Check for updates

Received: 16 January 2025 | Revised: 21 March 2025 | Accepted: 21 March 2025

DOI: 10.1111/dom.16374

ORIGINAL ARTICLE

WILEY

Oppositely biased glucagon-like peptide-1 receptor agonism does not differentially affect lipid metabolism in APOE*3-Leiden CETP mice

Melanie Modder MSc[1,2] | Alejandra Tomas PhD[3] | Salwa Afkir BSc[1,2] | Amanda C. M. Pronk BSc[1,2] | Trea C. M. Streefland BSc[1,2] | Reshma A. Lalai BSc[1,2] | Robin van Eenige PhD[1,2] | Patrick C. N. Rensen PhD[1,2] | Ben Jones PhD[4] | Sander Kooijman PhD[1,2]

[1]Division of Endocrinology, Department of Medicine, Leiden University Medical Center, Leiden, The Netherlands

[2]Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands

[3]Section of Cell Biology and Functional Genomics, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK

[4]Section of Endocrinology and Investigative Medicine, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK

Correspondence
Sander Kooijman, Leiden University Medical Centre, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.
Email: s.kooijman@lumc.nl

Abstract

Aims: [D[3],G[40],K[41].C16 diacid]exendin-4 (acyl-ExD3) and [F[1],G[40],K[41].C16 diacid]exendin-4 (acyl-ExF1) are oppositely biased glucagon-like peptide-1 (GLP-1) receptor agonists that preferentially promote β-arrestin recruitment or G protein-induced signalling, respectively. The latter is more favourable in glycaemic control and induces a steeper reduction in body weight in diet-induced obese mice. Here, we compared the effects of G protein-biased agonist acyl-ExF1 to those of β-arrestin-biased agonist acyl-ExD3 on lipid metabolism in hyperlipidaemic mice.

Materials and methods: APOE*3-Leiden.CETP mice were treated with saline, acyl-ExD3 or acyl-ExF1 via intraperitoneal injections for 6 weeks or intracerebroventricular infusion for 18 days. Body weight and composition were monitored at regular intervals, as were plasma glucose, triglyceride and cholesterol levels. At endpoint, mice were injected with very low-density lipoprotein (VLDL)-like particles containing glycerol tri[^{3}H]oleate to study triglyceride-derived fatty acid uptake by peripheral tissues including brown and white adipose tissue (BAT and WAT).

Results: Upon peripheral treatment, body weight gain was prevented and plasma glucose levels were reduced by acyl-ExF1, but circulating lipids were not affected by either acyl-ExF1 or acyl-ExD3. In contrast, central administration of either agonist strongly reduced plasma triglyceride and cholesterol levels, but did not affect glucose levels. Acyl-ExD3 and acyl-ExF1 increased [^{3}H]oleate uptake by adipose tissue, reaching statistical significance for the uptake by BAT and WAT, respectively, compared to vehicle treatment.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.
© 2025 The Author(s). *Diabetes, Obesity and Metabolism* published by John Wiley & Sons Ltd.

Conclusion: The oppositely biased GLP-1 receptor agonists acyl-ExD3 and acyl-ExF1 do not differentially affect lipid metabolism in APOE*3-Leiden.CETP mice, while effects on glucose homeostasis and prevention of body weight gain are more pronounced upon peripheral acyl-ExF1 treatment.

KEYWORDS
animal pharmacology, antidiabetic drug, antiobesity drug, dyslipidaemia, GLP-1 analogue

1 | INTRODUCTION

The world-wide incidence of obesity and diabetes is still growing. The two diseases predispose individuals to many severe cardiometabolic complications. In the early 80s, glucagon-like peptide-1 (GLP-1) was identified by two separate research groups from Boston and Denmark as a peptide that promotes glucose-stimulated insulin release from pancreatic β-cells.[1–6] However, with a biological half-life of 2–3 min, there seemed to be limited therapeutic potential. This view changed with the discovery of exendin-4 as a full GLP-1 receptor agonist.[7] Importantly, exendin-4 appeared to be resistant to enzymatic degradation by peptidases due to multiple amino acid substitutions and therefore has a longer half-life than endogenous GLP-1.

Over the years, several modifications of GLP-1 analogues have been undertaken to further improve their pharmacokinetics, including linkage to an Fc fragment of human IgG4 (i.e., dulaglutide) or conjugation to a fatty acid chain capable of binding non-covalently to albumin (i.e., liraglutide and semaglutide) to reduce renal clearance. In addition, replacement of an alanine residue near the cleavage site for DPP-4 by the unnatural amino acid 2-aminoisobutyric acid further prevents degradation (i.e., semaglutide).[8] Most strikingly, the GLP-1 receptor agonists currently used for treating patients with type 2 diabetes turned out to consistently promote weight loss in addition to improving glucose homeostasis. Some of the newest generation of GLP-1-based medications have also been approved for the treatment of overweight and obesity.[9,10]

Another more recent approach to enhance drug therapeutic efficacy is through refinement of ligand-induced intracellular signalling profiles of the GLP-1 receptor, a G protein-coupled receptor (GPCR). GPCRs transduce signals via activation of G proteins and subsequent induction of several second messengers. The primary intracellular signalling molecule coupling GLP-1 receptor activation to downstream effects is cyclic adenosine monophosphate (cAMP).[11,12] On the other hand, additional effector proteins are involved in regulating amplitude and duration of signalling, including β-arrestins, which are recruited to the receptor upon activation and induce receptor desensitization and internalization.[13,14] Interestingly, GLP-1 analogues with selectively lowered β-arrestin recruitment but preserved G protein-induced cAMP production show reduced GLP-1 receptor desensitization and degradation in pancreatic β-cells, leading to improvements in sustained insulin release compared with standard GLP-1 receptor agonists.[15–17] This group of so called 'G protein-biased agonists' is typified by exendin-phe1 (ExF1), a peptide variant of exendin-4 that exhibits reduced β-arrestin recruitment and more sustained glucoregulatory effects compared with exendin-4 in mice.[17] A further variant, exendin-asp3 (ExD3), showed bias in the opposite direction, that is, favouring β-arrestin over G protein recruitment, and was associated with faster downregulation of the GLP-1 receptor. Moreover, C-terminal acylation of these two biased GLP-1 receptor agonists prolonged their pharmacokinetics, while preserving their opposite signal bias. As such, [F^1,G^{40},K^{41}.C16 diacid]exendin-4 (acyl-ExF1) demonstrated extended insulinotropic effects in diet-induced obese (DIO) mice compared with [D^3,G^{40},K^{41}.C16 diacid]exendin-4 (acyl-ExD3), and additionally suppressed appetite and promoted more weight loss in chronic treatment settings.[18] It is speculated that the high clinical efficacies of both tirzepatide and orforglipron at least partly reflect their G protein-biased signalling profiles at the GLP-1 receptor.[19–21]

On top of being an effective treatment option for type 2 diabetes and obesity, evidence from preclinical work, non-randomized and randomized clinical trials supports the protective effects of GLP-1 receptor agonists against hyperlipidaemia and atherosclerotic cardiovascular disease.[22–27] A systematic review and meta-analysis of 35 clinical trials showed that various GLP-1 receptor agonists reduce plasma cholesterol levels and some, including liraglutide, reduce plasma triglyceride levels in patients with type 2 diabetes.[22] In addition, semaglutide has been demonstrated to lower fasted and postprandial plasma triglyceride levels in individuals with obesity.[25] Our group also demonstrated that a 12-week exenatide treatment in lean adults lowered plasma triglycerides and total cholesterol levels, to which increased nutrient uptake by brown adipose tissue might have contributed.[24] We have additionally explored the effects of GLP-1 receptor agonism on cardiometabolic health in APOE*3-Leiden.CETP mice, a well-established model for human lipoprotein metabolism that is prone to atherosclerosis development.[28,29] We demonstrated that treatment of these mice with a long-acting murine GLP-1 receptor agonist (i.e., GLP-140) alleviated hyperlipidaemia and tended to attenuate atherosclerotic lesion severity.[28] Here, we set out to compare treatment with G protein-biased acyl-ExF1 to treatment with β-arrestin-biased acyl-ExD3 with respect to their effects on lipid metabolism.

