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Original article

No effect of liraglutide on high density lipoprotein apolipoprotein AI kinetics in patients with type 2 diabetes

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ABSTRACT

Aim: The catabolism of high density lipoprotein (HDL) apolipoprotein AI (apoAI) is accelerated in patients with type 2 diabetes (T2D), related to hypertriglyceridemia, insulin resistance and low plasma adiponectin levels. Since liraglutide is likely to partly correct these abnormalities, we hypothesized that it might have a beneficial effect on HDL apoAI kinetics in patients with T2D.

Methods: An *in vivo* kinetic study of HDL apoAI was performed in 10 patients with T2D before and after 6 months of treatment with 1.2 mg/day of liraglutide, using a bolus of l-[1–^{13}C]leucine followed by a 16-hour constant infusion.

Results: Liraglutide reduced BMI (34.9 ± 4.7 *vs* 36.6 ± 4.9 kg/m^2, $P = 0.012$), HbA1c (7.1 ± 1.1 *vs* 9.6 ± 2.6%, $P = 0.003$), HOMA-IR (5.5 ± 1.9 *vs* 11.6 ± 11.2, $P = 0.003$), fasting triglycerides (1.76 ± 0.37 *vs* 2.48 ± 0.69 mmol/l, $P < 0.001$) and triglycerides during kinetics (2.34 ± 0.81 *vs* 2.66 ± 0.65 mmol/l, $P = 0.053$). Plasma HDL cholesterol and adiponectin concentrations were unchanged (respectively 0.97 ± 0.26 *vs* 0.97 ± 0.19 mmol/l, $P = 1$; 3169 ± 1561 *vs* 2618 ± 1651 µg/l, $P = 0.160$), similar to triglyceride content in HDL (5.13 ± 1.73 vs 5.39 ± 1.07%, $P = 0.386$). Liraglutide modified neither HDL apoAI fractional catabolic rate (0.35 ± 0.11 vs 0.38 ± 0.11 pool/day, $P = 0.375$), nor its production rate (0.44 ± 0.13 vs 0.49 ± 0.15 g/l/day, $P = 0.375$), nor its plasma concentration (1.26 ± 0.19 vs 1.29 ± 0.14 g/l, $P = 0.386$).

Conclusion: Six months of treatment with 1.2 mg/day of liraglutide had no effect on the kinetics of HDL apoAI in patients with T2D. The lack of decrease in triglyceride content in HDL related to an only moderate decrease in triglyceridemia, probably greatly explains these results. Insufficient improvement of insulin sensitivity and adiponectinemia may also be implied.

Introduction

Cardiovascular risk is increased in patients with type 2 diabetes (T2D) compared to nondiabetic individuals [1,2]. The dyslipidemia observed in T2D, characterized by hypertriglyceridemia, low high-density lipoprotein cholesterol (HDL-C) concentration, qualitative lipoprotein abnormalities and dysfunctional HDL, greatly contributes to this increased risk [3].

The decrease in HDL cholesterol level is the consequence of the acceleration of the catabolism of HDL particles, which has been evidenced in humans by *in vivo* kinetic studies showing accelerated catabolism of HDL apoAI [4–6]. It is well recognized that the enrichment of HDL in triglycerides (TGs), consecutive to hypertriglyceridemia, plays an important role in HDL apoAI hypercatabolism [5,7-10].

An accelerated HDL apoAI catabolism has also been observed in obese patients with normal triglyceridemia, suggesting that factors independent of hypertriglyceridemia and associated with obesity are also implicated in apoAI hypercatabolism [11,12]. Insulinemia, HOMA-IR, and BMI have been shown to be correlated with apoAI fractional catabolic rate (FCR) in several studies in patients with metabolic syndrome (with or without diabetes) [5,9,11,13]. Interestingly, our group also revealed a strong negative correlation between plasma adiponectin

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concentration and HDL apoAI FCR, both in healthy subjects and in patients with metabolic syndrome [9]. This association was independent of obesity, insulin resistance, and the content of TGs within HDL particles. Both adiponectin and HDL TGs/cholesterol ratio explained 62 % of the variance of apoAI FCR, and adiponectin on its own explained 43 %. These data suggest that adiponectin is likely to reduce HDL apoA-I catabolism and that the low plasma adiponectin levels observed in T2D may be involved in the accelerated catabolism of HDL.

