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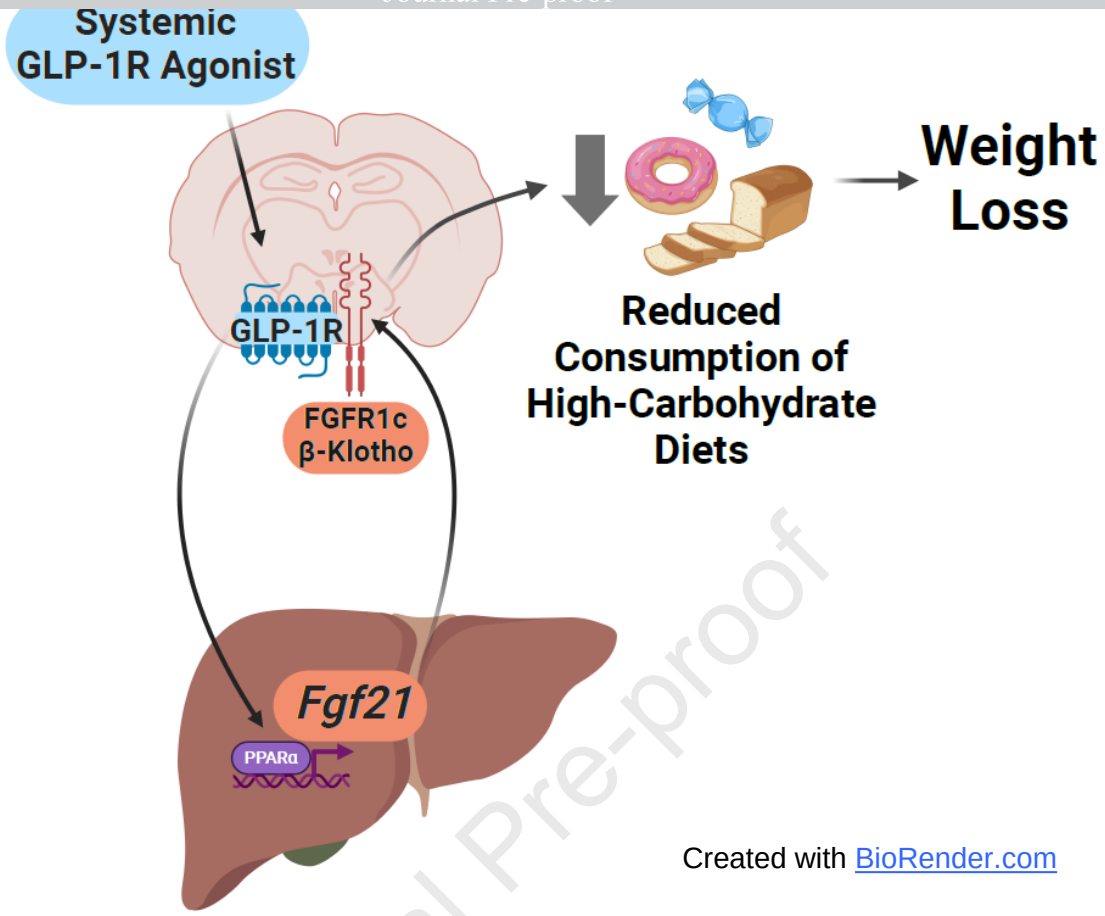
## Graphical Abstract



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# Fibroblast Growth Factor-21 is Required for Weight Loss Induced by the Glucagon-like Peptide-1 Receptor Agonist Liraglutide in Male Mice fed High Carbohydrate Diets

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**Abbreviations:** GLP-1R: Glucagon-like peptide-1 receptor; GLP-1RA: GLP-1R agonist; FGF21: Fibroblast Growth Factor-21; LC: low carbohydrate; HC: high carbohydrate; HFHS: high fat, high sugar; Klb: β-klotho; PPARα: peroxisome proliferator-activated receptor alpha; EE: Energy expenditure.

**Abstract**

**Objective:** Glucagon-like peptide-1 receptor (GLP-1R) agonists (GLP-1RA) and fibroblast growth factor-21 (FGF21) confer similar metabolic benefits. GLP-1RA induce FGF21, leading us to investigate mechanisms engaged by the GLP-1RA liraglutide to increase FGF21 levels and the metabolic relevance of liraglutide -induced FGF21.

**Methods:** Circulating FGF21 levels were measured in fasted male C57BL/6J, neuronal GLP-1R knockout, β-cell GLP-1R knockout, and liver peroxisome proliferator-activated receptor alpha knockout mice treated acutely with liraglutide. To test the metabolic relevance of liver FGF21 in response to liraglutide, chow-fed control and liver *Fgf21* knockout (Liv$^{Fgf21-/-}$) mice were treated with vehicle or liraglutide in metabolic chambers. Body weight and composition, food intake, and energy expenditure were measured. Since FGF21 reduces carbohydrate intake, we measured body weight in mice fed matched diets with low- (LC) or high-carbohydrate (HC) content and in mice fed a high-fat, high-sugar (HFHS) diet. This was done in control and Liv$^{Fgf21-/-}$ mice and in mice lacking neuronal β-klotho (Klb) expression to disrupt brain FGF21 signaling.