2 | MATERIALS AND METHODS

2.1 | Mouse experiments

APOE*3-Leiden.CETP mice were generated as previously described.[30] Animals were housed under standard conditions in groups (max. five

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

mice per cage) in a 12:12 h light/dark cycle with ad libitum access to food and water unless stated otherwise. Four weeks before the start of the treatment, the regular chow diet was switched to a Western-type diet (containing 15% cacao butter, 1% corn oil and 0.15% cholesterol; ssniff, Soest, Germany) to induce dyslipidaemia. Since only female APOE*3-Leiden.CETP mice develop dyslipidaemia upon Western-type diet feeding, experiments were restricted to female mice. After the 4-week dietary run-in period, mice were block-randomized into three intervention groups that were balanced for 4-h fasted plasma triglycerides and total plasma cholesterol, age, body weight and lean and fat body mass (EchoMRI 100-Analyzer; EchoMRI, Houston, Texas).[31]

In experiment 1, 8–10-week-old female mice ($n = 8$ per group) were intraperitoneally injected every 48 h with saline, acyl-ExD3 (20 nmol/kg) or acyl-ExF1 (20 nmol/kg) for a period of 6 weeks. At every injection moment, mice (individual) and food (per cage) were weighed. At weeks 2, 4 and 6, body composition was determined, and 4-h fasted blood samples were collected via the tail vein, from which plasma was isolated using heparin-coated capillaries. At the end of the experiment, plasma clearance and organ uptake of intravenously injected very-low-density lipoprotein (VLDL)-mimicking particles were assessed in 4-h fasted mice. Additional pieces of organs and tissues were collected for further histological assessment.

In experiment 2, 14–18-week-old female mice ($n = 11$ per group) were intracerebroventricularly infused with saline, acyl-ExD3 (0.75 nmol/day) or acyl-ExF1 (0.75 nmol/day) for a period of 18 days. Mice were anaesthetized and placed in a stereotaxic frame, and cannulas (Brain Infusion Kit 3, ALZET Cupertino, CA, USA) were implanted into the left ventricle of the brain (−0.45 mm anteroposterior, −1.00 mm lateral and 2.2 mm dorsoventral from bregma). The cannulas were connected to osmotic minipumps (Model 1004, Alzet, CA, USA) by a catheter and the minipumps were implanted subcutaneously on the back. The minipump continuously released 0.11 µL/h of the designated peptide (resulting in 0.75-nmol compound/day). Mice were weighed daily throughout the study and food was weighed daily per mouse from day 6 onwards. At day 0 and 18 after implantation, body composition was determined, and 4-h fasted blood samples were collected via the tail vein, from which plasma was isolated using heparin-coated capillaries. At the end of the experiment, plasma clearance and organ uptake of intravenously injected VLDL-mimicking particles were assessed in 4-h fasted mice. Additional pieces of organs and tissues were collected for further histological assessment.

All mouse experiments were reviewed and approved by the Animal Welfare Body Leiden (IvD Leiden) and executed under a licence (i.e., AVD11600202010187) granted by the Central Authority for Scientific Procedures on Animals (CCD) in accordance with the Dutch Act on Animal Experimentation and EU Directive 2010/63/EU.

2.2 | Plasma measurements

Plasma levels of glucose, triglycerides and total cholesterol were determined by colorimetric measurements using commercially available kits (Roche Diagnostics, Mannheim, Germany). For the quantification of high-density lipoprotein-cholesterol (HDL-C), 20% polyethylene glycol in 200 mM glycine-buffered saline (pH 10) was added to plasma to precipitate apolipoprotein B (ApoB) containing lipoproteins, and (HDL-specific) total cholesterol was measured in the supernatant as described above. Plasma levels of non-HDL-C were calculated by subtracting plasma HDL-C from plasma total cholesterol.

2.3 | In vivo organ uptake of VLDL-mimicking particles and glucose

VLDL-mimicking particles (1 mg triglycerides per mouse in 200-µL saline) containing glycerol tri[^{3}H]oleate (experiment 1 and 2) and [^{14}C] cholesteryl oleate (experiment 1) were prepared as described before.[32] The particles were injected into the tail vein, and blood was collected from the tail vein at $t = 0$, 2, 5, 10 and 15 min after injection to determine plasma decay. Subsequently, mice were killed via CO_2 asphyxiation, perfused transcardially with ice-cold PBS for 5 min before organs were collected and weighed and partly (approx. 50–150 mg) dissolved in 0.5 mL Solvable (6NE9100, PerkinElmer, Waltham, USA) at 56°C overnight, after which 5.0 mL Ultima Gold (6 013 329, PerkinElmer, Waltham, USA) was added. ^{3}H-activity and ^{14}C-activity were measured with a scintillation counter (Tri-Carb 2910 TR, PerkinElmer, Waltham, USA) and expressed as a percentage of the injected dose per gram tissue.

2.4 | Adipose tissue histology

Formalin-fixed paraffin-embedded interscapular brown adipose tissue and subcutaneous white adipose tissue sections (5 µm thickness) were prepared for haematoxylin and eosin staining using standard protocols. In addition, uncoupling protein-1 (UCP-1) and tyrosine hydroxylase (TH) content in interscapular brown adipose tissue were determined using immunostainings. As primary antibodies, rabbit polyclonal anti-mouse UCP-1 antibody (1:2000; U6382; Merck, Darmstadt, Germany) or rabbit polyclonal anti-TH antibody (1:2000; ab112; Abcam, Cambridge, UK) were used, respectively, with DAKO EnVision anti-rabbit antibodies (1:2000; K400311-2; Agilent, Santa Clara, USA) as secondary antibodies. The immunoperoxidase complexes on the secondary antibodies were visualized with Nova Red (SK-4800, Vector Laboratories Inc., Burlingame, USA). The areas occupied by intracellular lipid vacuoles, UCP-1 protein or TH within the tissues were quantified using Image J software (version 1.52a; National Institutes of Health, Bethesda, Maryland).

2.5 | Statistical analysis

Normality was confirmed in all data, and statistical outliers were removed using Grubb's test. Differences between groups were

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

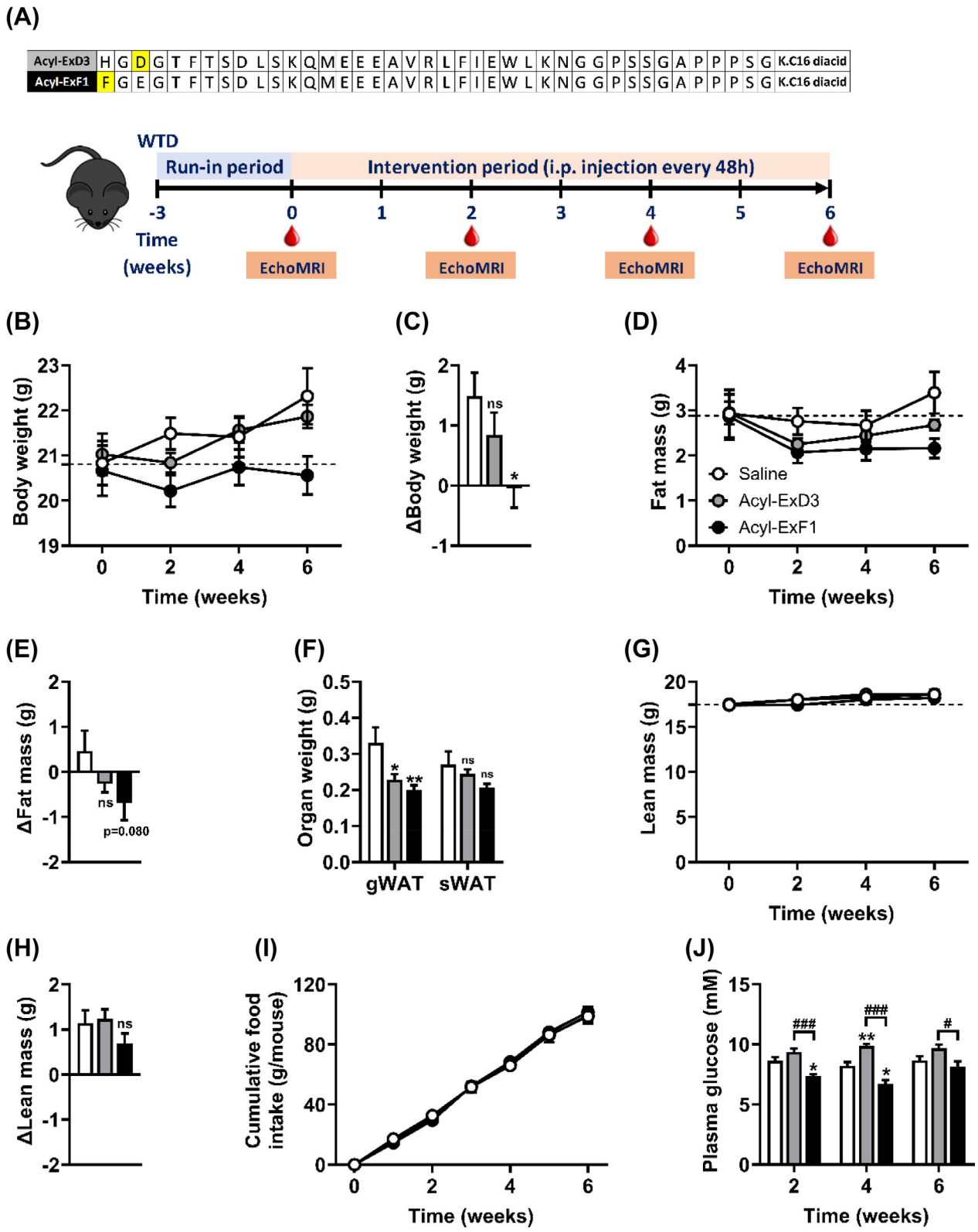


FIGURE 1 Legend on next page.

