baseline values Statistical calculations were performed using the SPSS software package (Chicago, IL). Continuous post-treatment data were compared with baseline data using the nonparametric Wilcoxon matched-pair signed-rank test. Continuous data between 2 groups were compared using the nonparametric Mann-Whitney *U* test. Correlations between quantitative parameters were calculated by the nonparametric Spearman test. A 2-tailed probability level of 0.05 was accepted as statistically significant.

Results

Glucose and Lipid Parameters

The clinical and biological characteristics of the patients at baseline and after 6 months of treatment with liraglutide are shown in Table 1. Treatment with liraglutide 1.2 mg/d was well tolerated in all patients. Treatment with liraglutide resulted in significant decreases in body weight, fasting glucose, glycohemoglobin A1c, homeostasis model assessment–insulin resistance, fasting triglycerides, total cholesterol, and ApoB. A slight decrease in LDL-cholesterol was also observed but did not reach statistical significance (P=0.09).

ApoB48 Kinetic Parameters

Treatment with liraglutide induced a significant reduction in ApoB48 pool (65±38 versus 162±87 mg; P=0.005; Table 2). The isotopic enrichment curves of ApoB48, expressed as percentage of plateau, presented in Figure 1, show a faster increase in isotopic enrichment of ApoB48 after liraglutide

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Table 1. Clinical and Biological Characteristics of the Patients at Baseline and After a 6-Month Treatment With Liraglutide

	Baseline	After 6-Mo Treatment With Liraglutide	*P* Value
Age, y	48.6±10.9		
Sex (male/female)	5/5		
BMI, kg/m^2	36.6±4.9	34.9±4.7	0.021
Body weight, kg	104.9±19.7	100.5±19.6	0.021
Fasting glucose, mmol/L	11.11±4.68	6.36±1.27	0.005
HbA1c, %	9.6±2.6	7.1±1.1	0.009
HOMA-IR	11.6±11.2	5.5±1.9	0.015
Total cholesterol, mmol/L	5.09±0.43	4.56±0.58	0.028
Triacylglycerol, mmol/L	2.48±0.69	1.76±0.37	0.005
LDL-cholesterol, mmol/L)	3.04±0.47	2.75±0.56	NS (0.09)
HDL-cholesterol, mmol/L	0.97±0.19	0.97±0.26	NS
ApoB, g/L	1.09±0.11	0.93±0.13	0.011
ApoA-I, g/L	1.35±0.15	1,33±0.19	NS
ApoC-III, mg/L	150±38	153±32	NS

Data are expressed as mean±SD. Apo indicates apolipoprotein; BMI, body mass index; HbA1c, glycohemoglobin A1c; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment–insulin resistance score; LDL, low-density lipoprotein; and NS, not significant.

treatment indicating a significantly faster FCR (5.12±1.35 versus 3.69±0.75 pool d^{-1}; *P*=0.005; Table 2). The isotopic enrichment plateau levels were similar during the baseline and treatment phases (5.36±1.09% versus 4.95±0.74%). Liraglutide treatment also significantly reduced the ApoB48 PR (3.02±1.33 versus 6.14±4.27 mg kg^{-1} d^{-1}; *P*=0.009; Table 2). The reduction of ApoB48 pool, the reduction of the ApoB48 PR, or the increase in ApoB48 FCR were not correlated with body weight reduction.

Oral Lipid Tolerance Tests in Mice

First, to verify that liraglutide had the same potential lowering effect on postprandial lipidemia in mice as observed in humans, we treated Balb/c mice with either saline or liraglutide (1 mg/kg of body weight) for 1 week. Liraglutide-treated mice and control mice did not show different fasting plasma glucose levels (95.75±7.0 versus 97.25±6.98 mg/dL). However, oral lipid tolerance tests (Figure 2) showed that mice treated with 1 mg/kg of liraglutide for 1 week presented significantly reduced postprandial lipidemia (at time 1, 2, 3, and 4 hours post oral fat load) with an AUC for triglycerides significantly lower compared with control animals (6.20±0.26 versus 9.39±1.17 mMol/L×8 h; *P*=0.043), suggesting the same lowering effect as observed in humans.