Glucagon like peptide 1 (GLP-1) receptor agonists have been widely prescribed in patients with T2D for several years. A metaanalysis recently reported that there is significant reduction in the risk of major adverse cardiovascular events, myocardial infarction, cardiovascular mortality, and all-cause mortality in patients with T2D treated with liraglutide, a GLP-1 receptor agonist [14]. Liraglutide has been shown to significantly decrease plasma levels of both fasting and postprandial TGs in several clinical trials [15–17]. Our team has shown that liraglutide reduces postprandial hypertriglyceridemia by increasing apoB48 FCR and by reducing the apoB48 production rate (PR) in patients with T2D [18]. In addition, liraglutide reduces very low density lipoprotein 1 (VLDL1) and low density lipoprotein (LDL) pool sizes by accelerating the catabolism of these lipoproteins [19]. At the same time, GLP-1 receptor agonists improve insulin sensitivity both by inducing weight loss and by a direct effect [20,21]. Finally, liraglutide is likely to significantly increase circulating adiponectin concentrations [15,22,23].

Given the ability of liraglutide to partly correct hypertriglyceridemia, insulin resistance and low plasma adiponectin level in patients with T2D, and since these abnormalities are implicated in the modifications of HDL apoAI metabolism commonly observed in these patients, we hypothesized that liraglutide treatment could have a beneficial effect on HDL apoAI kinetics in patients with T2D. This prompted us to perform an *in vivo* kinetic study of HDL apoAI in patients with T2D at baseline and after 6 months of treatment with 1.2 mg/day of liraglutide.

Patients, materials and methods

Study population

This prospective, single-center study was approved by our regional ethics committee (Dijon, France), and written informed consent was obtained from all patients before inclusion (https://www.clinicaltrials.gov. Unique identifier: NCT02721888). Ten patients with T2D and typical diabetic dyslipidemia (defined by TGs $>$ 1.70 mmol/l and/or HDL-C $<$ 1.03 mmol/l in men and $<$ 1.29 mmol/l in women) were included in this study. Treatment with liraglutide was indicated in all cases because of poorly controlled diabetes (HbA1c $>$ 7 %). These patients were treated with oral glucose-lowering agents (metformin alone in five patients, metformin and sulfonylureas in four patients, metformin and acarbose in one patient) for at least 6 months and had stable HbA1c during the previous 6 months. Exclusion criteria were: low density lipoprotein cholesterol (LDL-C) $>$ 4.90 mmol/l, creatinine clearance $<$ 30 ml/min, liver injury (aspartate aminotransferase or alanine aminotransferase $>$ 3 $\times$ the upper limit of normal range), hyper- or hypothyroidism, use of drugs known to affect lipid metabolism (corticosteroids, retinoids, antiproteases, estrogen, cyclosporin, glitazones, statins, fibrates, cholestyramine, ezetimibe, nicotinic acid, n-3, or phytosterols), treatment with dipeptidyl peptidase 4 inhibitors during the 3 previous months, or previous treatment with thiazolidinediones or any GLP-1 agonists.

