Chapter 4

GLP-1 Mimeticbody, a GLP-1 receptor agonist, decreases VLDL-TG production rate and plasma cholesterol concentration in high-fat-fed APOE*3-Leiden mice

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In preparation
Abstract

GLP-1 Mimetibody (GLP-1M) is a glucagon-like peptide-1 (GLP-1) receptor agonist that incorporates a GLP-1 peptide analogue linked to the Mimetibody™ platform, which has been shown to inhibit VLDL triglyceride production. In the present study, we further evaluate the potential of this compound to modulate lipid metabolism. High-fat-fed APOE*3-Leiden transgenic mice were administered daily i.p. injections of GLP-1M or placebo for 4 weeks. Plasma cholesterol concentrations, lipoprotein profiles, VLDL-TG and VLDL-[35S]apoB production rates and expression of genes involved in hepatic lipid metabolism were determined. GLP-1M lowered total plasma cholesterol levels (GLP-1M: 3.7 ± 0.6; Control: 4.6 ± 0.7 mM, P<0.05) and lipoprotein fractionation showed increased cholesterol levels in the HDL fraction. Moreover, GLP-1M decreased the production rate of VLDL-TG (GLP-1M: 163 ± 33; Control 264 ± 49 µmol/kg/h, P<0.05) and VLDL-[35S]apoB (GLP-1M: 67x10^3 ± 18x10^3 dpm/ml/h, P<0.05). Finally, GLP-1M decreased the hepatic expression of apob (-36%, P<0.05 vs. control). In conclusion, these results indicate that GLP-1M improves VLDL metabolism in high-fat-fed APOE*3-Leiden mice and suggest the potential to ameliorate dyslipidemia in patients with type 2 diabetes.
Introduction

Glucagon-like peptide-1 (GLP-1), an incretin hormone released by the gut in response to meals, stimulates insulin release, inhibits glucagon secretion, slows gastric emptying, and induces satiety\(^1\). Although these features hold promise for the treatment of type 2 diabetes mellitus, native GLP-1 cannot be used as a drug because of its short plasma half-life (several minutes). GLP-1 analogues that are resistant to hydrolysis by dipeptidyl peptidase IV (DPP-IV) have been developed to circumvent this problem\(^6\). Indeed, clinical studies show that they effectively improve glucose metabolism in T2DM patients\(^7\) and several compounds are currently on the verge of marketing as tools to treat the disease. Byetta™ (exendin-4 (Ex-4)) was approved in this context in 2005\(^5\)\(^9\)\(^10\).

Lipid metabolism is also disturbed in patients with T2DM. Increased very low density lipoprotein triglyceride (VLDL-TG) concentrations and decreased high-density lipoprotein (HDL) cholesterol in plasma significantly contribute to the long term risk of macrovascular disease in these patients\(^11\)\(^12\). We have recently shown that chronic treatment with the GLP-1 receptor agonist, CNTO736, apart from its glycemic effects, inhibits VLDL-TG production by the liver in C57Bl/6 mice maintained on a high fat diet\(^13\). Interestingly, Ex-4 did not impact on VLDL-TG production in this insulin resistant mouse model, despite the fact that both Ex-4 and CNTO736 equally and profoundly ameliorated glycemic control. Therefore, CNTO736 may have compound-specific effects on VLDL metabolism that potentially reinforce its capacity to prevent macrovascular disease in high fat-fed mice (and humans). However, several issues need to be clarified before firm conclusions can be drawn with respect to the potential of the drug to modify VLDL-TG metabolism and, thereby, reduce macrovascular risk.

The objective of this study was to further explore the inhibitory effect of CNTO736 on VLDL-TG production. We set out to compare the chronic effects of CNTO736 with the latest version of the molecule, from now on referred to as GLP-1 Mimetibody follow-up molecule (GLP-1M), and Ex-4 on lipid metabolism in APOE*3-Leiden transgenic mice. These mice express the human APOE*3-Leiden gene, resulting in a lipoprotein profile reminiscent of that of patients with dysbetalipoproteinemia, marked by elevated plasma cholesterol and triglyceride levels that are mainly confined to the VLDL/LDL-sized lipoprotein fraction\(^14\). In contrast to other mice, APOE*3-Leiden transgenics respond to various hypolipidemic drugs in a similar way as humans\(^15\) and are therefore considered a “human-like” animal model for studies evaluating the impact of drugs on hyperlipidemia and atherosclerosis\(^14\)\(^16\)\(^17\).
Methods

Animals and diet. Male APOE*3-Leiden transgenic mice (10-11 weeks old) were housed in a temperature and humidity-controlled environment and were fed a high-fat diet (44 energy% fat derived from bovine fat, Hope Farms, Woerden, The Netherlands) with free access to water for 12 weeks to induce insulin resistance\textsuperscript{18}. All animal experiments were approved by the Animal Ethics Committee from the Leiden University Medical Center, Leiden, The Netherlands.