Then, to study more specifically the effect of liraglutide on the production of intestinally derived lipoproteins, we performed an additional lipid tolerance test in mice after Triton WR1339 administration to block the lipolysis of triglyceride-rich lipoproteins. Mice treated with 1 mg/kg of liraglutide for 1 week still presented significantly reduced postprandial lipidemia at 1 and 3 hours of the oral lipid tolerance test (borderline significant at 2 hours) with a triglyceride increase from baseline to 3 hours during the lipid tolerance test significantly lower compared with control animals (9.3±5.6 versus 16.7±4.7 mMol/L×3 h; *P*=0.039; Figure 3). These data suggest that liraglutide in mice reduces the production of intestinally derived lipoproteins similarly to what is observed in humans.

During the lipid tolerance test in mice after Triton WR1339, the triglyceride/ApoB ratio was not significantly different between the liraglutide-treated mice and the control mice at baseline, 2, 3, 4, 6, and 8 hours after the oral fat load. Furthermore, the AUC or the incremental AUC for triglyceride/ApoB ratio was not statistically different between the 2 groups. Only at time 1 hour of the oral lipid tolerance test, liraglutide treated mice showed a slightly lower triglyceride/ApoB ratio compared with control mice (6.4±4.7 versus 11.9±2.5 mMol/mg; *P*=0.048). These data indicate that treatment with liraglutide is not associated with major changes in size of the chylomicrons secreted.

Adipose Tissue LPL Activity in Mice

Adipose tissue LPL activity was significantly increased in liraglutide-treated mice compared with control mice (0.724±0.207 versus 0.454±0.159 mU/min per milligram of tissue; *P*=0.024).

In Vitro Gene Expression

To estimate if the effects of liraglutide are mediated by mechanisms directly targeting the intestine, we chose to use an in vitro approach to treat the jejunum directly and to suppress indirect effects of liraglutide treatment. For that purpose, precision-cut jejunum slices were treated with liraglutide, and the mRNA expression of 3 genes implicated in intestine lipoprotein secretion was measured. Figure 4 shows that at concentrations of 10 or 20 µM, liraglutide induced a significant decrease in ApoB48, DGAT1 (diacylglycerol-O-acyltransferase 1), and MTP gene expression.

Discussion

Our in vivo kinetic study has shown that treatment with liraglutide induces a significant reduction of the ApoB48 pool because of both a significant reduction of ApoB48 production and a significant increase in ApoB48 catabolism. The decrease in postprandial hyperlipidemia was confirmed in mice after a short period of treatment with liraglutide, and we have also confirmed, in vitro, that liraglutide significantly reduces the expression of genes involved in chylomicron

Table 2. Kinetic Parameters of ApoB48 in the Patients at Baseline and After 6-Month Treatment With Liraglutide

	Baseline	After 6-Mo Treatment With Liraglutide	*P* Value
ApoB48 pool, mg	162±87	65±38	*P*=0.005
ApoB48 FCR, pool d^{-1}	3.69±0.75	5.12±1.35	*P*=0.005
ApoB48 PR, mg kg^{-1} d^{-1}	6.14±4.27	3.02±1.33	*P*=0.009

Data are expressed as mean±SD. ApoB indicates apolipoprotein B; FCR, fractional catabolic rate; and PR, production rate.

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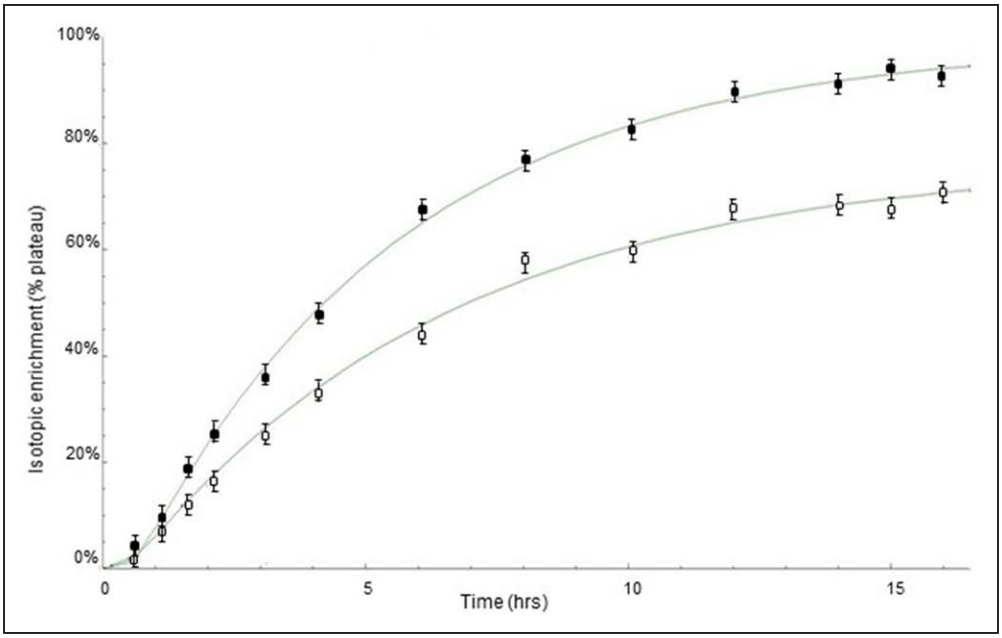