**Results:** Liraglutide increases FGF21 levels independently of decreased food intake via neuronal GLP-1R activation. Lack of liver *Fgf21* expression confers resistance to liraglutide-induced weight loss due to attenuated reduction of food intake in chow-fed mice. liraglutide-induced weight loss was impaired in Liv$^{Fgf21-/-}$ mice when fed HC and HFHS diets but not when fed a LC diet. Loss of neuronal Klb also attenuated liraglutide -induced weight loss in mice fed HC or HFHS diets.

**Conclusions:** Our findings support a novel role for a GLP-1R-FGF21 axis in regulating body weight in a dietary carbohydrate-dependent manner.

## 1. Introduction

Obesity is one of the largest health challenges in recent decades. Nearly 1 in 3 U.S. adults are overweight and more than 2 in 5 have obesity [1,2]. In addition to their direct action in the pancreas to stimulate insulin secretion, glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonists (GLP-1RA) comprise a class of drugs that also promote weight loss [3–9]. This weight loss effect is primarily due to a reduction in food intake resulting from GLP-1RA acting on several regions of the brain [10–14]. The mechanisms by which brain GLP-1R activation promotes weight loss remain unclear.

Fibroblast growth factor-21 (FGF21) is a hormone produced mainly by the liver in response to metabolic challenges including low protein and high carbohydrate consumption [15–20]. Interestingly, metabolic effects of FGF21 overlap with many of those associated with GLP-1R activation such as improved glycemic control [21–24], weight reduction [21,25–27], and suppression of carbohydrate intake [28–30]. Indeed, GLP-1R activation induces FGF21 production [31–36]. However, since GLP-1RA reduce caloric intake, and reduced caloric intake stimulates FGF21 production [37–39], GLP-1RA-induced stimulation of FGF21 production could just be secondary to reduced food intake. Furthermore, the contribution of FGF21 to the metabolic benefits of GLP-1RA has not been thoroughly addressed. Given the clinical benefits of GLP-1RA and FGF21, the therapeutic implications of a GLP-1R-FGF21 axis merit investigation.

Here we demonstrate that the therapeutic GLP-1RA liraglutide acts on neuronal GLP-1R to increase circulating FGF21 levels in a food intake-independent manner. We also show that liraglutide-induced FGF21 is required for the full weight-lowering effect of GLP-1R activation in chow-fed mice. Since FGF21 is a feedback inhibitor of carbohydrate intake [28,29], we hypothesized that FGF21 specifically reduces the intake of carbohydrates in response to GLP-1RA treatment. We support this by showing that mice lacking liver *Fgf21* (Liv$^{Fgf21\text{-}/\text{-}}$) are resistant to liraglutide-induced weight loss only when fed high-carbohydrate diets and not when fed low-

carbohydrate diets. Lastly, we show that central FGF21 signaling is required for FGF21 to mediate the weight loss action of liraglutide.

## 2. Materials and Methods

*2.1. Animal models and husbandry.* Only male mice were studied. C57BL/6J mice (The Jackson Laboratory, Inc.) were used in FGF21 measurement studies. Mice lacking *Glp1r* in glutamatergic (Vglut2$^{Glp1r-/-}$) and *Wnt1*-expressing (Wnt1$^{Glp1r-/-}$) neurons were generated by crossing *vGlut2*-Cre or *Wnt1*-Cre mice, respectively, with *floxed-Glp1r* mice as described previously [13,14]. β-cell$^{Glp1r-/-}$ mice were generated by crossing MIP-CreERT with *floxed-Glp1r* mice as previously described [40]. Liver peroxisome proliferator-activated receptor alpha (PPARα; Liv$^{Ppar-/-}$) mice were generated by crossing *Alb*-Cre mice with *floxed-Pppara* mice (a generous gift from Dr. Dan Kelly, University of Pennsylvania). Liv$^{Fgf21-/-}$ mice were generated by crossing *Alb*-Cre mice with *floxed-Fgf21* mice (The Jackson Laboratory, Inc.). Mice lacking β-klotho (Klb) in forebrain (Camk2a$^{Klb-/-}$) or glutamatergic (Vglut2$^{Klb-/-}$) neurons were generated by crossing *Camk2a*-Cre and *vGlut2*-Cre mice, respectively with *floxed-Klb* mice (*Camk2a*-Cre and *floxed-Klb* mice were a generous gift from Dr. Steven Kliewer, University of Texas Southwestern, and were provided by Dr. Christopher Morrison, Pennington Biomedical Research Center), as previously described [41,42]. MGI identification numbers for all mice are provided in **Table 1**. Mice were housed on a 12 h/12 h light/dark cycle (0600-1800h). They had *ad libitum* access to distilled water and were maintained on a chow diet (57.9% calories provided by carbohydrates, 28.7% protein, 13.4% fat; 3.36 kcal/g; 5L0D, LabDiet, St. Louis, MO) from the time of weaning unless specified otherwise.