Study design

Two kinetic studies were performed in each patient: the first one before initiation of liraglutide treatment and the second one after 6 months of liraglutide treatment. On the day following the baseline kinetic study, treatment was started at 8:00 A.M. at an initial dose of liraglutide 0.6 mg/day, which was uptitrated to 1.2 mg/day after 1 week. The dose of 1.2 mg/day was maintained throughout the remainder of the study. On the day before the kinetic study, after a 12-h fast, each patient was admitted to the diabetes 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/day, 55 % carbohydrate, 38 % fat, and 7 % protein) was fractionated into small portions, which were provided every 2 h starting 6 h before the tracer infusion up to the end of the study, to avoid variations in apolipoprotein plasma concentration, similarly to previous kinetic studies [5,6]. The endogenous labeling of apolipoproteins was carried out by administration of l-[1–^{13}C]leucine (99 atom%; Eurisotop, Saint Aubin, France) dissolved in a 0.9 % NaCl solution. At 8:00 A.M., each patient received intravenously a primed infusion of 0.7 mg/kg of tracer immediately followed by a 16-h constant infusion of 0.7 mg/kg/h. Blood samples were collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15, and 16 h after the primed infusion. Serum was separated by centrifugation at 3000 g for 10 min at 4 °C, and stored at −80 °C until experiments. To avoid the influence of acute exercise on lipid metabolism, all patients were instructed to refrain from strenuous exercise 3 days before the kinetic study.

Isolation of apolipoproteins

VLDL1 (Svedberg flotation 60–400) were isolated from plasma by gradient ultracentrifugation using a SW41 rotor in a L90 apparatus (Beckman Instruments, Palo Alto, CA) [24]. HDL were isolated from the LDL (Svedberg flotation 0–12) infranant by sequential ultracentrifugation using a 50.4 rotor at density range between 1.063 and 1.21 g/ml. VLDL1 and HDL fractions were then dialyzed against a 10 mmol/l ammonium bicarbonate buffer, pH 8.2, containing 0.01 % (w/v) EDTA and 0.013 % (w/v) sodium azide. VLDL1 and HDL fractions were delipidated 1 h at −20 °C using 10 vol of diethylether-ethanol 3:1. [5]. Apolipoproteins from each lipoprotein fraction (VLDL1 apoB100 and HDL apoA-I) were isolated by preparative discontinuous SDS-PAGE on a 3 % (w/v) and 15 % (w/v) gel. After staining with Coomassie blue R-250, apolipoprotein bands were excised from polyacrylamide gels and hydrolyzed in 6 M HCl at 110 °C for 16 h. Samples were then centrifuged to remove polyacrylamide. Supernatants were lyophilized in a Speed Vac (Savant Instrument, Farmingdale, NY). Lyophilized samples were dissolved in 50 % (v/v) acetic acid and applied to an AG-50W-X8 200–400 mesh cation exchange resin (Bio-Rad, Richmond, CA), and amino acids were recovered by elution with 4 N NH_4OH. After lyophilization, they were derivatized to N-acetyl O-propyl esters.

Determination of l-[1–^{13}C]leucine enrichment

Enrichment by gas chromatography mass spectrometry analyses were measured on a TRACE 1300 gas chromatograph connected to an ISQ LT single-quadrupole mass detector (ThermoFisher Scientific, Waltham, MA) equipped with a chemical ionization source operating in positive mode. Derivatized samples were dissolved in ethyl acetate, and 1 ml was injected at 250 °C using the split mode (ratio 5:1, 6 ml/min). Separation was carried out on an HP-5MS 30-m 3 250-mm 3 0.25-mm column (Agilent Technologies) using helium (1.2 ml/min) as a carrier gas. The elution program was set up from 100 °C to 300 °C at 20 °C/min. Temperatures of the transfer line and the ion source were fixed at 280 °C and 275 °C, respectively. Methane (1.5 ml/min) was used as the reacting reagent. Data were acquired in selected ion monitoring mode. N-acetyl n-propyl [^{13}C]leucine (charge/mass ratio 217.2) to N-acetyl n-propyl-leucine (charge/mass ratio 216.2) response ratios were calculated using Chromeleon 7.2.9 software (ThermoFisher Scientific).