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

determined by one-way analysis of variance (ANOVA) with post hoc Tukey's test. For measurements with sequential sampling, two-way ANOVA or mixed-effects models with post hoc Tukey's test were used or deltas over time were calculated and compared by one-way ANOVA with post hoc Tukey's test. Statistical analyses were performed with GraphPad Prism software, version 9.3.1 (GraphPad, La Jolla, California). $p < 0.05$ was considered statistically significant; p-values between 0.05 and 0.10 are referred to as trend towards statistical significance, and data are presented as means ± SEM.

3 | RESULTS

3.1 | G protein-biased GLP-1 receptor signalling lowers plasma glucose levels compared with β-arrestin-biased signalling

To compare the effect of two oppositely biased GLP-1R agonists on hyperlipidaemia, we treated female APOE*3-Leiden.CETP mice with intraperitoneal injections of vehicle (saline), acyl-ExD3 and acyl-ExF1 for a period of 6 weeks (Figure 1A). A dose of 20 nmol/kg every 48 h was used, which was previously shown to improve glucose metabolism and lower body weight in DIO mice.[18] We first assessed how both agonists affected body composition. In the rather lean APOE*3--Leiden.CETP mice, G protein-biased GLP-1 receptor agonist acyl-ExF1 prevented body weight gain over the 6 weeks treatment period and tended to lower fat mass, while the body weight and fat mass development of the mice treated with the β-arrestin-biased GLP-1 receptor agonist acyl-ExD3 was not different from the saline-treated animals (Figure 1B–E). Both GLP-1 receptor agonists lowered gonadal white adipose tissue weight (Figure 1F), but did not significantly affect lean mass or food intake (Figure 1G–I). In line with previous results in DIO mice,[18] we observed lowered plasma glucose levels in acyl-ExF1 treated mice compared with those receiving acyl-ExD3 treatment (Figure 1J).

3.2 | Peripheral administration of biased GLP-1 receptor agonists does not affect plasma lipid levels

We next set out to determine whether the G protein-biased GLP-1 receptor agonist acyl-ExF1 would also reduce hyperlipidaemia to a greater extent than treatment with the β-arrestin-biased agonist acyl-ExD3. Somewhat unexpectedly, neither of the GLP-1 receptor agonists lowered plasma triglyceride or cholesterol levels (Figure 2A,B). At the end of the 6-week treatment period, mice were injected with VLDL-like particles labelled with glycerol tri[^{3}H]oleate ([^{3}H]TO) and [^{14}C]cholesteryl oleate ([^{14}C]CO), to evaluate plasma clearance and organ uptake of triglyceride-derived fatty acids and VLDL remnants, respectively. In line with unchanged plasma lipid levels, we found no difference between the groups in the clearance rate of [^{3}H]TO or [^{14}C]CO from the plasma (Figure 2C,E). In contrast to our previous study with the long-acting GLP-140, we also did not find indications of increased lipid uptake and combustion by adipose tissue upon treatment with acyl-ExF1 (Figure 2D). In fact, treatment with acyl-ExD3 strongly reduced [^{3}H]oleate uptake by brown adipose tissue compared with saline treatment. However, this effect did not result in an altered uptake of VLDL-like particle remnants by the liver as demonstrated by equal hepatic [^{14}C]CO uptake across the groups (Figure 2F). Histological assessment of brown adipose tissue did also not reveal effects of acyl-ExD3 treatment on morphology (Figure 2G). Interestingly, treatment with acyl-ExF1 did increase sympathetic activity in the tissue evidenced by higher TH content, but this effect was compensated for as it was not accompanied by any changes in lipid content or [^{3}H]oleate uptake (Figure 2C,G–I). In conclusion, 6 weeks of treatment with intraperitoneal injections of acyl-ExD3 or acyl-ExF1 in APOE*3-Leiden.CETP mice did not substantially affect lipid metabolism to alleviate hyperlipidaemia.

3.3 | Central infusion of oppositely biased GLP-1 receptor agonists comparably reduces body weight and fat mass

We previously demonstrated the beneficial effects on lipid metabolism of treatment with prototypical exendin-4 when directly administered to the brain, due to increased uptake of triglyceride-derived fatty acids by white and brown adipose tissue for thermogenesis.[33] Therefore, we continued by studying the response to treatment with acyl-ExF1 and acyl-ExD3 following intracerebroventricular infusion. To this end, cannulas attached to osmotic minipumps were implanted into the left lateral ventricle of female APOE*3-Leiden.CETP mice. The minipumps ensured continuous delivery of saline, acyl-ExD3 or acyl-ExF1 via the cannulas for a period of 18 days (Figure 3A). A dose of 0.75 nmol/day of the compounds was delivered to the brain ventricle, similar to the dose of prototypical exendin-4 that improved lipid metabolism in our previous study.[33] Under these circumstances, both GLP-1 receptor agonists comparably reduced body weight

FIGURE 1 Acyl-ExF1 treatment lowers plasma glucose levels compared with saline and acyl-ExD3 treatment in APOE*3-Leiden.CETP mice. Over a period of 6 weeks, female APOE*3-Leiden.CETP mice were intraperitoneally injected every 48 h with saline (white), acyl-ExD3 (grey; 20 nmol/kg) or acyl-ExF1 (black; 20 nmol/kg) (A). Throughout the study (B) body weight, (D) fat mass, and (G) lean mass were monitored and (C, E, H) deltas between weeks 0 and 6 were calculated ($n = 7–8$ per group). For each cage, (I) food intake was determined and expressed as cumulative intake ($n = 2$ cages per group). At the endpoint, (F) gonadal white adipose tissue (gWAT) and subcutaneous (s)WAT were weighed ($n = 7–8$ per group). At week 2, 4 and 6, (J) plasma glucose levels were measured after 4 h of fasting ($n = 7–8$ per group). Values are presented as means ± SEM. ns: $p > 0.10$, $^*p < 0.05$, $^{**}p < 0.01$ compared with saline and $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ compared between acyl-ExD3 and acyl-ExF1 according to one-way ANOVA (C, E, F, H) or mixed-effects analysis (J) and following Tukey's multiple-comparison test.