*2.2. Acute GLP-1RA administration*. Weight-matched C57BL/6J, Vglut2$^{Glp1r-/-}$, β-cell$^{Glp1r-/-}$, Liv$^{Ppar-/-}$, and Wnt1$^{Glp1r-/-}$ mice and respective background-matched control mice were fasted for 4 h at the start of the light cycle and randomly assigned to receive either vehicle (0.9% saline), liraglutide (400 μg/kg, SubQ), exendin-4 (10 μg/kg, IP), or semaglutide (120 μg/kg). Body weight and tail

blood were collected at 0 and 7 h following treatment. Separate cohorts of C57BL/6J mice underwent this protocol following 7 days of treatment with exendin-4 (10 µg/kg, *b.i.d.*) or liraglutide (200 µg/kg, *b.i.d.*). Mice were fasted for 4h prior to an acute injection of vehicle or the respective GLP-1R agonist and collection of tail blood immediately prior to and 7 h post injection as described above.

*2.3. Pair-feeding studies*. Weight-matched C57BL/6J mice were randomly assigned to receive either vehicle (0.9% saline) or liraglutide (200 µg/kg, *b.i.d.*) while having *ad libitum* access to food or while being pair-fed to weight-matched liraglutide-treated mice for 48 h. Access to food for pair feeding was based on average feeding patterns of liraglutide-treated mice determined by prior metabolic cage experiments. For the dark cycle, mice were given access to 0.2 g of food at 1800h (lights off), 0.2 g at 0000h, and 0.4 g at 0500h (1h before lights on). For the light cycle, mice were given 0.4 g at 0800h. Food intake and body weight were monitored throughout the study period. Tail blood was collected at 0 and 48 h following treatment.

*2.4. Metabolic chamber experiments with chronic liraglutide administration.* 15-17-week-old weight-matched control and Liv$^{Fgf21-/-}$ mice fed a chow diet were individually housed for 5-7 days before being placed in a Promethion metabolic system (Sable Systems, Inc.). Following a 5-7 day acclimation period, mice were randomly assigned to receive vehicle (0.9% saline) or liraglutide (200 µg/kq *b.i.d.*) for 11 days. Food intake, energy expenditure were continuously recorded. Daily body weight was measured manually. Body composition measurements were obtained by NMR (Minispec 235 LF90II-TD NMR Analyzer, Bruker) at the start and end of the treatment period.

*2.5. Low and high carbohydrate diet experiments.* 10-12-week-old control and Liv$^{Fgf21-/-}$ mice were placed on a low fat, low carbohydrate or low fat, high carbohydrate diet (D08091802 or D12450J, Research Diets, Inc., respectively) for 4 weeks or a high fat, high sugar diet (D12451, Research Diets, Inc.) for 4 weeks or 1 week. Mice were randomly assigned to receive vehicle (0.9% saline) or liraglutide (200 µg/kg *b.i.d.*) for 14 days while being maintained on their respective diet. Some mice were dosed with liraglutide following feeding on a 60% high fat, low carbohydrate diet

(D12492, Research Diets, Inc.) for 4 weeks starting at 10-12 weeks of age. Body weight was measured daily. Body composition was measured at the start and end of the treatment period.

*2.6. Central Klb knockout studies.* 10-12-week-old control and Camk2a$^{Klb-/-}$ male mice were placed on the low fat, high carbohydrate diet for 4 weeks. 14-15-week-old control and Vglut2$^{Klb-/-}$ were placed on the high fat, high sugar diet for 1 week. Mice were randomly assigned to receive vehicle (0.9% saline) or liraglutide (200 µg/kg *b.i.d.*) for 14 days while being maintained on their respective diet. Body weight was measured daily. Body composition was measured at the start and end of the treatment period.

*2.7. FGF21 measurements*. Tail blood was collected in EDTA-coated microvette tubes (16.444.100, Sarstedt Inc.) and centrifuged at 4,000 x g at 4°C for 20 minutes. Plasma was collected and stored at -20°C until analysis. Plasma FGF21 was measured using a commercially available mouse FGF21 ELISA kit (ab212160, Abcam).

*2.8. RNA isolation and qPCR*. Total RNA was extracted from tissues using DirectZol RNA Mini-prep kit (R2051, Zymogen). cDNA was synthesized by reverse transcription using the iScript cDNA Synthesis Kit (1708891, Bio-Rad). Real time PCR reactions were performed using TaqMan Real-Time PCR Assays (Mm00840165_g1, Thermo Fisher Scientific) and TaqMan Fast Advanced Master Mix (4444556, Applied Biosystems).