Modeling

ApoAI data were analyzed with the Simulation Analysis and

Modeling (SAAM) II program (SAAM Institute, Inc., Seattle, WA). The protocol for modeling was the same as that of reference 6. We used the following monoexponential function: A(t) = Ap (1-exp[-k(t-d)]), where A(t) is the apoAI enrichment at time t, Ap is the apoAI enrichment at the plateau, d is the delay between the beginning of the experiment and the appearance of tracer in apoAI, and k is the apoAI fractional synthetic rate [6]. It was assumed that the majority of apoAI is synthesized by the liver [25]. Thus, the enrichment of the leucine precursor pool for apoAI is the same as that for VLDL apoB, and it was assumed to correspond to the tracer-to-tracee ratio at the plateau of the VLDL1 apoB curve. Because our study was performed in the steady state, the FCR equals the fractional synthetic rate. ApoAI PR was calculated as the product of its FCR and ApoAI plasma concentration, as previously done for obese patients with T2D [5]. The pool size of HDL apoAI was calculated by averaging apoAI plasma measurements at four different times (0, 2, 6, and 10 h after the beginning of the tracer infusion). HDL apoAI was estimated from plasma measurements because chylomicron apoAI concentration can be neglected, and thus doing, the approximation is far less important than measuring apoAI in the HDL fractions after ultracentrifugation, in which recovery is not 100 % since some apoAI is lost in infranatant.

Biochemical analysis

Glycemia, total cholesterol, HDL-C, TGs, total proteins, and apoAI were quantitated on a Vista analyzer with dedicated reagents (Siemens Healthcare Diagnostics, Deerfield, IL). LDL-C was calculated using the Friedewald formula since serum TG levels were below 3.88 mmol/l. HbA 1c was measured by high-performance liquid chromatography with a G8 HPLC Analyzer (Tosoh Bioscience, Tokyo, Japan). Free cholesterol and phospholipids on HDL were measured on the Vista analyzer using reagents from Diasys (Condom, France). Esterified cholesterol was calculated as the difference between total and free cholesterol expressed in grams per liter and multiplied by 1.68. Insulin was quantified with a chemiluminescent method on an IMMULITE 2000 XPi analyzer (Siemens Healthineers) with dedicated reagents. TGs during kinetics are the mean of triglyceridemia 0, 2, 6, and 10 h after the beginning of the tracer infusion. The HOMA-IR score, which was used to estimate the degree of insulin resistance, was calculated using the following equation: fasting serum glucose (mmol/l) x fasting serum insulin (mU/l) / 22.5. Plasma adiponectin was measured with a sandwich enzyme immunoassay (Quantikine ELISA, R&D Systems, Minneapolis, Minn).

Statistical analysis

Data are reported as means ± SD. Statistical calculations were performed using the Jasp software package (version 0.17.2.0). Due to the small sample size, continuous data were compared between T6 months and baseline using the non-parametric Wilcoxon signed-rank test. For triglyceridemia, BMI, body weight, fasting glycemia, HbA1c and HOMA, literature usually reports a decrease induced by liraglutide. Thus, Wilcoxon test was performed based on this hypothesis for these parameters (unilateral test). For others parameters a bilateral test was used. Correlations were analyzed using the univariate Spearman test. A probability level of 0.05 was accepted as statistically significant.

Results

Clinical and biological parameters

The clinical and biological parameters of the patients with T2D at baseline and after 6 months of treatment with liraglutide are presented in Table I. Liraglutide induced a significant decrease in BMI (−4.6 %, $P = 0.012$), fasting glucose (−42.8 %, $P < 0.001$), HbA1c (−2.5 points when expressed in%, $P = 0.003$), HOMA-IR (−52 %, $P = 0.003$) and fasting TGs (−29 %, $P < 0.001$). Fasting LDL-C and HDL-C concentrations were not modified. The decrease in TGs during the kinetics, was only 12 % on average and was at the limit of significance ($P = 0.053$).

Table I
Clinical and biological characteristics of the patients at baseline and after 6 months of treatment with liraglutide.

