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EXENDIN-4 DECREASES HEPATIC VLDL-TG PRODUCTION INDEPENDENTLY OF DIETARY FAT INTAKE AND CENTRAL GLP-1 RECEPTOR SIGNALING

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In preparation

ABSTRACT

Chronic subcutaneous treatment with the synthetic glucagon-like peptide-1 (GLP-1) receptor agonist exendin-4 (EX4) lowers hepatic very low-density lipoprotein-triglyceride (VLDL-TG) production in high-fat diet-fed mice, in addition to reducing body weight (BW) and plasma glucose levels. In the current study, we investigated whether this effect of EX4 is dependent on dietary fat intake and central GLP-1 receptor signaling. To study the role of dietary fat intake, male C57Bl/6J mice were fed either a high-fat diet (HFD; 24% w/w bovine fat) or low-fat diet (LFD; 4.2% w/w bovine fat) for three weeks before the start of chronic subcutaneous EX4 or vehicle treatment. EX4 decreased plasma levels of TG (-43%, $P<0.001$) and total cholesterol (-19%, $P<0.05$) in mice fed a LFD, but did not affect plasma lipid levels in HFD-fed animals. Furthermore, EX4 caused a strong reduction in hepatic VLDL-TG production in both LFD-fed mice (-37%, $P<0.001$) and HFD-fed mice (-23%, $P=0.08$) compared to their respective controls. To study the role of central GLP-1 signaling, mice were fed the HFD for three weeks before peripheral EX4 or vehicle administration with concomitant central infusion of the GLP-1 receptor antagonist exendin-9 (EX9) or vehicle. Central EX9 administration did not affect the EX4-induced decrease in hepatic VLDL-TG production in these mice. EX4 lowers hepatic VLDL-TG production irrespective of dietary fat content and without involvement of central GLP-1 receptor signaling.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has reached epidemical proportions. T2DM is hallmarked by insulin resistance, pancreatic β-cell dysfunction and glucose intolerance [1]. In addition, T2DM is associated with dyslipidemia caused by various disturbances in plasma lipid and lipoprotein metabolism, including increased hepatic apolipoprotein B (apoB) and triglyceride (TG) production [2].

Glucose homeostasis is maintained by the interaction between various hormones, including insulin and glucagon-like peptide-1 (GLP-1). Upon food intake, the incretin hormone GLP-1 is secreted by intestinal L cells and the brain in order to stimulate insulin secretion by the pancreas [3]. In addition, GLP-1 decreases food intake [4], inhibits pancreatic glucagon secretion and slows down gastric emptying [5]. GLP-1 mediates its effects via the GLP-1 receptor, a 7-transmembrane-spanning G-protein-coupled receptor that is abundantly expressed in various tissues [6], including the gastrointestinal tract, pancreatic islands, kidneys, heart and central nervous system [7]. Because of these effects, activation of the GLP-1 receptor is considered an interesting therapeutic target for the treatment of T2DM. As GLP-1 is rapidly degraded by dipeptidylpeptidase-4 (DPP-4) in plasma, current GLP-1-based therapies for the treatment of T2DM include inhibitors of DPP-4 as well as synthetic GLP-1 receptor agonists that are resistant to DPP-4-mediated degradation [3], both resulting in an increased GLP-1 receptor agonistic activity.

Recently, we showed that the synthetic GLP-1 receptor agonist exendin-4 (EX4), improves lipid metabolism in mice. Specifically, EX4 decreased hepatic VLDL-TG production in high-fat diet (HFD) fed wild-type and APOE*3-Leiden mice, both on a C57Bl/6J background [8]. We showed that EX4 decreased hepatic lipogenesis, which may underlie the reduction in VLDL-TG production. However, in addition to reducing VLDL production by the liver, EX4 decreases intestinal chylomicron secretion in both rodents [9] and humans [10]. As chylomicron remnants are a major source of hepatic TG, which in turn is the chief substrate regulating hepatic apoB secretion [11], increased dietary fat uptake by the intestine might subsequently lead to increased VLDL production by the liver. Therefore, in theory, EX4 may reduce VLDL-TG production secondarily to the reduction in intestinal TG absorption, and subsequent flux of TG to the liver.