*2.9. Statistics*. Data were analyzed using GraphPad Prism 9 Software (GraphPad Software, Inc., La Jolla, CA). Unpaired t-tests, one-way ANOVA or mixed-effects analysis followed by Holm-Sidak's multiple comparisons was used when appropriate and indicated in the figure legends. $P < 0.05$ was considered statistically significant. Values represent mean ± SEM. Energy expenditure (EE) data were analyzed using the EE analysis of covariance (ANCOVA) analysis provided by the NIDDK Mouse Metabolic Phenotyping Centers (MMPC, www.mmpc.org) using their Energy Expenditure Analysis page (http://www.mmpc.org/shared/regression.aspx) and supported by grants DK076169 and DK115255.

*2.10. Study approval*. All animal studies were approved by the Institutional Animal Care and Use Committees at Vanderbilt University, University of Michigan, Duke University, University of Iowa, and the Toronto Center for Phenogenomics, Mt. Sinai Hospital.

## 3. Results

*3.1. GLP-1RA increase FGF21 independently of its food intake-suppressing effects.* GLP-1RA induce FGF21 levels in several mouse models [31–36]. However, since fasting or a state of nutrient deficit stimulates FGF21 production [37–39], increased FGF21 could be secondary to the food intake suppressive effects of GLP-1RA. We first tested whether the GLP-1RA liraglutide increased FGF21 levels independently of its effects on food intake by administering vehicle or liraglutide to 4 h-fasted, male C57BL/6J mice and measuring plasma FGF21 levels at 0 and 7 h following treatment. All mice remained without food for the duration of the study period (**Figure 1A**). Plasma FGF21 levels were significantly higher 7 h following in liraglutide- vs. vehicle-treated mice (**Figure 1B**). A similar effect was observed in mice treated with the GLP-1RA exendin-4 and semaglutide (**Supplemental Figure 1A** and **1B**). Elevated circulating FGF21 levels in response to liraglutide were associated with a significant increase in *Fgf21* mRNA in the liver (**Figure 1C**), consistent with previous studies showing that circulating FGF21 is predominantly secreted from this organ [39]. We also treated *ad libitum*-fed male C57BL/6J mice with vehicle or liraglutide for 2 days and added a third group of mice pair-fed to weight match the liraglutide-treated group (**Figure 1D** and **1E**). Circulating FGF21 levels were significantly higher in liraglutide-treated mice compared to vehicle-treated mice and mice pair-fed to weight match the liraglutide-treated group (**Figure 1F**). These results demonstrate that GLP-1RA increase FGF21 independently of either their food intake-suppressive effects or their ability to promote weight loss.

We also assessed whether chronic GLP-1RA treatment elevates circulating FGF21 levels in fasted mice. Following a 7-day treatment with either exendin-4 or liraglutide, we show that GLP-

1RA treatment increases circulating FGF21 levels in fasted mice 7 h after the last GLP-1R agonist dose (**Figure 1G** and **1H**).

*3.2. Central nervous system GLP-1R and liver PPARα are required for liraglutide to increase plasma FGF21*. While plasma FGF21 is primarily derived from the liver [39], the GLP-1R is not expressed in hepatocytes [43–45]. We, therefore, tested whether neuronal or pancreatic β-cell GLP-1R expression is required for liraglutide to increase FGF21 levels. Using the same protocol as in Figure 1A, we administered vehicle or liraglutide to fasted control mice and mice lacking the GLP-1R in neurons targeted by the *Wnt1-Cre2* driver (Wnt1$^{Glp1r-/-}$) or in glutamatergic neurons (Vglut2$^{Glp1r-/-}$). *Wnt1-Cre2* targets neurons within the central nervous system, including the hypothalamus and brain stem, as well as the enteric nervous system [46]. Importantly, both Wnt1$^{Glp1r-/-}$ and Vglut2$^{Glp1r-/-}$ mice have been previously shown to be resistant to the weight-lowering effects of GLP-1RA [14,13]. Liraglutide failed to induce FGF21 in both Wnt1$^{Glp1r-/-}$ (**Supplemental Figure 2A** and **2B**) and Vglut2$^{Glp1r-/-}$ (**Figure 2A** and **2B**) mice. In contrast, the stimulatory effect of liraglutide on FGF21 remained intact in mice lacking the GLP-1R in pancreatic β-cells (**Fig. 2C** and **2D**), another major site of GLP-1R expression and actions [45]. To verify that liraglutide-induced circulating FGF21 originates from the liver, we administered vehicle and liraglutide to fasted control mice and mice lacking liver PPARα, a key regulator of liver FGF21 production. Plasma FGF21 levels were increased in liraglutide-treated control mice but not in liraglutide-treated liver *Ppara* knockout (Liv$^{Ppara-/-}$) mice (**Figure 2E** and **2F**). Taken together, these findings suggest that liraglutide engages neuronal GLP-1R to induce FGF21 production, and increased FGF21 production requires liver PPARα expression.