Drugs. GLP-1M was constructed by fusing a GLP-1 peptide analogue to a flexible Gly/Ser linker and a fragment of a V region heavy chain (VH) domain linked directly to the CH2 and CH3 domains of an Fc as described previously for CNTO736\textsuperscript{19}. Ex-4 (molecular weight = 4186.57 g/mol) was purchased from Sigma (St. Louis, MO).

Treatment. After 8 weeks of high-fat-feeding, mice were divided into 6 groups, matched for body weight and fasting plasma triglyceride (TG) concentration. The groups received daily subcutaneous (s.c.) doses of GLP-1M (0.3, 0.1, or 0.03 mg/kg, dissolved in PBS), CNTO736 (0.3 mg/kg, dissolved in PBS), Ex-4 (7.1 µg/kg, dissolved in PBS), or PBS in a volume of 100 µl at 08.00 a.m. during the last 4 weeks on diet. At 08.00 a.m. on the last day of the 12 week high-fat diet period, animals were given a last s.c. dose and the experiments were initiated 7 hours later.

Plasma lipid and lipoprotein analysis. Blood was collected by tail bleeding into chilled paraoxon-coated capillary tubes (Sigma) to prevent ongoing in vitro lipolysis\textsuperscript{20}. The tubes were placed on ice and centrifuged, and the obtained plasma was snap-frozen in liquid nitrogen and stored at -20°C. Plasma was assayed for glucose, FFA, TG, and cholesterol using commercially available enzymatic kits (Instruchemie, Delfzijl, The Netherlands), and insulin concentrations were measured by ELISA (Mercodia AB, Uppsala, Sweden). Lipid distribution over plasma lipoproteins by fast-performance liquid chromatography was determined as described before\textsuperscript{21}. Fractions of 50 µl were collected and assayed for TG and cholesterol as described above.

Hepatic VLDL particle production. Mice were fasted for 7 hours during the light period and anesthetized with an intraperitoneal injection of acepromazine 6.25 mg/kg acepromazine (Alfasan, Woerden, The Netherlands), 6.25 mg/kg midazolam (Roche, Mijdrecht, The Netherlands), and 0.3125 mg/kg fentanyl (Janssen-Cilag, Tilburg, The Netherlands). Mice received an intravenous injection of 100 µl PBS containing 150 µCi Tran\textsuperscript{35}S label (GE Healthcare, Little Chalfont, U.K.) which binds to newly produced
apolipoprotein B (apoB) required for the generation of liver-derived VLDL. After 30 min, the animals received a 15% (by volume) intravenous injection of Triton WR-1339 (500 mg/kg body weight; Tyloxapol, Sigma) to prevent systemic lipolysis of newly secreted hepatic VLDL-TG. Blood samples were drawn before (time point 0) and 10, 20, 40, and 60 min after injection. Plasma was assayed for TG as described above. After the last sampling, mice were sacrificed by cervical dislocation and exsanguinated via the retro-orbital plexus for isolation of VLDL. Livers were immediately removed from the mice and snap-frozen in liquid nitrogen for determination of hepatic lipid content. VLDL was quantitatively isolated from plasma after density gradient ultracentrifugation at d<1.006g/ml by aspiration. TG and total cholesterol were determined as described above. Phospholipids were determined using a standard commercial kit (Spinreact, Sant Esteve de Bas, Spain). VLDL-apoB was selectively precipitated with 2-propanol and counted for incorporated 35S.