In addition, we recently showed that subcutaneously (s.c.) administered GLP-1 reduces hepatic glucose production in mice partly dependent on activation of central GLP-1 receptors, since concomitant intracerebroventricular (i.c.v.) infusion of the GLP-1 receptor antagonist exendin-9 (EX9) partly blunted this effect [12]. We hypothesized that EX4 may thus not only reduce hepatic glucose production dependent on central GLP-1 receptor signaling, but also VLDL-TG production.

In the current study, we aimed to further unravel the mechanisms by which EX4 reduces hepatic VLDL production, including the role of dietary fat intake and central GLP-1 receptor signaling. Therefore, we investigated whether EX4 lowers hepatic VLDL production in mice fed a low-fat diet (LFD) as compared to HFD. In addition, we investigated whether concomitant i.c.v. infusion of EX9 in HFD-fed mice abolishes the effect of peripheral administered EX4.

MATERIALS AND METHODS

Animals

For all experiments, 12 weeks old male C57Bl/6J mice were used, housed in a temperature and humidity-controlled environment with free access to food and water. Experiments were performed after 4 h of fasting at 12:00 pm with food withdrawn at 8:00 am. Body weight (BW) was measured weekly during experiments. All animal experiments were approved by the Animal Ethics Committee of the Leiden University Medical Center, Leiden, The Netherlands.

Experiments

Two experiments were performed, both individually designed to investigate a specific aspect of the general research aim.

In the first experiment, animals were fed either a HFD (24% (w/w) fat, derived from bovine fat; AB Diets, Woerden, The Netherlands) or a LFD (4.2% (w/w) fat, derived from bovine fat; AB Diets, Woerden, The Netherlands). For macronutrient composition, we refer to Supplemental Table 1. After a diet run-in period of three weeks, mice were divided into four different groups, based on body weight and plasma glucose and TG levels. An osmotic minipump (model 1004, Alzet DURECT Corp., Cupertino, CA) was implanted s.c. in the left back region under light isoflurane anesthesia for the continuous delivery of exendin-4 (Sigma, St. Louis, MO; 50 μg/kg BW/day dissolved in PBS) or PBS as a control for 4 weeks, while HFD or LFD feeding was continued. At the end of the treatment period, plasma metabolic parameters and hepatic VLDL production was determined as described below.

In the second experiment, we implanted mice with a brain infusion kit in the left lateral ventricle of the brain as described before [12]. The brain infusion kit was connected to a subcutaneously positioned osmotic minipump in the left back region, to enable continuous infusion of either artificial cerebrospinal fluid (aCSF; control) or the GLP-1 receptor antagonist EX9 (Sigma, St. Louis, MO; 0.72 nmol/kg/day, dissolved in aCSF) for a total of three weeks. All animals received aCSF during the first week of recovery. After a week of recovery, the animals received a second osmotic minipump in the right back region, to enable concomitant continuous administration of either EX4 (50 μg/kg BW/day) or vehicle (PBS) for the remaining three weeks of i.c.v. drug administration. This setup rendered four experimental groups: PBS+aCSF (control), EX4+aCSF (EX4), PBS+EX9 (EX9) and EX4+EX9. At the end of the treatment period, plasma metabolic parameters and hepatic VLDL production was assessed as described below.

Blood sampling and plasma analyses

Blood was collected by tail bleeding into chilled capillary tubes coated with paraoxon (Sigma, St. Louis, MO) to prevent ongoing *in vitro* lipolysis [13]. The tubes were placed on ice and centrifuged, and the obtained plasma was snap-frozen in liquid nitrogen and stored at -20°C for further measurements. Plasma was assayed for glucose, TG and total cholesterol (TC) levels using commercially available enzymatic kits according to the manufacturers' protocols (Glucose: Glucose hexokinase kit, Instruchemie, Delfzijl, The Netherlands; TG and TC: no. 11488872 and 236691, Roche Molecular Biochemicals, Indianapolis, IN).