*3.3. FGF21 is partially required for liraglutide-induced weight loss*. To investigate the contribution of FGF21 to the weight-lowering effects of GLP-1R activation, we chronically administered vehicle or liraglutide to chow-fed control and liver *Fgf21* knockout (Liv$^{Fgf21-/-}$) mice. Pre-treatment body weights were not different between any of the groups (**Supplemental Figure 3A**). Chow-fed Liv$^{Fgf21-/-}$ mice were partially resistant to liraglutide-induced weight loss (**Figure 3A** and **3B**). This

was due, in part, to an attenuated reduction in food intake, particularly during the first day of treatment in Liv$^{Fgf21-/-}$ mice (**Figure 3C**). The total caloric intake throughout the treatment period was significantly reduced in control mice but not in Liv$^{Fgf21-/-}$ mice (**Figure 3D**). There was no significant difference in energy expenditure (EE) between genotypes and treatment groups (**Figure 3E** and **3F**). However, because there was a significant difference in pre-treatment EE between the liraglutide-treated groups (**Figure 3E**), we calculated the change in EE relative to the pre-treatment period. Liraglutide treatment produced a small decrease in EE in control mice but a larger, more sustained decrease in EE during treatment in Liv$^{Fgf21-/-}$ mice (**Supplemental Figure 3B**). The significant body weight difference between liraglutide-treated control and Liv$^{Fgf21-/-}$ mice (**Figure 3G**) was associated with an attenuated, albeit not significant, reduction in fat-free (i.e., lean) mass in Liv$^{Fgf21-/-}$ mice (**Figure 3H**). Changes in fat mass did not differ between liraglutide-treated control and Liv$^{Fgf21-/-}$ mice (**Figure 3I**). Circulating FGF21 levels were almost undetectable in both vehicle- and liraglutide-treated Liv$^{Fgf21-/-}$ mice compared to the already low levels in control mice (**Supplemental Figure 3C**), verifying the validity of this model. This chronic liraglutide dosing regimen did not significantly elevate circulating FGF21 levels in chow-fed mice (**Supplemental Figure 3C**). However, these measurements were made in *ad lib* fed mice, and as we show in **Figure 1H**, chronic liraglutide does stimulate circulating FGF21 levels in fasted mice.

*3.4. FGF21 mediates liraglutide-induced weight loss specifically in the context of high-carbohydrate diets by engaging the central nervous system*. Since FGF21 suppresses carbohydrate intake and sweet preference in rodents [28,29] and has been associated with these phenotypes in humans [47–52], we hypothesized that FGF21 contributes specifically to a reduction in body weight by liraglutide in the presence of high carbohydrate diets. To test this hypothesis, we placed control and Liv$^{Fgf21-/-}$ mice on calorically matched, low fat diets with either low (LC) or high carbohydrate (HC) content (30%, LC and 70%, HC, respectively) for 4 weeks followed by a 2-week treatment with vehicle or liraglutide while mice remained on their respective

diet. Regardless of diet and treatment, circulating FGF21 levels were almost undetectable in Liv$^{Fgf21-/-}$ mice (**Supplemental Figure 4A** and **4B**). Liraglutide reduced body weight to a greater degree in HC-fed control mice (**Figure 4C** and **4D**) than in LC-fed control mice (**Figure 4A** and **4B**). Importantly, only HC-fed Liv$^{Fgf21-/-}$ mice were significantly resistant to liraglutide-induced weight loss (**Figure 4C** and **4D**), suggesting that FGF21 contributes to the weight loss effects of liraglutide in mice maintained on low fat, high carbohydrate diets. Next, we examined the relevance of these findings in the context of a high fat, high sugar (HFHS) diet (45% fat; 35% carbohydrate [50% of which is sucrose]). Control and Liv$^{Fgf21-/-}$ mice were fed the HFHS diet for 1 or 4 weeks followed by treatment with vehicle or liraglutide for 2 weeks. It is important to note that mice did not gain weight when fed the HFHS diet for 1 week. The 1-week HFHS cohort is therefore included to control for any potential effects of the diet-induced weight gain observed in mice fed the same diet for 4 weeks. As seen in **Figures 4E** - **4H**, when maintained on either 4 weeks (**Figure 4E** and **4F**) or 1 week (**Figure 4G** and **4H**) of HFHS diet prior to treatment, Liv$^{Fgf21-/-}$ mice lost less weight than their control counterparts when dosed with liraglutide. Importantly, when fed a high fat diet (HFD) containing lower carbohydrate content (60% fat, 20% carbohydrate), liraglutide-induced weight loss was not significantly attenuated in Liv$^{Fgf21-/-}$ mice (**Figure 4I** and **4J**). Taken together, these results support our hypothesis that FGF21 mediates the weight lowering actions of liraglutide specifically in the context of high carbohydrate diets.