	Baseline	After 6 months treatment with liraglutide	*P*
Age (years)	48.6 ± 10.9		
Male/female (n)	5/5		
BMI (kg/m^2)	36.6 ± 4.9	34.9 ± 4.7	0.012
Body weight (kg)	104.9 ± 19.7	100.5 ± 19.6	0.024
Fasting glucose (mmol/l)	11.11 ± 4.68	6.36 ± 1.27	< 0.001
HbA1c (%)	9.6 ± 2.6	7.1 ± 1.1	0.003
HOMA-IR	11.6 ± 11.2	5.5 ± 1.9	0.003
Total cholesterol (mmol/l)	5.09 ± 0.43	4.56 ± 0.58	0.003
LDL-cholesterol (mmol/l)	3.04 ± 0.47	2.75 ± 0.56	0.105
HDL-cholesterol (mmol/l)	0.97 ± 0.19	0.97 ± 0.26	1
Fasting triglycerides (mmol/l)	2.48 ± 0.69	1.76 ± 0.37	< 0.001
Triglycerides during kinetics (mmol/l)	2.66 ± 0.65	2.34 ± 0.81	0.053
Adiponectin (µg/l)	2618 ± 1651	3169 ± 1561	0.160

Results are means ± SD.
For BMI, body weight, fasting glucose, HbA1c, HOMA-IR and triglycerides, Wilcoxon test was performed based on the hypothesis that the value for these parameters should decrease (unilateral test).
Triglycerides during kinetics are the mean of triglyceridemia 0, 2, 6, and 10 h after the beginning of the tracer infusion.

Lipoprotein composition

The composition of HDL before and after 6 months of treatment with liraglutide is shown in Table II. Liraglutide treatment did not induce any change of the proportion of esterified cholesterol, free cholesterol, phospholipids, triglycerides or total proteins in HDL. The TG-to-cholesteryl ester ratio remained unchanged.

HDL apoAI kinetic parameters

HDL apoAI kinetic parameters are shown in Table III. Liraglutide treatment did not induce any change in the HDL apoAI pool, FCR or PR.

Table II
HDL composition at baseline and after 6 months of treatment with liraglutide.

	Baseline	After 6 months treatment with liraglutide	*P*
Esterified cholesterol (%)	17.63 ± 1.88	15.93 ± 2.67	0.131
Free cholesterol (%)	2.36 ± 0.22	2.33 ± 0.47	0.557
Phospholipids (%)	23.02 ± 2.29	23.91 ± 1.33	0.322
Triglycerides (%)	5.39 ± 1.07	5.13 ± 1.73	0.386
Total proteins (%)	51.61 ± 2.70	52.60 ± 2.31	0.557
Triglyceride/esterified cholesterol	0.31 ± 0.07	0.34 ± 0.18	0.922

Results are means ± SD.
Results are expressed as the percent of total mass.

Table III
ApoAI kinetics parameters at baseline and after 6 months of treatment with liraglutide.

	Baseline	After 6 months treatment with liraglutide	*P*
Plasma pool (g/l)	1.29 ± 0.14	1.26 ± 0.19	0.386
FCR (pool/day)	0.38 ± 0.11	0.35 ± 0.11	0.375
PR (g/l/day)	0.49 ± 0.15	0.44 ± 0.13	0.375

Results are mean ± SD.
FCR: Fractional Catabolic Rate; PR: Production Rate.

Correlations

At baseline, apoAI FCR and apoAI PR were correlated with none of the following parameters: fasting triglyceridemia, triglyceridemia during kinetics, HDL-C, HDL TG, HDL TG/CE ratio, BMI, HOMA, HbA1c fasting glycemia and plasma adiponectin. After 6 months of liraglutide, no significant correlation was observed between these parameters and apoAI FCR or apoAI PR. For the difference between T6 months and baseline for the different parameters cited above, no significant correlation was oberved with the difference in apoAI FCR or apoAI PR.

Discussion

T2D is characterized by a low plasma HDL-C and an increase in HDL catabolism which can be evidenced by accelerated HDL apoAI catabolism. Since hypertriglyceridemia, insulin resistance and low adiponectin level had been suggested to play a great role in this hypercatabolism, we thought that the partial corrections of these abnormalities in patients with T2D treated with 1.2 mg/day of liraglutide could lead to a partial correction of HDL apoAI hypercatabolism. Contrary to our hypothesis, we observed no change in HDL apoAI kinetic parameters despite moderately decreased triglyceridemia and improved insulin sensitivity.