Figure 1. Kinetic curves of ApoB48 (apolipoprotein B48) obtained during a primed constant infusion of L-D8-Valine enrichment values, expressed as percentage of plateau, in the diabetic patients before (white square) and after 6-mo treatment with liraglutide 1.2 mg/d (black square). The curves were obtained by pluricompartimental modeling. Data are shown as mean±SEM.

synthesis in intestinal tissue. All these data indicate that liraglutide reduces postprandial lipidemia dramatically by modifying ApoB48 kinetics significantly and that these effects may possibly be because of a direct influence of liraglutide on the intestine.

GLP-1 receptor agonists have been shown to reduce postprandial lipidemia in healthy subjects or patients with type 2 diabetes mellitus. In healthy men, the postprandial increase in triglyceride levels was completely abolished by intravenous infusion GLP-1.[29] A single subcutaneous injection of exenatide (10 µg) has been shown to markedly reduce the postprandial elevation of triglycerides and ApoB48 in individuals with impaired glucose tolerance and recent-onset type 2 diabetes mellitus.[12] In patients with type 2 diabetes mellitus, 3 weeks of treatment with liraglutide as compared to placebo significantly reduced postprandial excursions of triglycerides and ApoB48 after a fat-rich meal independently of gastric emptying.[13] One subcutaneous injection of exenatide (10 µg) has been shown, compared with placebo, to reduce ApoB48 production, in normolipidemic and normoglycemic men.[18] Our results, which show a dramatic reduction of the ApoB48 pool after 6 months of treatment with liraglutide, are thus in accordance with previously reported data. However, to date, the effect of GLP-1 receptor agonists on the kinetics of ApoB48 in patients with type 2 diabetes mellitus remained unknown. Moreover, no data on the action of liraglutide, one of the most potent GLP-1 receptor agonists, on the metabolism of ApoB48 were available. Our study has shown, for the first time, that liraglutide significantly modifies aopB48 metabolism. Liraglutide decreases production and enhances catabolism of ApoB48, which leads to a significant reduction of plasma concentrations.

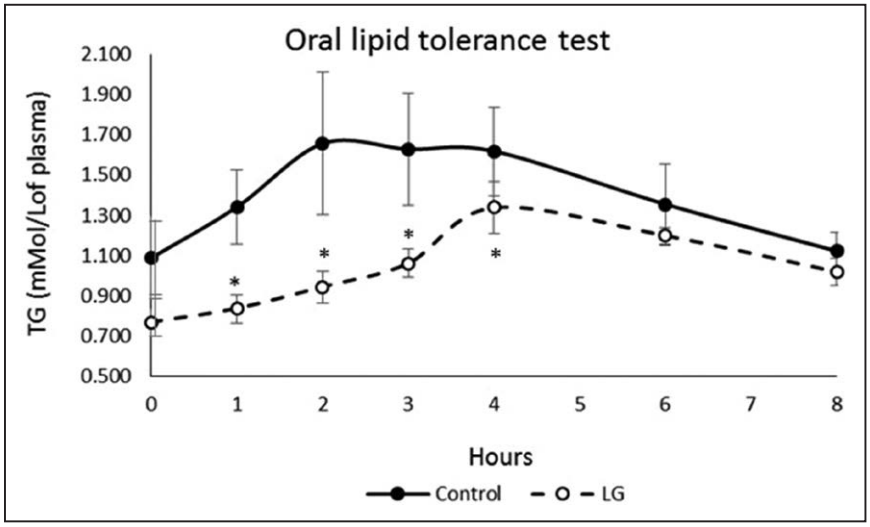

Figure 2. Effect of liraglutide (LG) on postprandial lipemia in mice. Mice were treated for 1 wk with saline (control) or 1 mg/kg of LG. Postprandial lipemia was assayed using an oral lipid tolerance test. Results are expressed as means±SEM. *P<0.05 vs control conditions.