We next examined the target engaged by FGF21 to facilitate the weight-lowering effects of liraglutide. FGF21 signals to a receptor complex comprised of FGF receptor 1c (FGFR1c) and its co-receptor, β-klotho (Klb) [53–55]. As FGFR1c is ubiquitously expressed, Klb expression confers tissue specificity for FGF21 [56]. Previous studies show that obese mice lacking *Klb* expression in the forebrain (Camk2a$^{Klb-/-}$) are resistant to the suppressive effects of recombinant FGF21 on body weight and energy expenditure [41]. More recent studies show that mice lacking *Klb* expression in glutamatergic neurons (Vglut2$^{Klb-/-}$) are resistant to FGF21-induced reduction in sugar intake and sweet preference [57]. We, therefore, tested the hypothesis that FGF21 acts in

these neuronal populations to facilitate the weight loss induced by liraglutide. When placed on the same HC diet as that used in **Figures 4C** and **4D**, liraglutide-treated Camk2a$^{Klb-/-}$ mice displayed partial resistance to weight loss compared to liraglutide-treated control mice (**Figure 5A** and **5B**). Similarly, Vglut2$^{Klb-/-}$ mice maintained on 1 week of the same HFHS diet used in **Figure 4G** and **4H** were also resistant to the weight-lowering effects of liraglutide compared to control mice (**Figure 5C** and **5D**). These findings suggest that central FGF21 signaling is required for the full effect of liraglutide-induced weight loss, supporting our hypothesis that liraglutide-induced FGF21 signals to the central nervous system to facilitate the weight-lowering actions of liraglutide.

## 4. Discussion

In this study, we identify a novel brain GLP-1R-liver FGF21 axis that mediates the weight-lowering effect of GLP-1RA in the presence of diets with high carbohydrate content. These results could provide insight into the clinically observed variability in weight loss following GLP-1R agonist treatment. While liraglutide and semaglutide can promote up to 10% and 15% weight loss, respectively, many individuals lose significantly less weight in response to these drugs [58–60]. Results from this preclinical study suggest the hypothesis that macronutrient content and FGF21-related factors (e.g., FGF21-resistant states, FGF21 polymorphisms) could influence the weight loss efficacy of GLP-1RA and thereby may help explain the significant heterogeneity in the magnitude of weight loss following GLP-1RA treatment in humans.

GLP-1RA such as exendin-4 and liraglutide have been previously shown to increase circulating levels of FGF21 [31–36]. However, these studies did not address whether GLP-1RA stimulation of FGF21 production arises indirectly from GLP-1RA-induced reduction in food intake – a known stimulus of hepatic FGF21 secretion [37–39]. Here, we demonstrate that liraglutide increases FGF21 production independently of its effects on food intake. Although the liver is the primary source of circulating FGF21 [39], the GLP-1R is not expressed in hepatocytes [43–45]. This strongly suggests an indirect mechanism for GLP-1R agonist-mediated stimulation of FGF21

levels. Utilizing multiple tissue-specific knockout mouse models, we show that neuronal GLP-1R expression, specifically in glutamatergic neurons and those within the cellular target domains of *Wnt1Cre2*, is required for the stimulatory effect of liraglutide on FGF21. Systemic liraglutide engages several brain regions such as the arcuate and paraventricular hypothalamic nuclei, subfornical organ, area postrema, and the nucleus tractus solitarius [61]. Future studies will target *Glp1r* deletion in specific cell types (e.g., pro-opiomelanocortin neurons in the arcuate nucleus [62,63]) or broadly within these regions to identify the neuroanatomical location(s) responsible for liver FGF21 induction. Findings from these experiments will provide an important foundation for subsequent studies investigating how neuronal GLP-1R activation stimulates FGF21 production by the liver. One possibility is that GLP-1RA-induced liver FGF21 production is mediated by direct autonomic projections from the brain to the liver. Central GLP-1R activation has also been shown to increase sympathetic outflow to adipose depots in rodents [64–67], which could stimulate lipolysis and subsequent release of free fatty acids, a potent stimulator of liver PPARα and FGF21 production. Another potential mechanism by which central GLP-1R activation could stimulate hepatic FGF21 production is via the hypothalamic-pituitary-adrenal (HPA) axis. It is well-established that GLP-1RA, administered centrally or peripherally, activate the HPA axis to increase circulating corticosterone levels in both rodents and humans [68–70]. Glucocorticoids (GC) can in turn induce *Fgf21* expression in the liver via hepatic GC receptor activation [71]. Future studies utilizing pharmacological and surgical methods to disrupt these pathways will be important to delineate the role of autonomic innervation and/or the HPA axis in mediating the GLP-1R-FGF21 interaction.

Our finding that liraglutide-induced reduction in body weight is attenuated in chow-fed Liv$^{Fgf21-/-}$ mice indicates that FGF21 is a component of the anorectic effect of GLP-1RA. Among its many metabolic actions, FGF21 plays an important role in regulating carbohydrate intake and preference. Liver FGF21 production is stimulated by high carbohydrate intake [29,72,73], and this, in turn, acts as a feedback inhibitor of subsequent carbohydrate consumption [28,29]. Here