**Hepatic lipid content.** Liver tissue samples were homogenized in PBS (approx. 10% wet w/v), and the protein content was measured according to the method of Lowry et al. For analysis of liver lipids, 0.4 ml aliquots of tissue were homogenized with 1.5 ml methanol-chloroform (2:1). The homogenate was then centrifuged (2,000 rpm for 5 min) and 0.5 ml chloroform and 0.5 ml distilled water were added to the liquid phase, and the samples were vortexed. Phase separation was accomplished by centrifugation (2,000 rpm for 5 min), where after the bottom phase was removed to a new tube and the samples were dried in a nitrogen flow at 50ºC. The pellet was dissolved in 100 µl 2% Triton X-100 in chloroform. TG and cholesterol concentrations were determined as described above.

**Real-time PCR.** Real-time polymerase chain reaction (RT-PCR) was used to measure mRNA expression levels of liver genes known to play important roles in TG and cholesterol homeostasis (fatty acid synthase (fas), acetyl-Coenzyme A carboxylase alpha (acaca), apoB, microsomal triglyceride transfer protein (mttp), ATP-binding cassette, sub-family A (ABC1), member 1 (abc1), sterol regulatory element binding protein 1 (srebf1), srebf2, farnesoid X-activated receptor (fxr), low density lipoprotein receptor (ldlr), diacylglycerol O-acyltransferase 1 (dgat1), cytochrome P450, family 7, subfamily a, polypeptide 1 (cyp7a1), liver X receptor α (lxlra), lxlrb, peroxisome proliferative activated receptor, alpha (ppara), pparγ, and acyl-Coenzyme A oxidase (acox1). Therefore, 2 additional groups of APOE*3-Leiden mice were used, which were not subjected to Triton injection, to isolate livers. These animals were also maintained on a high fat diet for 12 weeks. During the last 4 weeks on diet, mice received daily s.c. injections of GLP-1M (0.3 mg/kg) or PBS. At the end of the treatment period of 4 weeks, mice were fasted for 7 hours during the light period, sacrificed by cervical dislocation, and liver samples were frozen in liquid nitrogen.
Total RNA was extracted from liver samples using Trizol. The amount of RNA was determined by Nanodrop. The quality was checked by the ratio of absorption at 260 nm and absorption at 280 nm. cDNA was synthesized from 2 µg of total RNA using RevertAid™ First Strand cDNA synthesis kit (Fermentas, Hanover, MD, U.S.A.). For RT-PCR, primer assays were ordered by Qiagen, Venlo, the Netherlands. PCR amplification was performed in a total reaction volume of 25 µl. The reaction mixture consisted of QuantiFast SYBR Green PCR Master Mix (Qiagen), Primer assay, nuclease free water, and cDNA. An identical cycle profile was used for all genes: 95°C for 5 min, followed by 95°C for 10 sec and 60°C for 30 sec for 40 cycles. Data were analyzed using a comparative critical threshold (Ct) method in which the amount of target normalized to the amount of endogenous control (GAPDH/HPRT/Cyclophilin) and relative to the control samples is given by $2^{-\Delta\Delta\text{Ct}}$. For each gene, all samples were run together in duplex allowing relative comparisons of the samples of a given gene.

**Statistical analysis.** Differences between groups were determined with the Kruskal–Wallis non-parametric test for $k$ independent samples. When significant differences were found, the Mann–Whitney non-parametric 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 mean ± SD.

**Results**

**Plasma metabolites, body weight, and hepatic content.** Body weight, fasting plasma glucose, insulin, TG, and cholesterol concentrations are shown in table 1. Chronic treatment did not alter body weight in any group. Fasting plasma glucose concentration was significantly decreased for all drug-treated groups compared to baseline values after chronic administration. Insulin levels increased during the last 4 weeks on diet for the control group and GLP-1M (0.03 and 0.1 mg/kg), whereas no difference was found for the other groups. TG levels decreased in all treated groups compared to baseline values. However, significance was found for the lowest dose of GLP-1M only. The groups did not differ with respect to fasting plasma FFA levels. Plasma cholesterol levels significantly decreased after 4 weeks of treatment with 0.3 mg/kg GLP-1M and CNTO736 compared to baseline values.

Lipoprotein fractionation by fast performance liquid chromatography showed no differences in distribution for TG concentration (figure 1a). However, cholesterol concentration was considerably lower in the VLDL fraction of 0.3 mg/kg GLP-1M and CNTO736 treated animals compared to controls. In addition, cholesterol concentration
was increased in the HDL fraction for the GLP-1M and CNTO736 treated mice compared to controls (Figure 1b).

Hepatic TG and total cholesterol content did not differ between groups (table 2).