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

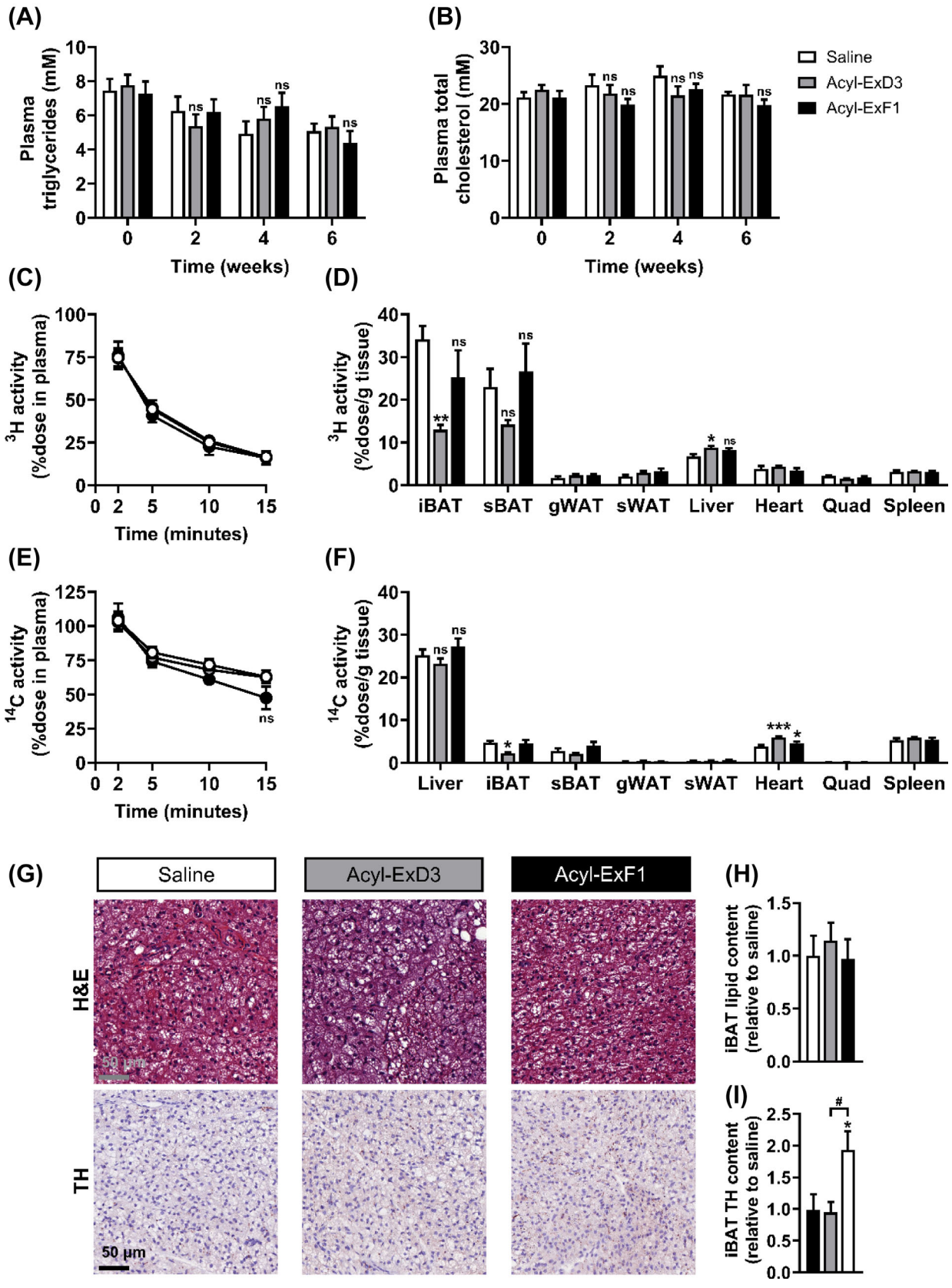


FIGURE 2 Legend on next page.

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(Figure 3B), explained by lowered fat mass (Figure 3C) and white adipose tissue depot weights (Figure 3D), without changing lean mass (Figure 3E). In line with the peripheral injections, both GLP-1 receptor agonists did not significantly affect average food intake (Figure 3F). It should be noted, though, that food intake could only be monitored in the period from day 6 onwards due to recovery of animals from the surgery. As the largest reduction in food intake likely happened during the first days of treatment, we might not have been able to detect this effect. Intracerebroventricular infusion of acyl-ExF1 and acyl-ExD3 did not alter plasma glucose levels (Figure 3G), indicating no leakage of the agonists into the periphery where they would have potentiated glucose-stimulated insulin secretion.

3.4 | Central infusion of biased GLP-1 receptor agonists lowers plasma lipid levels and increases VLDL-like particle-derived fatty acid uptake by adipose tissue

Central acyl-ExD3 as well as acyl-ExF1 treatment similarly lowered plasma triglyceride and cholesterol levels (Figure 4A,B). As HDL-cholesterol levels were not affected (Figure 4C), the reductions in plasma total cholesterol were explained by lower plasma non-HDL-cholesterol levels (Figure 4D). In order to determine which organs contributed to the reduced plasma triglyceride levels, we again injected mice with VLDL-like particles labelled with [^{3}H]TO at the endpoint of the study. Intracerebroventricular infusion of both the G protein-biased agonist acyl-ExF1 and the β-arrestin-biased agonist acyl-ExD3 accelerated plasma clearance of [^{3}H]TO compared with saline (Figure 4E). Whilst acyl-ExD3 significantly increased [^{3}H]oleate uptake by brown adipose tissue and tended to increase [^{3}H]oleate uptake by white adipose tissue, acyl-ExF1 significantly increased [^{3}H]oleate uptake by white adipose tissue (Figure 4F). In addition, central administration of acyl-ExD3 increased, and administration of acyl-ExF1 tended to increase [^{3}H]oleate uptake by the liver (Figure 4F). In conclusion, central acyl-ExF1 and acyl-ExD3 treatment comparably improved body composition and alleviated dyslipidaemia as a result of increased triglyceride-derived fatty acid uptake by adipose tissue and the liver. Increased uptake of triglyceride-derived fatty acids by white adipose tissue, while its tissue weight is lower, points towards a shift from lipid storage to lipid cycling and/or oxidation. To obtain a more detailed understanding of the activated processes, we continued with histological examination of the tissue. Central administration of the β-arrestin-biased GLP-1 receptor agonist acyl-ExD3 clearly reduced cell size in white adipose tissue, and G protein-biased agonist acyl-ExF1 only tended to decrease white adipocyte cell size (Figure 5A,B). In addition, seven out of nine mice centrally treated with acyl-ExD3 showed positive areas of thermogenesis marker UCP1 in their white adipose tissue, while this was only the case for two of nine mice treated with saline and 3 out of 8 mice treated with acyl-ExF1 (Figure 5A), suggesting acyl-ExD3 treatment might have induced white adipose tissue browning. In brown adipose tissue, we observed no significant effect on lipid content, UCP1 levels or TH levels as a proxy for sympathetic innervation (Figure 5C–F). We summarized the findings of our experiments in a mechanistic figure (Figure 5G).

4 | DISCUSSION

Acyl-ExD3 and acyl-ExF1 are two oppositely biased GLP-1 receptor agonists favouring β-arrestin recruitment or G protein-induced cAMP production, respectively. The latter is superior with respect to restoring glycaemic control and reducing body weight in DIO mice.[18] In the current study, we show that acyl-ExD3 and acyl-ExF1 do not differentially affect lipid metabolism in APOE*3-Leiden.CETP mice, a model for human lipoprotein metabolism. After peripheral treatment, circulating lipids were not affected by acyl-ExD3 or acyl-ExF1, while central administration of either agonist strongly reduced plasma triglyceride and cholesterol levels, accompanied by increased lipid uptake by adipose tissue and liver.

We first showed that peripheral acyl-ExF1 treatment prevents weight gain in APOE*3-Leiden.CETP mice, along with improved fasted plasma glucose levels, as opposed to acyl-D3, which did not improve body weight or glucose levels. These results demonstrate that the more favourable properties of cAMP-biased agonist acyl-ExF1 on glycaemic control and body weight are preserved in lean, non-diabetic mice. However, intraperitoneal injections of neither acyl-ExD3 nor acyl-ExF1 alleviated dyslipidaemia in APOE*3-Leiden.CETP mice. This is different from the results of our earlier study in the same mouse model in which the long-acting GLP-140 lowered plasma triglycerides and cholesterol after 4 weeks of subcutaneous treatment.[28] On the other hand, treatment of APOE*3-Leiden.CETP mice with non-esterified exendin-4 also did not result in lower plasma lipid levels.[29]

FIGURE 2 Acyl-ExF1 and acyl-ExD3 do not affect plasma lipid levels in APOE*3-Leiden.CETP mice. Over a period of 6 weeks, female APOE*3-Leiden.CETP mice were intraperitoneally injected every 48 h with saline (white), acyl-ExD3 (grey; 20 nmol/kg) or acyl-ExF1 (black; 20 nmol/kg). At weeks 0, 2, 4 and 6 levels of plasma (A) triglycerides and (B) total cholesterol were measured after 4 h of fasting ($n = 7–8$ per group). At the endpoint, mice were injected with VLDL-like particles double-labelled with glycerol tri[^{3}H]oleate and [^{14}C]cholesteryl oleate to assess plasma decay of (C) [^{3}H]oleate and (E) [^{14}C]cholesteryl oleate and uptake of (D) [^{3}H]oleate and (F) [^{14}C]cholesteryl oleate by interscapular brown adipose tissue (iBAT), subscapular (s)BAT, gonadal white adipose tissue (gWAT), subcutaneous (s)WAT, liver, heart, quadriceps and spleen ($n = 7–8$ per group). In iBAT, after (G) haematoxylin & eosin (H&E) staining and immunostaining, (H) lipid content, and (I) tyrosine hydroxylase (TH) content was quantified ($n = 7–8$ per group). Values are presented as means ± SEM. ns: $p > 0.10$, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with saline and $^{\#}p < 0.05$ compared between acyl-ExD3 and acyl-ExF1 according to one-way ANOVA (A, B, D, F, H, I) or two-way ANOVA (C, E) and following Tukey's multiple-comparison test.