we hypothesized that GLP-1RA-induced FGF21 acts in a similar manner and facilitates the decreased intake of carbohydrate-rich diets in response to GLP-1RA treatment. This hypothesis was supported by our finding that loss of liver *Fgf21* expression selectively attenuated liraglutide-induced weight loss in mice fed a high carbohydrate diet whether they were low or high in fat content. Contrasting the latter, loss of liver *Fgf21* expression did not attenuate liraglutide-induced weight loss in mice fed a high fat (60%), low carbohydrate diet, further suggesting that the contribution of *Fgf21* to weight loss by liraglutide is dependent on dietary carbohydrate content. Supporting this, a previous study showed that loss of liver *Fgf21* expression did attenuate liraglutide-induced weight loss in mice fed a similar 60% high fat diet as the one used here but that was supplemented with fructose. Thus, we speculate that the additional carbohydrate content from the fructose supplementation unmasked a role for loss of *Fgf21* not observed in our studies using non-fructose supplemented high fat diet. Interestingly, we also demonstrate that in control mice, liraglutide reduces body weight to a greater degree in mice fed a high-carbohydrate diet. These findings may be clinically relevant since they raise the testable hypothesis that dietary carbohydrate content can modify the effectiveness of GLP-1RA as weight loss drugs. Moreover, since several variants in the *hFGF21* gene locus have been associated with effects on carbohydrate intake and sweet preference in humans [47–52], results from our studies propose a potential significance of these genetic variants in influencing an individual's weight loss response to GLP-1RA.

Weight loss in response to GLP-1RA is primarily attributed to reduced food intake. Although GLP-1RA stimulate sympathetic outflow to adipose tissues in rodents [64–66], a consistent effect on energy expenditure has not been clearly established [74]. On the other hand, pharmacological levels of FGF21 have been shown to increase energy expenditure in mice [21,25]. A previous study reported that liraglutide stimulates FGF21 production from adipose tissue-resident invariant natural killer T (iNKT) cells, and that this increased FGF21, in turn, promoted weight loss via increased energy expenditure [34]. In the present studies, we did not observe increased energy

expenditure in response to liraglutide in control or Liv$^{Fgf21-/-}$ mice. Interestingly, liraglutide-treated Liv$^{Fgf21-/-}$ mice displayed a sustained decrease in energy expenditure whereas this effect was attenuated in control mice. This suggests that an increase in food intake and a greater reduction in energy expenditure additively contribute to the attenuation of the weight-lowering effect of liraglutide in Liv$^{Fgf21-/-}$ mice. Furthermore, reduced weight loss in Liv$^{Fgf21-/-}$ mice was also associated with a slightly attenuated reduction in lean mass compared to control mice, although this was not significant. FGF21 has been suggested to promote muscle atrophy in response to fasting [75], so this is in line with our findings that loss of *Fgf21* expression provides a slight protection of lean mass in response to liraglutide-induced weight loss. Future studies will address the target tissues and mechanisms mediating the effects of liraglutide-induced increases in FGF21 levels on food intake and energy expenditure.

FGF21 signals via a FGFR1c-Klb dimer. Since the tissue specificity of FGF21 actions is conferred by expression of Klb, site-specific knockouts of *Klb* are used to disrupt FGF21 signaling in different cell types and tissues [53–55]. Disruption of *Klb* in forebrain regions expressing *Camk2a* render mice unresponsive to the pharmacological effects of FGF21 on body weight [41] and sweet preference [28]. In addition, *Klb* expression in glutamatergic neurons mediates the suppressive effect of FGF21 on carbohydrate intake [57] and body weight [76,77]. Our finding that liraglutide-induced weight loss is also diminished in mice lacking *Klb* in *Camk2a*-expressing cells suggests that forebrain neurons are targeted by FGF21 to reduce body weight in response to liraglutide. We further demonstrate that loss of *Klb* expression in glutamatergic neurons also attenuates liraglutide-induced weight loss in mice fed a high-fat, high-sugar diet. It must be noted that our findings in Camk2a$^{Klb-/-}$ and Vglut2$^{Klb-/-}$ mice were obtained using different diets and for different durations – HC for 4 weeks and HFHS for 1 week, respectively – so these results must be interpreted with caution. Future experiments will determine whether loss of *Klb* expression in these neuronal populations impairs GLP-1RA weight loss across multiple high carbohydrate diets. Nevertheless, our findings suggest that liraglutide-induced FGF21 promotes weight loss in the

presence of high carbohydrate diets via its actions on the central nervous system. Future studies will also use region-specific *Klb* knockout models to more precisely identify the brain region(s) mediating this effect. A key target is the ventromedial hypothalamus since loss of *Klb* expression in this brain region blocks the ability of FGF21 to reduce carbohydrate intake and sucrose preference [57]. The paraventricular nucleus of the hypothalamus is another potential site of liraglutide-induced FGF21 actions as loss of *Klb* expression in this region attenuates baseline preference for sucrose even in the absence of markedly increased FGF21 [57].

Besides decreasing absolute carbohydrate intake, FGF21 also reduces the preference for high carbohydrates and simple sugar [28–30]. GLP-1RA also decrease the rewarding value of simple sugar [78–81]. The present studies focused on absolute intake by providing mice with only one type of diet. By providing mice with simultaneous access to diets or solutions with different carbohydrate/simple sugar content we can determine whether GLP-1RA-mediated increases in FGF21 also affect dietary preferences.