Table 1. Plasma parameters in APOE*3-Leiden mice at the start and after 2 and 4 weeks of chronic injections with vehicle, GLP-1M (0.03, 0.1, or 0.3 mg/kg), CNTO736, or exendin-4.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>GLP-1M (0.03 mg/kg)</th>
<th>GLP-1M (0.1 mg/kg)</th>
<th>GLP-1M (0.3 mg/kg)</th>
<th>CNTO736 (0.3 mg/kg)</th>
<th>Exendin-4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>start</td>
<td>2 wks</td>
<td>4 wks</td>
<td>start</td>
<td>2 wks</td>
<td>4 wks</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>30.4 ± 3.5</td>
<td>30.3 ± 3.05</td>
<td>30.5 ± 3.5</td>
<td>30.8 ± 3.0</td>
<td>30.8 ± 3.12</td>
<td>31.2 ± 3.5</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>10.5 ± 2.0</td>
<td>11.0 ± 1.10</td>
<td>11.0 ± 1.0</td>
<td>9.8 ± 0.9</td>
<td>9.8 ± 0.8</td>
<td>2.1 ± 1.1</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.1 ± 0.2</td>
<td>1.5 ± 0.4</td>
<td>1.8 ± 0.6</td>
<td>0.9 ± 0.2</td>
<td>1.1 ± 0.3</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>1.1 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>1.2 ± 0.9</td>
<td>0.7 ± 0.2</td>
<td>1.3 ± 0.8</td>
</tr>
<tr>
<td>FFA (mM)</td>
<td>0.7 ± 0.2</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>5.0 ± 1.1</td>
<td>5.2 ± 1.0</td>
<td>4.6 ± 1.0</td>
<td>4.7 ± 1.0</td>
<td>4.9 ± 1.0</td>
<td>4.3 ± 1.0</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for at least 8 mice per group. *P<0.05 vs. start treatment. **P<0.01 vs. start treatment.

Figure 1. Effect of chronic treatment with vehicle, GLP-1M (0.03, 0.1, or 0.3 mg/kg), CNTO736, or exendin-4 on the distribution of triglyceride (TG; A) and total cholesterol (TC; B) among lipoproteins in APOE*3-Leiden mice.

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Table 2. Effect of chronic treatment with vehicle, GLP-1M (0.03, 0.1, or 0.3 mg/kg), CNTO736, or exendin-4 on hepatic lipid content.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Triglycerides (µg/mg protein)</th>
<th>Total cholesterol (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>563 ± 129</td>
<td>152 ± 50</td>
</tr>
<tr>
<td>GLP-1M (0.03 mg/kg)</td>
<td>490 ± 91</td>
<td>131 ± 22</td>
</tr>
<tr>
<td>GLP-1M (0.1 mg/kg)</td>
<td>478 ± 166</td>
<td>131 ± 29</td>
</tr>
<tr>
<td>GLP-1M (0.3 mg/kg)</td>
<td>486 ± 178</td>
<td>152 ± 56</td>
</tr>
<tr>
<td>CNTO736 (0.3 mg/kg)</td>
<td>534 ± 104</td>
<td>148 ± 26</td>
</tr>
<tr>
<td>Exendin-4</td>
<td>492 ± 120</td>
<td>155 ± 43</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for at least 8 mice per group.

VLDL production. Chronic treatment with GLP-1M and CNTO736 reduced plasma TG concentrations after Triton injection, a measure of VLDL-TG production rate, compared to control animals. Ex-4 treatment had no effect whatsoever on VLDL production (figure 2). Analysis of the composition of the VLDL particles revealed no differences between groups (table 3). The de novo total apoB production rate in newly synthesized VLDL particles was significantly lower in mice treated with 0.3 mg/kg GLP-1M and CNTO736 compared to controls. No effect on apoB production was found for Ex-4 treated animals compared to controls (figure 3).