Hepatic VLDL-TG and VLDL-apoB production

Four hour-fasted mice were anesthetized with 6.25 mg/kg BW Acepromazine (Alfasan, Woerden, The Netherlands), 6.25 mg/kg BW Midazolam (Roche, Mijdrecht, The Netherlands), and 0.31 mg/kg BW Fentanyl (Janssen-Cilag, Tilburg, The Netherlands). A basal blood sample was taken from the tail tip in a chilled capillary, and subsequently mice received an intravenous injection of 100 µl PBS containing 20 µCi Tran^{35}S label (MP Biomedicals, Eindhoven, the Netherlands) via the tail vein, resulting in incorporation of ^{35}S into newly produced VLDL-apoB. After 30 min, the animals received an intravenous injection of tyloxapol (500 mg/kg BW; Triton WR-1339, Sigma), as a 10% (w/w) solution in sterile saline, to prevent systemic lipolysis of newly secreted hepatic VLDL-TG [14].

Blood samples were taken from the tail tip into chilled capillaries at the indicated time points up to 90 min after tyloxapol injection. The tubes were kept on ice after which they were centrifuged at 4°C. Plasma TG concentration was determined as described above. At 120 min after tyloxapol injection, the animals were sacrificed and blood was collected by orbital bleeding for isolation of VLDL by density gradient ultracentrifugation [15]. ^{35}S-activity was measured in the VLDL fraction and VLDL-apoB production rate was calculated as dpm.h^{-1} [16].

Statistical analyses

Differences between two groups were determined with Mann-Whitney non-parametric tests for two independent samples. Differences between multiple groups were determined with the Kruskal-Wallis non-parametric test for k independent samples. When significant differences were found, the Dunn's Multiple Comparisons test was used as a follow-up test to determine differences between two independent groups. A p-value of less than 0.05 was considered statistically significant. Data are presented as means ± SEM.

RESULTS

Exendin-4 reduces hepatic VLDL-TG production independent of dietary fat intake.

To evaluate whether reduction of dietary fat intake curtails the effect of EX4 on hepatic VLDL production, mice were fed with either a HFD (24% w/w) or a LFD (4.2% w/w) for three weeks before concomitant treatment with either vehicle (control) or EX4 for 4 weeks. In HFD-fed animals, EX4 reduced BW gain and plasma glucose levels, whereas plasma TG and TC levels were unchanged (Table 1) compared to control animals. In addition, EX4 tended to reduce hepatic VLDL-TG production rate (Figure 1A) albeit that statistical significance was just not reached (-23%, P=0.08; Figure 1B). EX4 did not affect hepatic VLDL-apoB production rate (Figure 1C).
In LFD-fed animals, EX4 decreased both absolute BW and BW gain (Table 1) compared to control animals. Interestingly, EX4 did not affect plasma glucose levels, whereas it decreased both plasma TG levels (-43%, P<0.001) and TC levels (-19%, P<0.05) (Table 1). In addition, EX4 also potently decreases hepatic VLDL-TG production in LFD-fed mice (-37%, P<0.001; Figure 1D,E). Furthermore, like in HFD-fed animals, EX4 did not affect hepatic VLDL-apoB production rate (Figure 1F).

Table 1. Exendin-4 improves metabolic parameters in both high-fat and low-fat diet-fed mice. Body weight and plasma parameters in high-fat and low-fat diet-fed mice after 4 weeks of treatment with vehicle (control) or exendin-4 (EX4). Values are means ± SEM, n=8 per group. *P<0.05, **P<0.01, ***P<0.001 compared to control. BW, body weight; TG, triglycerides; TC, total cholesterol.

	High-fat diet		Low-fat diet	
	Control	EX4	Control	EX4
BW (g)	33.2 ± 0.8	31.4 ± 1.0	29.5 ± 0.6	27.0 ± 0.4**
BW gain (g)	+1.7 ± 0.2	-1.3 ± 0.6***	+1.1 ± 0.3	-0.4 ± 0.6*
Glucose (mM)	10.1 ± 0.4	7.4 ± 0.3***	9.2 ± 1.0	7.5 ± 0.7
TG (mM)	0.6 ± 0.0	0.6 ± 0.0	0.7 ± 0.0	0.4 ± 0.0***
TC (mM)	3.8 ± 0.3	3.8 ± 0.2	3.6 ± 0.2	2.9 ± 0.2*

The effect of exendin-4 on hepatic VLDL-TG production does not require central GLP-1 receptor signaling.