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

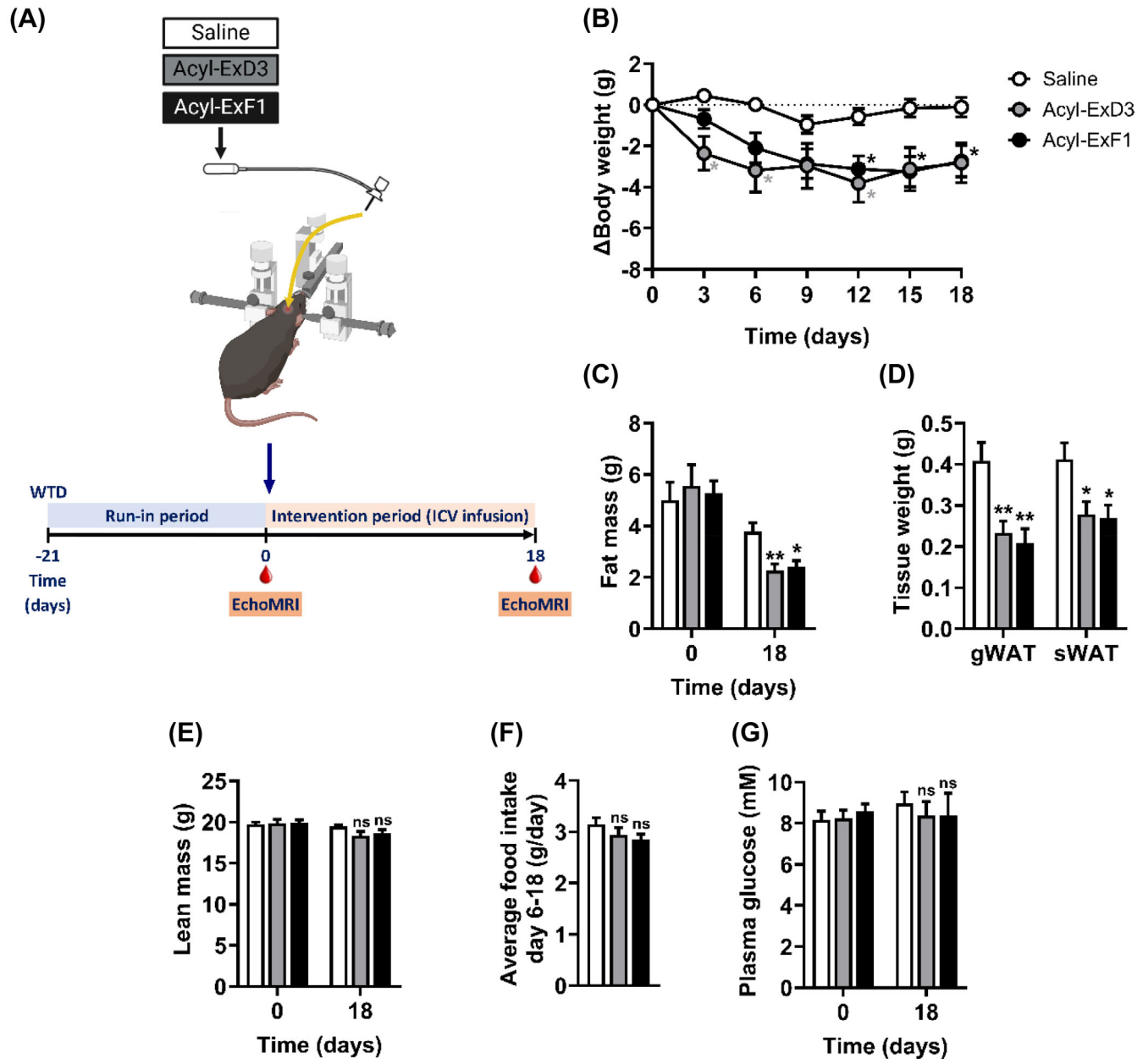


FIGURE 3 Central infusion of acyl-ExF1 and acyl-ExD3 lowers body weight and fat mass in APOE*3-Leiden.CETP mice. Over a period of 18 days, female APOE*3-Leiden.CETP mice were intracerebroventricularly (ICV) infused with saline (white), acyl-ExD3 (grey; 0.75 nmol/day) or acyl-ExF1 (black; 0.75 nmol/day) (A), created in BioRender. Throughout the study (B) body weight, (C) fat mass, and (E) lean mass were monitored ($n = 8–9$ per group). From day 6 onwards, food was weighed daily per mouse, from which (F) average food intake per day was calculated. At the endpoint, (D) gonadal white adipose tissue (gWAT) and subcutaneous (s)WAT were weighed ($n = 8–9$ per group). At days 0 and 18, (G) plasma glucose levels were measured after 4 h of fasting ($n = 8–9$ per group). Values are presented as means ± SEM. ns: $p > 0.10$, $^{*}p < 0.05$, $^{**}p < 0.01$ compared with saline (in panel B: Grey asterisk represents acyl-ExD3; black asterisk represents acyl-ExF1) according to two-way ANOVA (B) or one-way ANOVA (C-G) and following Tukey's multiple-comparison test.

More studies are needed to identify under what circumstances (e.g., type of agonist, dose, duration of treatment) GLP-1 receptor agonists can be employed to counteract dyslipidaemia.

Intracerebroventricular infusion of acyl-ExD3 and acyl-ExF1 induced weight loss and lowered adiposity in agreement with previous reports on central GLP-1 receptor agonism.[33–35] Additionally, in contrast to peripheral administration, both agonists decreased plasma triglycerides. The potential of GLP-1 receptor agonists to lower adiposity and plasma lipid levels can be partly attributed to enhanced sympathetic innervation of white and brown adipose tissue to promote thermogenesis. In that situation, VLDL-triglyceride-derived fatty acid uptake by these tissues increases to fuel more oxidation.[28,36–38] In accordance, the reduction in plasma triglycerides after central treatment with acyl-ExD3 and acyl-ExF1 was accompanied by more rapid