Figure 2. TG concentrations (A) and VLDL-TG production rate (B) in APOE*3-Leiden mice that received chronic injections with vehicle, GLP-1M (0.03, 0.1, or 0.3 mg/kg), CNTO736, or exendin-4 after Triton injection. Values represent mean ± SD for at least 8 mice per group. *P<0.05 vs. control. #P<0.05 vs. exendin-4.
Table 3. Lipid composition of nascent VLDL of APOE*3-Leiden mice that received chronic injections with vehicle, GLP-1M (0.03, 0.1, or 0.3 mg/kg), CNTO736, or exendin-4.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Cholesterol</th>
<th>Triglycerides</th>
<th>Phospholipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20 ± 3</td>
<td>67 ± 4</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>GLP-1M (0.03 mg/kg)</td>
<td>21 ± 4</td>
<td>67 ± 5</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>GLP-1M (0.1 mg/kg)</td>
<td>19 ± 5</td>
<td>68 ± 3</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>GLP-1M (0.3 mg/kg)</td>
<td>17 ± 5</td>
<td>71 ± 7</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>CNTO736 (0.3 mg/kg)</td>
<td>19 ± 5</td>
<td>73 ± 6</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>Exendin-4</td>
<td>19 ± 5</td>
<td>68 ± 7</td>
<td>13 ± 2</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for at least 8 mice per group.

Real-Time PCR. Chronic treatment with GLP-1M (0.3 mg/kg) resulted in a significant downregulation of *apob* and *ppary* (Figure 4). A trend towards downregulation was seen for *ldlr* (p=0.09) and *dgat1* (p=0.09) and a trend towards upregulation for *abca1* (p=0.10). However, the difference with the control group for these latter genes did not reach significance. No differences were found for the other genes (p>0.30).
Discussion

Here we show that GLP-1M, a GLP-1 receptor agonist, dose-dependently inhibits VLDL-TG release in diet-induced insulin resistant APOE*3-Leiden mice. The rate of appearance of apoB, an important component of VLDL particles, was diminished as well, indicating that the drug curtails the number of particles produced. The reduction of apoB output was corroborated by a decline of apoB mRNA expression in the liver. The composition of VLDL particles was not affected by GLP-1M. Remarkably, Ex-4 did not impact on VLDL-TG production, although it did reduce plasma glucose levels to an extent that was similar to that brought about by both other drugs. Finally, chronic treatment with GLP-1M (but not Ex-4) decreased circulating (fasting) cholesterol levels and shifted the distribution of cholesterol among distinct plasma lipoprotein fractions toward HDL.

GLP-1M appears to inhibit VLDL-TG production in APOE*3-Leiden mice maintained on a high fat diet. Changes in VLDL-TG production are due to alterations in the amount of TG per VLDL particle, by the number of particles secreted (as reflected by apoB output), or both. The lipid composition of nascent VLDL did not differ between groups. However, the VLDL-apoB production rate was significantly decreased by GLP-1M. These data suggest that GLP-1M particularly inhibits the production of particles, leaving their composition unaffected. Interestingly, overproduction of VLDL particles of normal size and composition is the hallmark of diabetic dyslipidemia in humans.

The effect of GLP-1M on VLDL-TG production may be attributable to a direct effect of the drug on apoB gene expression. The availability of apoB and substrate play key roles in the control of VLDL production and insulin inhibits VLDL particle assembly at multiple levels. GLP-1M did not reduce hepatic triglyceride stores to explain its impact on VLDL particle production. Our high-fat-fed mice were insulin resistant, as reflected by high plasma glucose and insulin levels in fasting condition, and insulin resistance is associated with increased VLDL-apoB production in mice and humans. The mechanistic details of insulin action on apoB metabolism are not completely known, but may involve the modulation of apoB mRNA translation. Thus, it is conceivable, that GLP-1M inhibits the production of VLDL particles in high-fat-fed mice by rescuing hepatic insulin sensitivity, thereby reducing the efficacy of apoB mRNA translation. However, Ex-4 did not affect VLDL-TG production, while it reduced circulating glucose concentrations to a similar extent as GLP-1M did. Moreover, in a previous study we showed that CNTO736 inhibits VLDL-TG release whereas Ex-4 (in a dose of 7.1 µg/kg similar to the dose used in the present study) does not, even though both drugs have similar effects on insulin sensitivity of glucose metabolism. It needs to be emphasized, that our current data suggest that the dose of Ex-4 we used may have been too low to fully mimic the impact of
the highest dose of GLP-1M on insulin sensitivity, given the (not significantly) lower plasma glucose levels during GLP-1M treatment. Nevertheless, we consider it unlikely that GLP-1M inhibits VLDL-TG production primarily through its beneficial effect on insulin action. GLP-1M clearly diminished apob expression in the liver. We speculate that this pharmacological feature of the drug is primarily responsible for its effect on VLDL particle production. Indeed, GLP-1 was shown to inhibit lymphatic apoB output, supporting a role of this peptide in the control of apoB production\textsuperscript{33}. The lack of effect of Ex-4 in this context warrants further study.