To evaluate whether the EX4-induced decrease in hepatic VLDL-TG production is mediated by central GLP-1 receptor signaling, HFD-fed mice were implanted with a brain infusion kit, ensuring chronic i.c.v administration of either EX9 or vehicle (control) for four weeks in combination with peripheral EX4 or vehicle (control) administration. As expected, EX4 reduced BW as compared to controls (Table 2). In contrast, EX9 and EX4+EX9 treated animals did not show a significant BW reduction

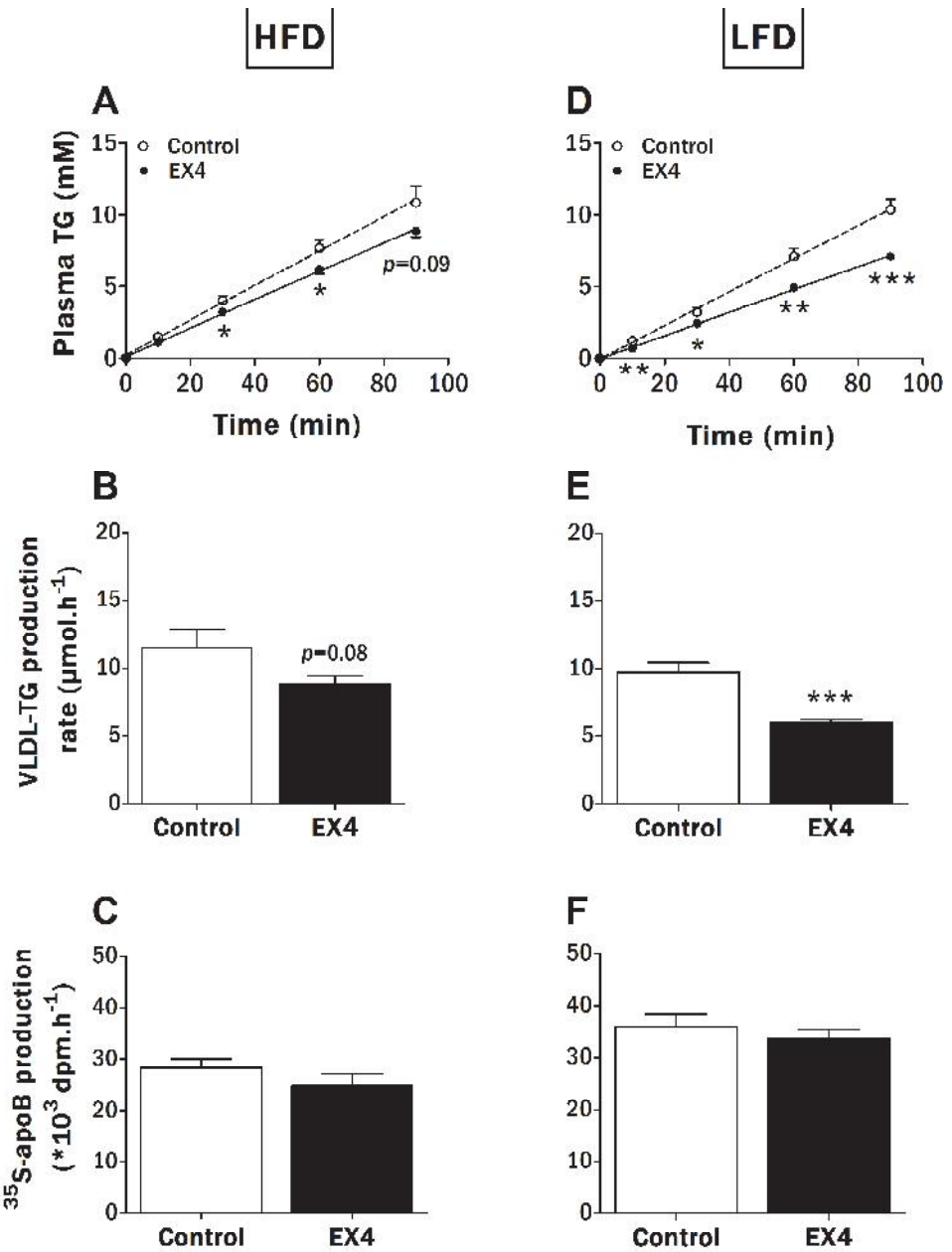

Figure 1. Exendin-4 decreases hepatic VLDL-TG production in both high-fat and low-fat diet-fed mice. Both HFD-fed (A-C) and LFD-fed (D-F) mice received either vehicle (control) or exendin-4 (EX4) via subcutaneous osmotic minipumps for four weeks, after which hepatic VLDL-TG production was assessed. After a 4 h fast, mice were anesthetized and received consecutive i.v. injections of Tran^{35}S (t=-30 min) and tyloxapol (t=0 min). Plasma triglyceride (TG) levels were determined at indicated time points (A,D). VLDL-TG production rates were calculated from the slopes of the individual TG-time graphs (B,E). At t=120 min, mice were exsanguinated and VLDL fractions were isolated from serum by ultracentrifugation. ^{35}S-apoB production was determined by scintillation counting of the isolated VLDL fraction (C, F). Values are means ± SEM (n = 6-8). ⋆ P<0.05, ⋆⋆ P<0.01, ⋆⋆⋆ P<0.001 compared to controls.

compared to control animals (Table 2). Although both BW gain and plasma glucose levels tended to be lower in EX4 treated animals, overall group median variance testing failed to reach statistical significance (P=0.16 and P=0.06 respectively; Table 2). Unpaired t-testing, however, did show a significant reduction in BW for EX4-treated animals (-138%, P<0.05) and a significant reduction in glucose levels for both EX4 (-23%, P<0.05) and EX4+EX9-treated animals (-32%, P<0.05) compared