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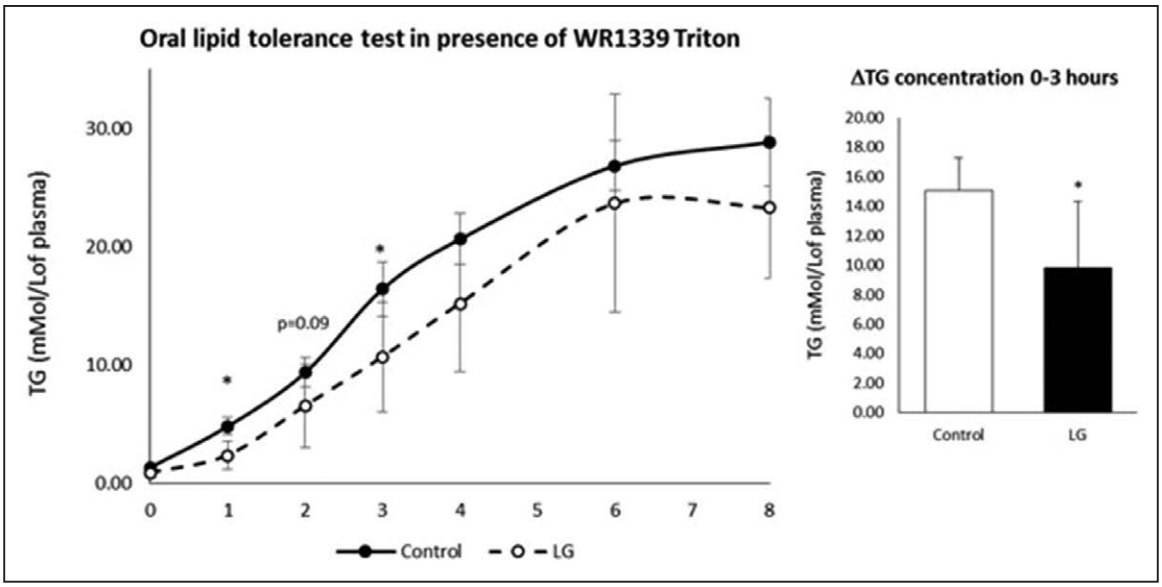

Figure 3. Effect of liraglutide (LG) on postprandial lipemia in mice after injection of WR1339 Triton. Mice were treated for 1 wk with saline (control) or 1 mg/kg of LG. Postprandial lipemia was assayed using an oral lipid tolerance test, after a single injection of WR1339 Triton (500 mg/kg of body weight), 1 h prior of lipid loading. ΔTG concentration is calculated as the variation of triglyceride concentrations between t=0 h and t=3 h during the oral lipid tolerance test performed in the presence of WR1339 Triton. Results are expressed as means±SEM. TG indicates triglycerides. **P*<0.05 vs control conditions.

Treatment with liraglutide has several effects, such as body weight reduction and changes in satiety and gastric emptying which may reduce ApoB48 production. However, the important effect of liraglutide on ApoB48 metabolism suggests that it may have a direct effect on the intestine. The presence of GLP-1 receptors in the intestinal tissue reinforces the hypothesis of a possible direct action in the intestine,[30–32] and the results that we observed in mice also suggest that liraglutide may have a direct effect in the intestine. Indeed, a short period of treatment with liraglutide induced a significant reduction in postprandial lipidemia in mice, and in vitro liraglutide reduced the expression of genes involved in the biosynthesis of chylomicrons, such as ApoB48, MTP, or DGAT1 in the intestine. To date, the direct effect of liraglutide on the expression of genes involved in chylomicron secretion has never been analyzed. Exenatide, another GLP-1 agonist, has been shown to suppress ApoB48 expression in isolated hamster enterocytes, indicating direct involvement of GLP-1 receptor signaling in intestinal lipoprotein particle expression.[33] All these data indicate that liraglutide likely has a direct effect on the intestine, reducing chylomicron production. The direct effect of liraglutide on lipid metabolism has also recently been suggested in a study showing a significant reduction of palmitate synthesis after a single injection of liraglutide in obese rats.[34]

If our data indicate that a direct effect of liraglutide in the intestine is likely, we cannot exclude the additional possibility that liraglutide acts via the central nervous system. Farr et al[35] showed that the intracerebroventricular administration of a GLP-1 receptor agonist in the Syrian hamster resulted in a significant reduction of postprandial triglycerides and ApoB48, suggesting a possible modulation of chylomicron secretion via an action in the central nervous system.