The dyslipidemia observed in our population was typical of the dyslipidemia usually observed in patients with T2D, characterized by a moderate hypertriglyceridemia and a low HDL-C level [3]. Liraglutide at the dose of 1.2 mg/day greatly improved insulin sensitivity and glycemic control, as usually observed [26]. It also induced a decrease in triglyceridemia, both in the fasting and fed states. HDL-C levels remained unchanged in our study. In the literature, liraglutide-induced increases in HDL-C concentration are variable and of low significance [27,28]. For plasma adiponectin concentrations, the moderate increase after 6 months of treatment with liraglutide did not reach significance, but it should be noted that other clinical studies also reported a lack of change in plasma adiponectin level on liraglutide [29].

The present kinetic study was performed similarly to those previously published by our team [5,6]. Feeding the T2D patients during the kinetics was of particular interest considering that this is the most frequent state for humans during a 24 h period and because GLP-1 receptor agonists have been shown to reduce postprandial triglyceridemia in patients with T2D [17]. Here, both FCR and PR of HDL apoAI were very similar to previously obtained measurements in patients with T2D, keeping in mind that they were much higher than in healthy control subjects [5,6].

Strong experimental arguments support the role of the enrichment in TG in the acceleration of HDL apoAI catabolism. Indeed, hypertriglyceridemia stimulates the transfer of TG molecules from apolipoprotein B-containing lipoproteins to HDL. Once in HDL, TG are hydrolyzed by hepatic lipase, leading to thermodynamically unstable HDL and structural modifications of apoAI, which facilitates its dissociation from the surface of HDL [7,8]. In the present study, the decrease in triglyceridemia on liraglutide treatment did not lead to a decrease in the TG content of HDL. The same observation has been reported for insulin therapy in patients with T2D, where insulin had no effect on HDL apoAI hypercatabolism, despite a decrease in triglyceridemia [5]. Triglyceridemia is actually a limiting factor for the exchange of neutral lipids between lipoproteins by the cholesteryl ester transfer protein only in normal triglyceridemia [30]. The threshold of 1.63 mmol/l has been suggested by Caixas et al. [31]. In line with this affirmation, our team observed no change in cholesteryl ester transfer protein activity in patients with T2D in which insulin therapy induced a decrease in triglyceridemia from 3.18 to 2.08 mmol/l [5]. In the present study, despite a liraglutide-induced decrease in triglyceridemia, it remained slightly elevated and above the threshold proposed by Caixas et al. [31], which is likely to explain the lack of decrease in the TG content in HDL. This probably importantly contributes to the persistence of apoAI FCR at its initial value.

After 6 months of treatment with liraglutide, the patients with T2D in the present study experienced a moderate decrease in BMI and considerably improved insulin sensitivity since HOMA dropped from 11.6 to 5.5 on average. However, the patients were still obese and HOMA remained far above the threshold for normality. An accelerated HDL apoAI catabolism has been observed in non diabetic obese patients with normal fasting triglyceridemia, suggesting that factors associated with obesity or insulin resistance are also implied in apoAI hypercatabolism [11,12]. This is likely to be important, since it can reach + 30 to 50%, compared to control subjects. In reference 11, HOMA was equal to 4.1 (*vs* 0.9 in the control group). The precise mechanisms underlying such an hypercatabolism remain to be clarified. If we look carefully at these studies, although normal, fasting triglyceridemia was higher in obese subjects than in controls [12], or postprandial triglyceridemia was higher in obese subjects despite similar fasting triglyceridemia [11]. Interestingly, in the latter study, HDL were TG-enriched. Thus, impairment of triglyceride metabolism, even if moderate, could partly explain the effect of insulin resistance on apoAI catabolism.