to controls. Plasma TG did not significantly differ between groups. Interestingly, plasma TC levels tended to be decreased in both EX4 and EX9 only groups, and combined EX4+EX9 treatment significantly decreased plasma TC levels as compared to control treatment (Table 2). Hepatic VLDL-TG production rate was decreased by EX4 treatment (-30%, P<0.05; Figure 2A,B) and combined EX4+Ex9 treatment (-43%, p <0.01; Figure 2A,B). Treatment with EX9 alone did not significantly affect hepatic VLDL-TG production. Hepatic VLDL-apoB production did not differ between groups (Figure 2C).

Table 2. Exendin-9 does not abrogate the exendin-4-induced improvement in metabolic parameters in high-fat diet-fed mice. Body weight and plasma parameters in high-fat diet-fed mice after vehicle (control) or exendin-4 (EX) treatment with or without concomitant central infusion of exendin-9 (EX9). Values are means ± SEM, n=4-8 per group. *p < 0.05 compared to control. BW, body weight; TG, triglycerides; TC, total cholesterol.

	Control	EX4	EX9	EX4 + EX9
BW (g)	30.3 ± 0.8	28.0 ± 0.4*	29.4 ± 0.4	28.0 ± 0.6
BW gain (g)	+0.8 ± 0.5	-1.1 ± 0.6	-1.0 ± 1.0	-1.0 ± 1.3
Glucose (mM)	9.5 ± 0.7	7.3 ± 0.4	8.1 ± 1.1	6.5 ± 0.4
TG (mM)	0.6 ± 0.1	0.5 ± 0.0	0.7 ± 0.1	0.6 ± 0.0
TC (mM)	3.2 ± 0.2	3.0 ± 0.3	3.0 ± 0.2	2.7 ± 0.2*

DISCUSSION

In this study, we showed that in male C57Bl/6J mice fed a HFD, chronic peripheral EX4 administration decreased hepatic VLDL-TG production, without affecting plasma TG and TC levels nor hepatic VLDL-apoB production. The decrease in hepatic VLDL-TG production did not depend on a high dietary fat content, as EX4 also decreased hepatic VLDL-TG production, as well as plasma TG and TC levels, in LFD-fed mice. Furthermore, central EX9 administration did not counteract the EX4-induced decrease in hepatic VLDL-TG production, indicating that this effect of EX4 is not mediated by central GLP-1 receptor signaling.