The inhibition of VLDL-TG production by GLP-1M did not result in decreased plasma TG levels, which is also true for CNTO736\textsuperscript{13}. Thus, other mechanisms that determine plasma VLDL-TG levels (e.g. rates of endothelial binding and subsequent lipolysis by LPL, and/or the rates of removal of small VLDL from the circulation or its conversion to IDL) must have been affected by GLP-1M as well. The drug did not affect the distribution of TG among the various lipoprotein subfractions, suggesting that it does not impact on VLDL to IDL conversion. Various factors, including the triglyceride and apoE content of VLDL particles, modulate epithelial binding and subsequent lipolysis\textsuperscript{34}. Although the TG content of VLDL particles was not affected by GLP-1M treatment, the drug may have modified other features of VLDL particles that determine clearance. Also, uptake of small VLDL particles by the liver may have been affected. Obviously, evaluation of these hypotheses requires further study.

Chronic treatment with 0.3 mg/kg GLP-1M and CNTO736 decreased total cholesterol levels in plasma, and Ex-4 (in the current dose) did not appear to exert this effect. Cholesterol is removed from the circulation by uptake of lipoproteins in various tissues via receptor mediated pathways\textsuperscript{35}. Uptake in the liver occurs primarily via the LDL receptor. Subsequently, cholesterol enters the entero-hepatic bile acid circulation to be partly disposed of via the faeces. Ldlr expression tended to be diminished by GLP-1M, suggesting that clearance via this receptor does not explain the drug’s effect on circulating cholesterol concentrations. Alternatively, excess cholesterol was directed towards the liver via HDL lipoproteins to be disposed of via bile. Hepatic cholesterol content was not affected by GLP-1M, suggesting that increased supply of (HDL) cholesterol was offset by enhanced removal via the bile\textsuperscript{36}. Preliminary studies indicate that both sterol and bile acid secretion were increased indeed by chronic treatment with GLP-1M (Parlevliet et al., data not shown).

GLP-1M (and CNTO736) shifted the distribution of cholesterol among distinct lipoprotein fractions toward HDL, suggesting that the drug somehow promotes efficient clearance of cholesterol from chylomicrons, VLDL, and IDL by extrahepatic tissues to form mature HDL. Again, Ex-4 in the current dose does not appear to exert this effect. The
export of hepatic cholesterol towards nascent HDL by ABCA1 is rate-limiting in the process of HDL formation. Abca1 expression in the liver was increased in GLP-1M treated animals as compared with placebo treated controls, but the difference did not reach statistical significance. HDL metabolism is also controlled by the concerted impact of a number of plasma proteins such as phospholipid transfer protein, lecithin-cholesterol acyltransferase, endothelial-bound enzymes LPL and hepatic lipase, and GLP-1M may modulate the production and/or activity of these peptides to shift cholesterol in plasma from VLDL towards HDL lipoproteins.

Our data suggest that GLP-1M may have powerful anti-atherogenic properties in insulin resistant subjects. Atherosclerosis is driven by increased (VLDL) cholesterol and triglyceride levels, often accompanied by low circulating HDL concentrations. HDL particles are anti-atherogenic, partly because of their role in reverse cholesterol transport, but also because of their anti-oxidative, anti-inflammatory, anti-thrombotic, and anti-apoptotic properties. Therefore, the reduction of plasma cholesterol concentration by GLP-1M as well as its capacity to increase HDL cholesterol levels holds promise as pharmacological features preventing atherosclerosis in high risk metabolic conditions.

The current study suggests that GLP-1M has properties not reported for a GLP-1 analogue thus far. Chronic administration of the drug in high-fat-fed APOE*3-Leiden mice clearly inhibited VLDL-TG production. Moreover, chronic GLP-1M administration decreased total cholesterol levels and increased HDL in the same experimental context. Chronic administration with Ex-4 did not impact on VLDL or cholesterol metabolism, suggesting that the GLP-1M effects on lipid metabolism are not mediated via its impact on insulin sensitivity.

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References


Chapter 4


Effect of GLP-1M treatment on VLDL triglyceride production