In addition to the enrichment of HDL in TG, low plasma adiponectin concentrations, which is a key feature of insulin resistance, have been shown to be associated with apoAI hypercatabolism. In multivariate regression analysis adiponectin appeared as an important independent determinant of HDL apoAI FCR [9]. However, to our knowledge, no precise mechanism allows to explain this statistical association. Beyond the lack of decrease in the enrichment of HDL in TG, the responsibility of insufficient weight loss or improvement in insulin sensitivity, as well as of the lack of significant increase in plasma adiponectin, for the persistence of apoAI hypercatabolism on liraglutide treatment, is difficult to establish, especially because these abnormalities are usually linked, even if changes in plasma adiponectin concentrations are not necessarily correlated with weight loss or the reduction in insulin resistance [15,29]. Looking at previous studies can help us to argue about these points. Ng et al. reported a 13 % reduction in apoAI FCR In patients with metabolic syndrome but normal glycemia after a 12 % weight loss and a BMI that dropped from 35.2 to 30.5 kg/m^2 which is more substantial than in our study [32]. In that study, triglyceridemia was reduced but remained slightly elevated (1.97 mmol/L on average). In addition, the HOMA score after weight loss reached 1.99 on average, which is almost normal, contrary to our patients, in whom the HOMA score was still 5.5 after liraglutide. Interestingly, plasma levels of adiponectin significantly increased in Ng's study, with a negative correlation between changes in adiponectin and apoAI FCR. In another study, apoAI FCR did not change after 16 weeks of exercice in obese subjects who lost 3.6 % of body mass with BMI that dropped from 31.6 to 30.5 kg/m^2, with a decrease in triglyceridemia of 12 % and unchanged plasma adiponectin [33]. Altogether, these results suggest that an important weight loss with an insulin sensitivity close to normal values, and/or increase in adiponectin are necessary to partially correct apoAI hypercatabolism, given that the correction remains of low significance in the presence of elevated triglyceridemia, even if the elevation is weak.

Here, the HDL apoAI PR was very similar to previous assessments in patients with T2D, given that it was significantly increased compared to

healthy controls [5]. The reasons for this increase are far from being elucidated. It may be a feedback response to the decrease in the circulating apoAI concentration relative to accelerated catabolism. Since insulin downregulates apoAI transcription, it may also be the consequence of insulin resistance [34]. Considering that liraglutide had no effect on apoAI FCR and since it only partially corrected insulin resistance, the lack of effect of liraglutide on apoAI PR may not be surprinsing.

As a limitation of this study, we acknowledge that the number of participating patients is small. But it is similar to that of many *in vivo* kinetic studies because these studies are demanding and time consuming. However there is little chance that a small sample size prenvented us to observe liraglutide-induced changes in HDL apoAI FCR or PR, since we did not observe any tendancy. Patients are paired to themselves, which reinforces statistical power. It must be noted that the changes usually induced by liraglutide on metabolic parameters (TGs, glycemia …) have been observed in the present study, despite small sample size.

In conclusion, 6 months of treatment with 1.2 mg/day of liraglutide changed neither HDL apoAI FCR, nor HDL apoAI PR. The lack of decrease in the enrichment of HDL in TG related to an only moderate decrease in triglyceridemia without normalization is likely to play an important role in the persistence of these abnormalities. The lack of normalization of insulin sensitivity (even if it was improved) and of significant changes of plasma adiponectin concentration may also contribute to the persistence of apoAI hypercatabolism. The persistence of HDL apoAI kinetics abnormalities in patients with T2D treated by liraglutide is consistent with the observation that serum HDL cholesterol concentration is unchanged or only weakly increased.

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CRediT authorship contribution statement

Laurence Duvillard: Writing – original draft, Methodology, Formal analysis, Data curation. **Jean-Paul Pais de Barros:** Formal analysis. **Alexia Rouland:** Writing – review & editing, Investigation. **Isabelle Simoneau:** Writing – review & editing, Investigation. **Damien Denimal:** Writing – review & editing, Formal analysis. **Benjamin Bouillet:** Writing – review & editing, Investigation. **Jean-Michel Petit:** Writing – review & editing, Investigation. **Bruno Vergès:** Writing – review & editing, Funding acquisition, Conceptualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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