We previously demonstrated that EX4 reduced VLDL-TG production in male C57Bl/6J mice, both wild-type and APOE*3-Leiden transgenic, explained by a reduction in hepatic lipogenesis as observed in the latter animal model [8]. However, as dietary lipid supply to the liver via chylomicrons is involved in VLDL-TG production [11] and EX4 reduces chylomicron production [9,10], we speculated that EX4 may also reduce VLDL-TG production secondary to a reduction in intestinal TG absorption. In support of this theory, we recently observed that EX4 did not decrease hepatic VLDL-TG production in APOE*3-Leiden.CETP female mice fed a diet high in cholesterol yet low in fat content (Wang *et al.*, unpublished data). However, we observed that EX4 likely caused an even more pronounced decrease in hepatic VLDL-TG production in LFD-fed animals in comparison to HFD-fed animals. Interestingly, the VLDL-TG production rates were comparable between both HFD and LFD control groups, indicating that the HFD itself had no additional effect on the VLDL-TG production rate as compared to the LFD. This is in seeming contrast to our previous study showing a 2.7-fold higher hepatic VLDL-TG production rate in APOE*3-Leiden mice fed the same HFD when compared to their chow-fed littermates [8]. This difference might be related to the composition of the control diets used, as the LFD used in the current study contains a considerably higher amount of carbohydrates as compared to the rodent chow used in the previous study (71% vs 55% w/w, respectively). Carbohydrates stimulate hepatic lipogenesis via activation of the carbohydrate response element binding protein [17], which could explain the higher basal VLDL-TG production rate observed upon LFD compared to chow feeding.

Notably, EX4 reduced plasma TG levels in LFD-fed mice, but not in HFD-fed mice (Table 1). This might be secondary to the stronger decrease in hepatic VLDL-TG production following EX4 treatment in LFD-fed animals, which may ultimately lead to a reduction in plasma TG levels. Alternatively, EX4 might increase TG clearance from the plasma in these animals, hereby reducing plasma TG levels. In favor of this notion, Lockie and colleagues [18] recently showed that stimulation of central GLP-1 receptor signaling directly stimulates thermogenesis in brown adipose tissue (BAT) in mice, suggesting that the decrease in plasma TG levels we observed in our LFD-fed animals might be caused by increased TG clearance by BAT. In this case, increased fat supply by HFD may thus overrule the TG clearance capacity of BAT and not result in lowering of plasma TG. However, a recent study in normolipidemic, normoglycemic men showed no effect of EX4 treatment on plasma TG clearance rates [10]. Therefore, to conclusively answer the proposed hypothesis, further research should be aimed at delineating the differences in TG fluxes, arising from both liver and intestine and directed towards oxidative tissues such as muscle and BAT, after EX4 treatment in both HFD and LFD-fed WT mice.

Although EX4 tended to lower hepatic VLDL-apoB production in both HFD and LFD-fed mice, this decrease was not significant in both groups. This suggests that in wild-type C57Bl/6J mice, EX4 decreases the lipidation of apoB within VLDL particles during synthesis rather than reducing whole-particle production by the liver. This is