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

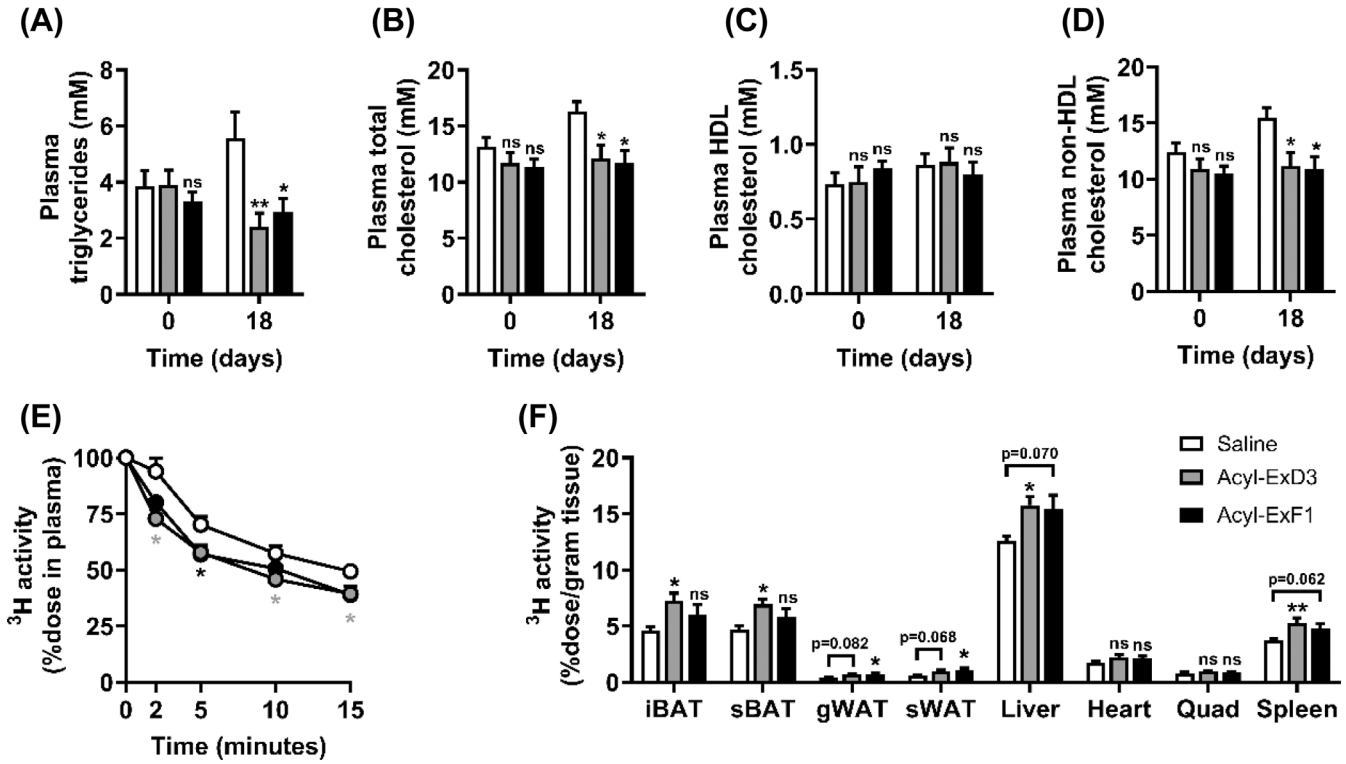


FIGURE 4 Central infusion of acyl-ExF1 and acyl-ExD3 alleviate dyslipidaemia and increase VLDL-derived FA uptake by adipose tissue. Over a period of 18 days, female APOE*3-Leiden.CETP mice were intracerebroventricularly infused with saline (white), acyl-ExD3 (grey; 0.75 nmol/day) or acyl-ExF1 (black; 0.75 nmol/day). At day 0 and 18 plasma levels of (A) triglycerides and (B) total cholesterol, (C) high-density lipoprotein (HDL) cholesterol and (D) non-HDL cholesterol were measured after 4 h of fasting ($n = 8–9$ per group). At the endpoint, mice were injected with VLDL-like particles labelled with glycerol tri[^{3}H]oleate to assess (E) plasma decay and (F) uptake of [^{3}H]oleate by interscapular brown adipose tissue (iBAT), subscapular (s)BAT, gonadal white adipose tissue (gWAT), subcutaneous (s)WAT, liver, heart, quadriceps and spleen ($n = 8–9$ per group). Values are presented as means ± SEM. ns: $p > 0.10$, *$p < 0.05$, **$p < 0.01$ compared with saline (in panel E: Grey asterisk represents acyl-ExD3; black asterisk represents acyl-ExF1) according to one-way ANOVA (A-D, F) or mixed-effects analysis (E) and following Tukey's multiple-comparison test.

clearance of VLDL-like particle-derived fatty acids from the plasma and increased uptake by adipose tissue. As accelerated delipidation of VLDL particles is coupled to increased hepatic uptake of cholesterol-rich VLDL remnants,[39,40] plasma levels of total and non-HDL-cholesterol were also lowered after 18 days of central acyl-ExD3 and acyl-ExF1 treatment. These effects of GLP-1 receptor agonists on lipoprotein metabolism have been previously demonstrated not only in mice but also in humans. As such, treatment with liraglutide and lixisenatide has been shown to substantially reduce postprandial plasma levels of triglycerides and triglyceride-rich lipoproteins, as well as remnant lipoprotein cholesterol, likely as a result of increased triglyceride-derived fatty acid clearance.[26,27,41]

Interestingly, the extent to which central acyl-ExF1 and acyl-ExD3 lowered adiposity and plasma lipid levels was not different between the GLP-1 receptor agonists, despite their opposite signalling bias. There are multiple possible explanations for this outcome. Firstly, the effects of GLP-1 receptor agonists on glucose metabolism, body weight control and thermogenesis are—at least in part—controlled by different cell types. The insulinotropic effects are mediated by GLP-1 receptor activation on pancreatic β-cells, hence the lack of glucose-lowering effects upon central administration of low-dose GLP-1 receptor agonists. On the other hand, body weight regulation is driven by activation of the GLP-1 receptors of the vagal nerve and various brain areas, including the hindbrain, arcuate nucleus, dorsomedial hypothalamus, lateral hypothalamic area and paraventricular nucleus.[42–46] Furthermore, GLP-1 receptors in the ventromedial nucleus of the hypothalamus are primarily responsible for regulating adipose tissue thermogenesis via the sympathetic nervous system.[47] We suspect GLP-1 receptor signalling bias, as identified in pancreatic β-cells, to be less relevant in neuronal populations, a phenomenon known as tissue bias, possibly partly explained by different levels of GLP-1 receptor expression in these different cell types.[48] However, it remains difficult to explain why peripheral administration did result in a differential protection against weight gain, while upon central administration the agonists appeared to be equally effective. Possibly, the continuous central infusion of GLP-1 receptor agonist via osmotic minipumps led to alterations in pharmacodynamics. An alternative explanation is that due to the substantial weight loss upon central infusion of either agonist, survival mechanisms kicked in to prevent further (differential) weight loss in these lean mice. Lastly, while the minimal β-arrestin recruitment and boosted cAMP production induced by acyl-ExF1 is a favourable combination for insulin production by

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

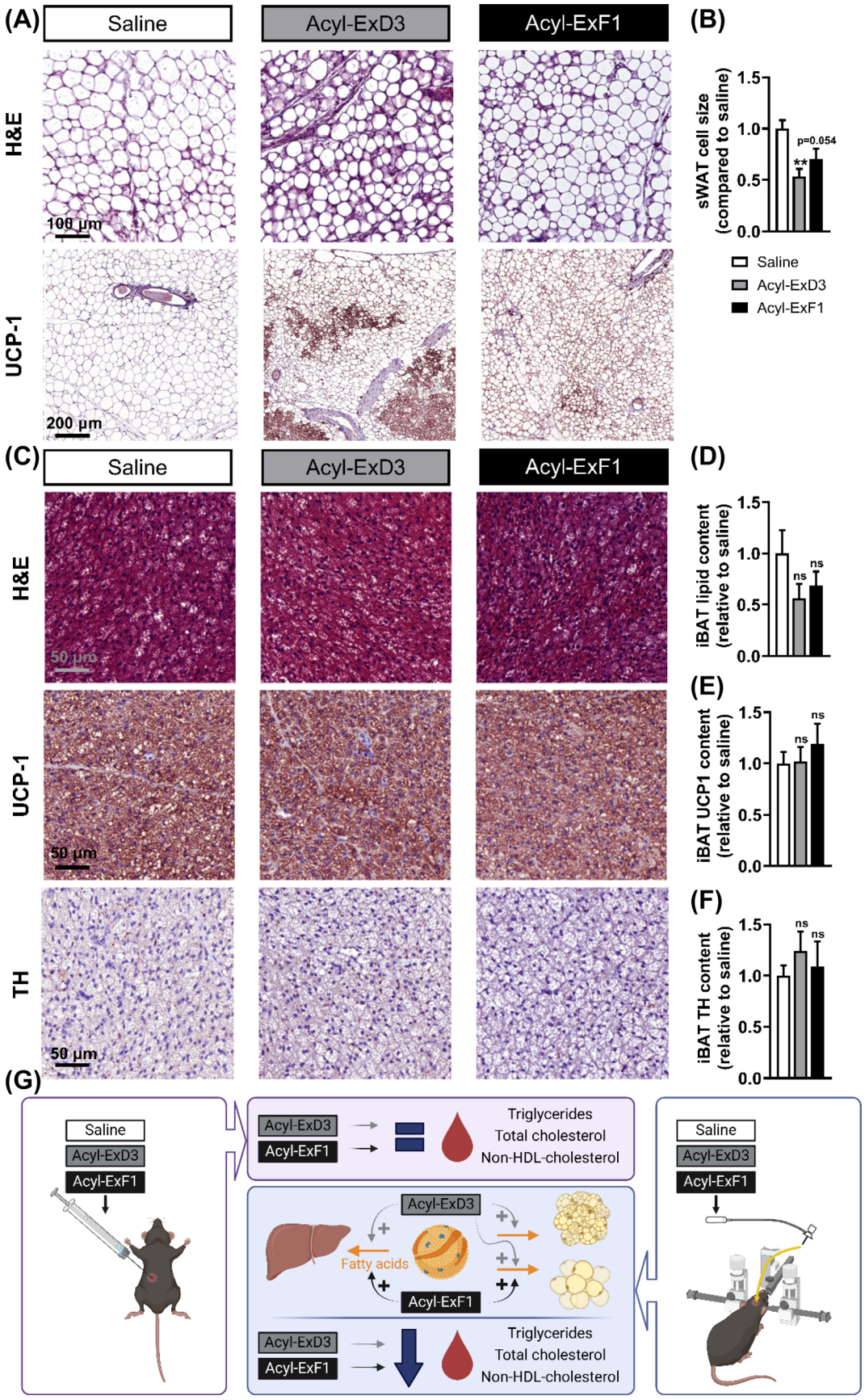


FIGURE 5 Legend on next page.

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

pancreatic β-cells, other outcomes of GLP-1 receptor signalling might be regulated by (the interplay of) alternative intracellular molecules, and/or by agonist-driven differences in GLP-1 receptor signal compartmentalization, including, potentially, distribution of intracellular signalling. Without knowledge of the differential effects of acyl-ExF1 and acyl-ExD3 on a wider range of signalling intermediates, this remains speculative but is a potential avenue for further study.