Another interesting observation is the enhancement of ApoB48 catabolism in our type 2 diabetic patients after 6 months of treatment with liraglutide. This suggests an increase in the activity of the LPL, the main enzyme involved in chylomicron catabolism. This is supported by the increased

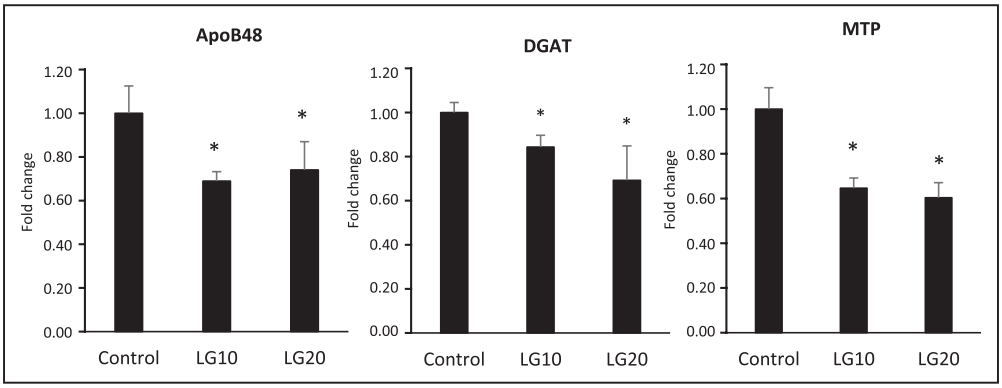

Figure 4. Effect of liraglutide (LG) on expression of ApoB48 (apolipoprotein B48), DGAT1 (diacylglycerol-O- acyltransferase 1), and MTP (microsomal transfer protein) genes. Jejunum slices were treated for 4 h either with LG (10 or 20 μmol/L) or vehicle (control) before gene expression measurement by real-time reverse transcriptase polymerase chain reaction analysis. Results are expressed as means±SEM (4 distinct experiments). **P*<0.05 vs control conditions.

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activity of LPL that we observed in mice after a short period of treatment with liraglutide. The reasons for this liraglutide-induced increase in ApoB48 catabolism are not totally clear. However, we may think that the weight loss and the improvement of insulin sensitivity induced by liraglutide could play a role in the acceleration of ApoB48 catabolism. Indeed, weight loss has been shown to increase adipose tissue LPL activity in obese subjects.[36] Moreover, the reduction of insulin resistance through exercise training induced a significant increase in plasma LPL activity in sedentary adults without weight loss.[37] In our study, diabetic patients showed both a mean reduction in body weight and insulin resistance, which could be responsible for the enhancement of ApoB48 catabolism via an increase in LPL activity. This increase in ApoB48 catabolism could also be because of the reduction in LPL inhibitory proteins, such as ApoC-III, angiopoietin-like 3 (ANGPTL3), or angiopoietin-like 4 (ANGPLT4). However, ApoC-III was not significantly decreased in our patients treated with liraglutide suggesting that the increase in ApoB48 catabolism is not likely to be induced by a decrease in ApoC-III.

Postprandial hyperlipidemia is an important feature of diabetic dyslipidemia which is observed in patients with type 2 diabetes mellitus.[1] It has been shown to be the result of an increase in chylomicron production[2] and a reduction in chylomicron clearance.[5] The increased chylomicron production, observed in type 2 diabetic patients, has been shown to be related to an increased rate of intestinal ApoB48 secretion[3] and an augmented expression of MTP within the intestine.[4] Interestingly, liraglutide treatment seems to somewhat correct the kinetic chylomicron abnormalities observed in type 2 diabetes mellitus by decreasing ApoB48 production and increasing ApoB48 catabolism. The reduction of MTP expression by liraglutide is also in line with its beneficial effect on postprandial hyperlipidemia.