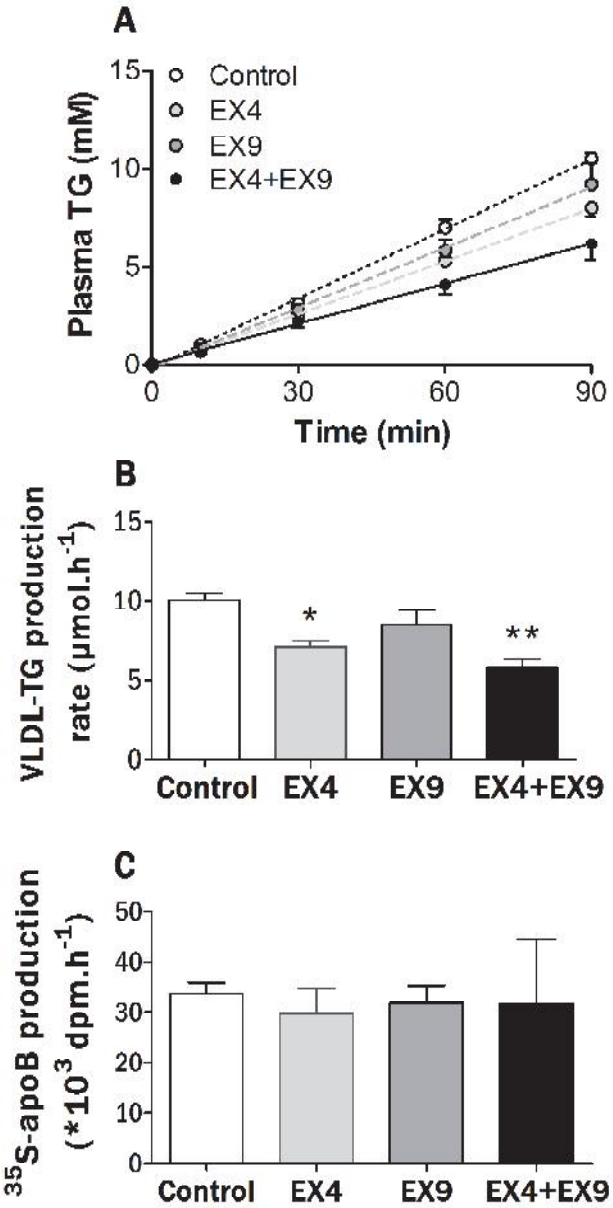

Figure 2. Exendin-4 decreases hepatic VLDL-TG production indpendent of central GLP-1 receptor signaling. HFD-fed mice received either vehicle (control) or exendin-4 (EX4) via subcutaneous osmotic minipumps for four weeks, while being continuously i.c.v. infused with vehicle (control) or exendin-9 (EX9), after which hepatic VLDL-TG production was assessed. After a 4 h fast, mice were anesthetized and received consecutive i.v. injections of Tran^{35}S (t=-30 min) and tyloxapol (t=0 min). Plasma triglyceride (TG) levels were determined at indicated time points (A). VLDL-TG production rates were calculated from the slopes of the individual TG-time graphs (B). At t=120 min, mice were exsanguinated and VLDL fractions were isolated from serum by ultracentrifugation. ^{35}S-apoB production was determined by scintillation counting of the isolated VLDL fraction (C). Values are means ± SEM (n = 2-8). * P<0.05, ** P<0.01 compared to controls.

in contrast to our previous findings in APOE*3-Leiden mice, where EX4 reduced both VLDL-TG and -apoB production [8]. This discrepancy might be explained by the differences in genetic background of the mouse strains. Hepatic VLDL-TG and VLDL-apoB production are both decreased in APOE*3-Leiden mice, and the VLDL particles produced contain relatively lower amounts of TG [19]. The differential effects of EX4 in APOE*3-Leiden versus WT mice might be related to these differences in hepatic lipid metabolism caused by the APOE*3-Leiden mutation.