In the current study, we used APOE*3-Leiden.CETP mice to study lipid metabolism, as these mice are a widely established model for humanized lipoprotein metabolism and have been shown to respond in a human-like manner to lipid-lowering therapies, including statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, increasing the translational value of our research.[49–51] Even though the mouse model highly resembles human lipid metabolism, caution should always be taken when extrapolating findings from mouse studies to humans. For example, we had to restrict our studies to females because only female APOE*3-Leiden.CETP mice develop hyperlipidaemia upon Western-type diet feeding, while male mice start storing lipids ectopically instead and are considered a better model for metabolic dysfunction-associated fatty liver disease (MAFLD) and steatohepatitis (MASH). Another challenge for translating findings from animals to humans arises when the outcomes differ across animal models. For example, while suppression of appetite is a major underlying mechanism for the weight-lowering effects of current GLP-1 therapeutics in humans, the extent to which GLP-1R agonists suppress food intake in animal models depends on many factors, including but not limited to the type of agonist and route of administration.[8] In addition, nutritional status is critical, as fasted animals show reduced cell surface expression of GLP-1R and therefore an attenuated response to GLP-1 compared with fed animals.[52] Lean mice as used in the current study defend their body weight and often show only transient (ranging from a couple of hours to days) reductions in food intake in response to GLP-1R agonists.[28] In contrast, (DIO) mice generally show continued suppression of food intake, as we have also demonstrated for treatment with acyl-ExD3 and acyl-ExF1.[18]

In conclusion, our results show that peripheral administration of two oppositely biased GLP-1 receptor agonists leads to differential regulation of glucose levels and protection from body weight gain in lean APOE*3-LeidenCETP mice. More favourable effects were found with optimized G protein-biased signalling compared with β-arrestin-biased signalling. However, no effect of either agonist on circulating lipid levels was observed. Somewhat unexpectedly, central administration of the two biased agonists equally reduced body weight and fat mass, and alleviated dyslipidaemia due to increased fatty acid uptake by white and brown adipose tissue. Thus, while the G protein-biased signalling increases the potency of GLP-1 receptor agonists for improving glucose regulation and lowering body weight, signalling bias seems to be less significant in the effects on lipid metabolism, at least in lean and hyperlipidaemic APOE*3-LeidenCETP mice.

ACKNOWLEDGEMENTS

The authors thank Maarten Schenke for his invaluable education on the performance of brain operations and Lisa Krul, Robert van Leeuwen and Tamara Zimmermann for excellent technical support.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.16374.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Melanie Modder https://orcid.org/0000-0002-8715-1586
Alejandra Tomas https://orcid.org/0000-0002-2290-8453
Ben Jones https://orcid.org/0000-0003-0461-2584

REFERENCES

1. Lund PK, Goodman RH, Dee PC, Habener JF. Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. *Proc Natl Acad Sci USA*. 1982;79:345-349. doi:10.1073/pnas.79.2.345
2. Lund PK, Goodman RH, Habener JF. Pancreatic pre-proglucagons are encoded by two separate mRNAs. *J Biol Chem*. 1981;256:6515-6518.
3. Mojsov S, Heinrich G, Wilson IB, Ravazzola M, Orci L, Habener JF. Preproglucagon gene expression in pancreas and intestine diversifies at the level of post-translational processing. *J Biol Chem*. 1986;261:11880-11889.
4. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. *J Clin Invest*. 1987;79:616-619. doi:10.1172/JCI112855
5. Orskov C, Holst JJ, Knuhtsen S, Baldissera FG, Poulsen SS, Nielsen OV. Glucagon-like peptides GLP-1 and GLP-2, predicted products of the glucagon gene, are secreted separately from pig small intestine but not pancreas. *Endocrinology*. 1986;119:1467-1475. doi:10.1210/endo-119-4-1467

FIGURE 5 Central infusion of acyl-ExF1 and acyl-ExD3 reduces adipocyte size. Over a period of 18 days, female APOE*3-Leiden.CETP mice were intracerebroventricularly infused with saline (white), acyl-ExD3 (grey; 0.75 nmol/day) or acyl-ExF1 (black; 0.75 nmol/day). In sWAT, (A, B) adipocyte size was determined after haematoxylin & eosin (H&E) staining and uncoupling protein-1 (UCP-1) content was revealed by immunostaining ($n = 8–9$ per group). In interscapular brown adipose tissue (iBAT), after (C) haematoxylin & eosin (H&E) staining and immunostaining, (D) lipid content, (E) UCP-1 content, and (F) tyrosine hydroxylase (TH) content was quantified ($n = 8–9$ per group). (A, C) Representative pictures are shown. (G) Graphical summary of findings. Values are presented as means ± SEM. ns: $p > 0.10$, $^{**}p < 0.01$ compared with saline according to one-way ANOVA and following Tukey's multiple-comparison test.