Postprandial hyperlipidemia has been shown to be a significant risk factor for cardiovascular disease[7–9] and is likely to promote atherosclerosis and the occurrence of cardiovascular events in patients with type 2 diabetes mellitus. The increase in postprandial triglycerides has been shown to be correlated with the increase in postprandial TNFα, IL6, and VCAM-1 values in patients with type 2 diabetes mellitus, indicating that postprandial lipidemia has a deleterious proinflammatory effect.[10] Furthermore, the magnitude of postprandial hypertriglyceridemia is strongly correlated with a reduction in flow-mediated dilatation in patients with type 2 diabetes mellitus, indicating that postprandial lipidemia plays a role in endothelial dysfunction.[11] Therefore, it seems that patients with type 2 diabetes mellitus would reduce their cardiovascular risks if they were able to reduce their postprandial hyperlipidemia. The present study shows that liraglutide has a significant impact on intestinal-derived lipoprotein metabolism by modifying, in a positive way, ApoB48 kinetics. The effect of liraglutide on postprandial lipid metabolism is likely to reduce the risk for cardiovascular events. It could partially explain the cardiovascular benefit of liraglutide observed in the LEADER trial (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results trial), in which 3.5 years of treatment with liraglutide was associated with a significant 13% reduction in the primary outcome (time to first major cardiovascular event: cardiovascular death, nonfatal myocardial infarction, nonfatal stroke; P=0.01), a 22% reduction in cardiovascular death (P=0.007) and a 15% reduction in total mortality (P=0.02).[38]

Metabolic studies performed in constant feeding state are not exactly identical to postprandial studies performed with an oral fat load. However, the study of postprandial metabolism of intestinal-derived lipoproteins is usually performed in humans in constant feeding state. This method is considered to evaluate correctly postprandial lipid metabolism.[39–41] We are confident that the different protocol designs performed in humans (constant feeding state) and in mice (oral fat load) do not confound interpretation of the results generated because they have been shown to give similar information in previous studies. For instance, it has been shown, in humans, that treatment with atorvastatin-induced a significant reduction of ApoB48 pool in a kinetic study performed in constant feeding[39] and a significant decrease in ApoB48 incremental AUC after an oral fat load.[42]

As a limitation of the study, we acknowledge that we performed an uncontrolled study without the inclusion of a placebo arm. However, because human in vivo kinetic studies are heavy and time-consuming studies, the absence of a placebo arm is frequent in human kinetic studies having analyzed the effect of a therapeutic intervention on lipid metabolism.[43–45] We do not think that our study design induced an important bias in our results. Indeed, the evolution of the body weight and the lipid parameters observed after 6-month treatment with liraglutide was similar to what is usually observed in clinical practice. As far as body weight reduction is concerned, the weight loss observed in our study was similar to the mean body weight reduction reported in other clinical studies with the same dose of liraglutide during a comparable period of treatment.[46,47] Furthermore, the reduction of ApoB48 pool, the reduction of the ApoB48 PR or the increase in ApoB48 FCR were not correlated with body weight reduction. Thus, body weight reduction is not likely to be responsible for the ApoB48 kinetic modifications observed on liraglutide treatment, in our study.

In summary, the present study provides evidence that treatment with liraglutide significantly reduces the ApoB48 pool, in patients with type 2 diabetes mellitus and typical diabetic dyslipidemia, by reducing ApoB48 production and increasing ApoB48 catabolism. Moreover, a short period of treatment with liraglutide dramatically reduces postload triglycerides in mice and, in vitro, liraglutide significantly reduces the expression of genes involved in chylomicron synthesis in intestinal tissue, suggesting a possible direct effect of liraglutide on the intestine.

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Disclosures

None.

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Highlights

- Liraglutide dramatically reduces ApoB48 (apolipoprotein B48) pool in patients with type 2 diabetes mellitus.
- Liraglutide significantly reduces ApoB48 production in patients with type 2 diabetes mellitus.
- Liraglutide significantly increases ApoB48 catabolism in patients with type 2 diabetes mellitus.
- Liraglutide, in vitro, reduces the expression of genes involved in the biosynthesis of chylomicrons (ApoB48, MTP [microsomal transfer protein], DGAT1 [diacylglycerol-O-acyltransferase 1]).

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