GLP-1 receptor agonism, *e.g.* via EX4 treatment, reduces plasma glucose levels and body weight gain in both humans [20,21] and rodents [22,23]. Its effects on plasma TG levels, however, are less well-defined. In line with previous rodent studies [24],(Parlevliet *et al.*, unpublished data) we report that EX4 treatment does not affect plasma TG or TC concentrations in HFD-fed mice (Table 1 and 2). Strikingly, EX4 did decrease plasma TG as well as TC levels in mice fed a LFD (Table 1). These results corroborate findings of our previous study, showing that EX4 decreased plasma TG and TC levels in APOE*3-Leiden.CETP female mice fed a high-cholesterol but low-fat diet (Wang *et al.*, *submitted*; see **chapter 6**). Interestingly, a recent study by Lee and colleagues [25] did show a reduction in serum TG as well as fatty acid levels in HFD-fed wild-type mice after repeated i.p. injections with EX4 given every other day for 10 weeks. This discrepancy might be explained by the longer treatment period applied in that study, in which long-term suppression of hepatic VLDL-TG production ultimately may have led to decreased plasma TG levels. Strikingly, daily i.p. injection of the GLP-1 analogue liraglutide for only four weeks decreased plasma TG and TC levels in WT mice fed a high-fat high-sugar diet for 8 weeks [26]. Therefore, it would be highly interesting to further investigate the effects of EX4 on lipid metabolism under various lipidemic conditions related to the dietary macronutrient content, as well as the time of onset of these effects, in a dedicated study. In view of this, it is interesting to note that two weeks of continuous EX4 treatment already reduced BW gain and hepatic VLDL-TG production in our current HFD-fed wild-type mouse model, whereas plasma TG and TC levels again were not affected (Supplemental Table 2, Supplemental Figure 1).

Regardless of the fat content of the diet, EX4 decreased VLDL-TG production which could be mediated via central GLP-1 receptor signaling, in view of our recent observation that GLP-1 reduces hepatic glucose production dependent on central signaling [12]. However, we here show that central GLP-1 receptor signaling is not a likely key mediator of the EX4-induced reduction of VLDL-TG production, as chronic blockade of central GLP-1 receptor signaling by i.c.v. administration of EX9 did not abolish the effects of peripheral administered EX4. In support of this, *in vitro* studies with primary human hepatocytes showed that EX4 reduces hepatocyte TG stores by activation of GLP-1 receptors present on these hepatocytes [27], underscoring a direct effect of EX4 on lipid metabolism in hepatocytes. Nogueiras and colleagues [28] did show that continuous central GLP-1 administration in mice decreased the hepatic expression of lipogenic genes, thus suggesting that increased central GLP-1

receptor signaling could decrease hepatic VLDL-TG production. This effect, though, was secondary to a decrease in body weight resulting from the inhibition of food intake caused by central GLP-1 receptor activation [28]. Interestingly, a recent study showed that acute i.c.v. administration of EX4, similar to acute intraperitoneal administration, reduced hepatic VLDL-TG production in mice fed a HFD for four weeks [29], showing that hepatic VLDL production can, at least acutely, be regulated by central GLP-1 receptor signaling. The underlying mechanism of action, however, remains elusive. Importantly, the brain can also be indirectly involved in the effects of peripherally administered EX4, as EX4 can bind and activate vagal sensory afferents in the hepatoportal region and in the liver tissue. These afferents project to neurons in the nucleus of the solitary tract of the brain stem, which in turn can activate ascending fibers to generate a response in the hypothalamus (*for review* [7]). Therefore, experiments combining either central or peripheral EX4 administration with hepatic denervations are essential to irrefutably determine the role of the brain in the EX4-mediated regulation of hepatic VLDL-TG production.

The current treatment strategy for T2DM encompasses pharmacological treatment aiming to alleviate insulin resistance and glucose intolerance, combined with restricting energy intake and increasing physical activity [30]. Our data clearly show that EX4 can reduce body weight, plasma glucose levels as well as hepatic lipid production regardless of dietary fat intake. As long-term adherence to lifestyle modifications remains a major drawback, EX4 might prove to have additional benefits in the treatment of T2DM, especially in patients displaying diabetic dyslipidemia.

In conclusion, the present study shows that the EX4-induced decrease in hepatic VLDL-TG production does not depend on a high dietary fat intake and that central GLP-1 receptor signaling is not a key regulator of this effect. As EX4 is a promising new therapy for T2DM patients displaying diabetic dyslipidemia, it is of high interest to further unravel the exact mechanism behind its lipid-lowering properties.

ACKNOWLEDGEMENTS

This work was supported by research grants from the Netherlands Diabetes Foundation (DFN2007.00.010 to P.C.N.R.) and the Netherlands Heart Foundation (2007B081 to P.C.N.R.). P.C.N. Rensen is an Established Investigator of the Netherlands Heart Foundation (2009T038 to P.C.N.R.).

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