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

6. Orskov C, Holst JJ, Poulsen SS, Kirkegaard P. Pancreatic and intestinal processing of proglucagon in man. *Diabetologia*. 1987;30:874-881. doi:10.1007/BF00274797
7. Eng J. Exendin peptides. *Mt Sinai J Med*. 1992;59:147-149.
8. Muller TD, Finan B, Bloom SR, et al. Glucagon-like peptide 1 (GLP-1). *Molecular Metabolism*. 2019;30:72-130. doi:10.1016/j.molmet.2019.09.010
9. Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like Peptide-1. *Cell Metab*. 2018;27:740-756. doi:10.1016/j.cmet.2018.03.001
10. Jensterle M, Rizzo M, Haluzik M, Janez A. Efficacy of GLP-1 RA approved for weight Management in Patients with or without diabetes: a narrative review. *Adv Ther*. 2022;39:2452-2467. doi:10.1007/s12325-022-02153-x
11. Drucker DJ, Philippe J, Mojsov S, Chick WL, Habener JF. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci USA*. 1987;84:3434-3438. doi:10.1073/pnas.84.10.3434
12. Hayes MR, Leichner TM, Zhao S, et al. Intracellular signals mediating the food intake-suppressive effects of hindbrain glucagon-like peptide-1 receptor activation. *Cell Metab*. 2011;13:320-330. doi:10.1016/j.cmet.2011.02.001
13. Moore CA, Milano SK, Benovic JL. Regulation of receptor trafficking by GRKs and arrestins. *Annu Rev Physiol*. 2007;69:451-482. doi:10.1146/annurev.physiol.69.022405.154712
14. Mores KL, Cassell RJ, van Rijn RM. Arrestin recruitment and signaling by G protein-coupled receptor heteromers. *Neuropharmacology*. 2019;152:15-21. doi:10.1016/j.neuropharm.2018.11.010
15. Koole C, Wootten D, Simms J, Miller LJ, Christopoulos A, Sexton PM. Differential impact of amino acid substitutions on critical residues of the human glucagon-like peptide-1 receptor involved in peptide activity and small-molecule allostery. *J Pharmacol Exp Ther*. 2015;353:52-63. doi:10.1124/jpet.114.220913
16. Zhang H, Sturchler E, Zhu J, et al. Autocrine selection of a GLP-1R G-protein biased agonist with potent antidiabetic effects. *Nat Commun*. 2015;6:8918. doi:10.1038/ncomms9918
17. Jones B, Buenaventura T, Kanda N, et al. Targeting GLP-1 receptor trafficking to improve agonist efficacy. *Nat Commun*. 2018;9:1602. doi:10.1038/s41467-018-03941-2
18. Lucey M, Pickford P, Bitsi S, et al. Disconnect between signalling potency and in vivo efficacy of pharmacokinetically optimised biased glucagon-like peptide-1 receptor agonists. *Molecular Metabolism*. 2020;37:100991. doi:10.1016/j.molmet.2020.100991
19. Wharton S, Blevins T, Connery L, et al. Daily Oral GLP-1 receptor agonist Orforglipron for adults with obesity. *N Engl J Med*. 2023;389:877-888. doi:10.1056/NEJMoa2302392
20. Willard FS, Douros JD, Gabe MBN, et al. Tirzepatide is an imbalanced and biased dual GIP and GLP-1 receptor agonist. *JCI Insight*. 2020;5:e140532. doi:10.1172/jci.insight.140532
21. Kawai T, Sun B, Yoshino H, et al. Structural basis for GLP-1 receptor activation by LY3502970, an orally active nonpeptide agonist. *Proc Natl Acad Sci USA*. 2020;117:29959-29967. doi:10.1073/pnas.2014879117
22. Sun F, Wu S, Wang J, et al. Effect of glucagon-like peptide-1 receptor agonists on lipid profiles among type 2 diabetes: a systematic review and network meta-analysis. *Clin Ther*. 2015;37:225-241 e228. doi:10.1016/j.clinthera.2014.11.008
23. Alexiadou K, Hartley A, Tan TM, Khamis R. The cardiovascular effects of GLP-1 receptor agonists beyond obesity and type 2 diabetes: an anti-atherosclerotic action. *Trends Cardiovasc Med*. 2024;34:552-557. doi:10.1016/j.tcm.2024.03.003
24. Janssen LGM, Nahon KJ, Bracké KFM, et al. Twelve weeks of exenatide treatment increases [(18)F]fluorodeoxyglucose uptake by brown adipose tissue without affecting oxidative resting energy expenditure in nondiabetic males. *Metabolism*. 2020;106:154167. doi:10.1016/j.metabol.2020.154167
25. Hjerpsted JB, Flint A, Brooks A, Axelsen MB, Kvist T, Blundell J. Semaglutide improves postprandial glucose and lipid metabolism, and delays first-hour gastric emptying in subjects with obesity. *Diabetes Obes Metab*. 2018;20:610-619. doi:10.1111/dom.13120
26. Matikainen N, Söderlund S, Björnson E, et al. Liraglutide treatment improves postprandial lipid metabolism and cardiometabolic risk factors in humans with adequately controlled type 2 diabetes: a single-centre randomized controlled study. *Diabetes Obes Metab*. 2019;21:84-94. doi:10.1111/dom.13487
27. Taskinen MR, Björnson E, Matikainen N, et al. Effects of liraglutide on the metabolism of triglyceride-rich lipoproteins in type 2 diabetes. *Diabetes Obes Metab*. 2021;23:1191-1201. doi:10.1111/dom.14328
28. van Eenige R, Ying Z, Tramper N, et al. Combined glucose-dependent insulinotropic polypeptide receptor and glucagon-like peptide-1 receptor agonism attenuates atherosclerosis severity in APOE*3-Leiden.CETP mice. *Atherosclerosis*. 2023;372:19-31. doi:10.1016/j.atherosclerosis.2023.03.016
29. Wang Y, Parlevliet ET, Geerling JJ, et al. Exendin-4 decreases liver inflammation and atherosclerosis development simultaneously by reducing macrophage infiltration. *Br J Pharmacol*. 2014;171:723-734. doi:10.1111/bph.12490
30. Westerterp M, van der Hoogt CC, de Haan W, et al. Cholesteryl Ester Transfer Protein Decreases High-Density Lipoprotein and Severely Aggravates Atherosclerosis in. *Arterioscler Thromb Vasc Biol*. 2006;26(11):2552-2559. doi:10.1161/01.ATV.0000243925.65265.3c
31. van Eenige R, Verhave PS, Koemans PJ, Tiebosch IACW, Rensen PCN, Kooijman S. RandoMice, a novel, user-friendly randomization tool in animal research. *PLoS One*. 2020;15:e0237096. doi:10.1371/journal.pone.0237096
32. Ying Z, Boon MR, Coskun T, Kooijman S, Rensen PC. A simplified procedure to trace triglyceride-rich lipoprotein metabolism in vivo. *Physiol Rep*. 2021;9:e14820.
33. Kooijman S, Wang Y, Parlevliet ET, et al. Central GLP-1 receptor signalling accelerates plasma clearance of triacylglycerol and glucose by activating brown adipose tissue in mice. *Diabetologia*. 2015;58:2637-2646. doi:10.1007/s00125-015-3727-0
34. Lockie SH, Heppner KM, Chaudhary N, et al. Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. *Diabetes*. 2012;61:2753-2762. doi:10.2337/db11-1556
35. Nogueiras R, Pérez-Tilve D, Veyrat-Durebex C, et al. Direct control of peripheral lipid deposition by CNS GLP-1 receptor signaling is mediated by the sympathetic nervous system and blunted in diet-induced obesity. *J Neurosci*. 2009;29:5916-5925. doi:10.1523/JNEUROSCI.5977-08.2009
36. Oliveira FCB, Bauer EJ, Ribeiro CM, et al. Liraglutide Activates Type 2 Deiodinase and Enhances β3-Adrenergic-Induced Thermogenesis in Mouse Adipose Tissue. *Front Endocrinol*. 2022;12:803363. doi:10.3389/fendo.2021.803363
37. Gutierrez AD, Gao Z, Hamidi V, et al. Anti-diabetic effects of GLP1 analogs are mediated by thermogenic interleukin-6 signaling in adipocytes. *Cell Rep Med*. 2022;3:100813. doi:10.1016/j.xcrm.2022.100813
38. Martins FF, Marinho TS, Cardoso LEM, et al. Semaglutide (GLP-1 receptor agonist) stimulates browning on subcutaneous fat adipocytes and mitigates inflammation and endoplasmic reticulum stress in visceral fat adipocytes of obese mice. *Cell Biochem Funct*. 2022;40:903-913. doi:10.1002/cbf.3751
39. Berbée JF, Boon MR, Khedoe P et al. Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. *Nat Commun*. 2015;6:1-11.
40. Liu C, Schönke M, Zhou E, et al. Pharmacological treatment with FGF21 strongly improves plasma cholesterol metabolism to reduce atherosclerosis. *Cardiovasc Res*. 2022;118:489-502. doi:10.1093/cvr/cvab076

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

41. Whyte MB, Shojaee-Moradie F, Sharaf SE, et al. Lixisenatide reduces chylomicron triacylglycerol by increased clearance. *J Clin Endocrinol Metab*. 2019;104:359-368. doi:10.1210/jc.2018-01176
42. Secher A, Jelsing J, Baquero AF, et al. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J Clin Invest*. 2014;124:4473-4488. doi:10.1172/JCI75276
43. Burmeister MA, Ayala JE, Smouse H, et al. The hypothalamic glucagon-like peptide 1 receptor is sufficient but not necessary for the regulation of energy balance and glucose homeostasis in mice. *Diabetes*. 2017;66:372-384. doi:10.2337/db16-1102
44. Adams JM, Pei H, Sandoval DA, et al. Liraglutide modulates appetite and body weight through glucagon-like peptide 1 receptor-expressing glutamatergic neurons. *Diabetes*. 2018;67:1538-1548. doi:10.2337/db17-1385
45. Alhadeff AL, Grill HJ. Hindbrain nucleus tractus solitarius glucagon-like peptide-1 receptor signaling reduces appetitive and motivational aspects of feeding. *Am J Physiol Regul Integr Comp Physiol*. 2014;307:R465-R470. doi:10.1152/ajpregu.00179.2014
46. Huang KP, Acosta AA, Ghidewon MY, et al. Dissociable hindbrain GLP1R circuits for satiety and aversion. *Nature*. 2024;632:585-593. doi:10.1038/s41586-024-07685-6
47. Beiroa D, Imbernon M, Gallego R, et al. GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes*. 2014;63:3346-3358. doi:10.2337/db14-0302
48. Steen A, Larsen O, Thiele S, Rosenkilde MM. Biased and g protein-independent signaling of chemokine receptors. *Front Immunol*. 2014;5:277. doi:10.3389/fimmu.2014.00277
49. Zhou E, Li Z, Nakashima H, et al. Beneficial effects of brown fat activation on top of PCSK9 inhibition with alirocumab on dyslipidemia and atherosclerosis development in APOE*3-Leiden.CETP mice. *Pharmacol Res*. 2021;167:105524. doi:10.1016/j.phrs.2021.105524
50. van der Vaart JI, van Eenige R, Rensen PCN, Kooijman S. Atherosclerosis: an overview of mouse models and a detailed methodology to quantify lesions in the aortic root. *Vasc Biol*. 2024;6:e230017. doi:10.1530/VB-23-0017
51. de Haan W, Vries-van r Weij J, van der Hoorn JWA, et al. Torcetrapib does not reduce atherosclerosis beyond atorvastatin and induces more proinflammatory lesions than atorvastatin. *Circulation*. 2008;117(19):2515-2522. doi:10.1161/CIRCULATIONAHA.107.761965
52. Ronveaux CC, de Lartigue G, Raybould HE. Ability of GLP-1 to decrease food intake is dependent on nutritional status. *Physiol Behav*. 2014;135:222-229. doi:10.1016/j.physbeh.2014.06.015

How to cite this article: Modder M, Tomas A, Afkir S, et al. Oppositely biased glucagon-like peptide-1 receptor agonism does not differentially affect lipid metabolism in APOE*3-Leiden CETP mice. *Diabetes Obes Metab*. 2025;27(6):3477-3489. doi:10.1111/dom.16374

14631326, 2025, 6, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16374 by Imperial College London, Wiley Online Library on [17/12/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License