## 5. Conclusions

The present studies identify FGF21 as a component of a novel brain-liver-brain crosstalk that plays a key role in mediating the food intake- and weight-suppressive benefits of the GLP-1RA liraglutide in the presence of high carbohydrate diets. More in-depth preclinical and clinical studies into the role of the brain GLP-1R-liver FGF21 crosstalk may shed light on the well-documented variability in response to GLP-1RA and thereby enable precision medicine tailoring of GLP-1-based therapeutics to different individuals based on their genetics and environment such as diet. Given the benefits and growing call for wider implementation of “food is medicine” interventions such as medically tailored meals (MTMs) for diet-related diseases [82,83], more in-depth understanding of how dietary composition modifies GLP-1RA efficacy would inform refinement of current and future therapeutic protocols for the use of MTMs and GLP-1-based therapeutics for chronic weight management. Moreover, our results support the novel notion that the anorectic

effect of GLP-1RA is comprised of discrete and differentially regulated actions of these compounds influenced by different dietary components. Better understanding of these pathways may drive development of novel strategies such as dual and/or biased agonists [84] to fully harness the therapeutic potential of the GLP-1 system. Lastly, while the current study focuses on food intake and body weight, GLP-1RA and FGF21 analogues share many other metabolic benefits including protection against cardiovascular diseases [85,86], hepatosteatosis [87,88], neurodegenerative diseases [89–91], and suppression of alcohol consumption [28,92–94]. Future studies examining the potential role of the GLP-1R-FGF21 axis in these therapeutic areas could inform the development of novel pharmacologic strategies for the treatment of these conditions.

**Author contributions**

TDVL and JEA designed the experiments and wrote the manuscript. TDVL, PF, ABW, BJE, NB-K, LLB, JK, JLB, MBB, MBP, JPR, AIS and KGN performed the experiments and/or analyzed the data. TDVL, JEC, LLB, DJD, NB-K, RJS, MJP and JEA edited the manuscript. All authors reviewed the final version.

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## References

[1] Fryar CD, Carroll MD, Afful J, 2020. Prevalence of Overweight, Obesity, and Extreme Obesity Among Adults Aged 20 and Over: United States, 1960–1962 Through 2017–2018. NCHS Health E-Stats.

[2] Stierman, B., Afful, J., Carroll, M.D., Chen, T.C., Davy, O., Fink, S., et al., 2021. National Health and Nutrition Examination Survey 2017–March 2020 Prepandemic Data Files Development of Files and Prevalence Estimates for Selected Health Outcomes. National Health Statistics Reports 2021(158), Doi: 10.15620/CDC:106273.

[3] Astrup, A., Rössner, S., Van Gaal, L., Rissanen, A., Niskanen, L., Al Hakim, M., et al., 2009. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. The Lancet 374(9701): 1606–16, Doi: 10.1016/S0140-6736(09)61375-1.

[4] Vilsbøll, T., Christensen, M., Junker, A.E., Knop, F.K., Gluud, L.L., 2012. Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. BMJ 344(7841), Doi: 10.1136/BMJ.D7771.

[5] Pi-Sunyer, X., Astrup, A., Fujioka, K., Greenway, F., Halpern, A., Krempf, M., et al., 2015. A Randomized, Controlled Trial of 3.0 mg of Liraglutide in Weight Management. New England Journal of Medicine 373(1): 11–22, Doi: 10.1056/NEJMOA1411892/SUPPL_FILE/NEJMOA1411892_DISCLOSURES.PDF.

[6] Davies, M., Færch, L., Jeppesen, O.K., Pakseresht, A., Pedersen, S.D., Perreault, L., et al., 2021. Semaglutide 2·4 mg once a week in adults with overweight or obesity, and type 2 diabetes (STEP 2): a randomised, double-blind, double-dummy, placebo-controlled, phase 3 trial. The Lancet 397(10278): 971–84, Doi: 10.1016/S0140-6736(21)00213-0.

[7] Wadden, T.A., Bailey, T.S., Billings, L.K., Davies, M., Frias, J.P., Koroleva, A., et al., 2021. Effect of Subcutaneous Semaglutide vs Placebo as an Adjunct to Intensive Behavioral Therapy on Body Weight in Adults With Overweight or Obesity: The STEP 3 Randomized Clinical Trial. JAMA 325(14): 1403–13, Doi: 10.1001/JAMA.2021.1831.

[8] Wilding, J.P.H., Batterham, R.L., Calanna, S., Davies, M., Van Gaal, L.F., Lingvay, I., et al., 2021. Once-Weekly Semaglutide in Adults with Overweight or Obesity. New England Journal of Medicine 384(11): 989–1002, Doi: 10.1056/NEJMOA2032183/SUPPL_FILE/NEJMOA2032183_DATA-SHARING.PDF.

[9] Vosoughi, K., Atieh, J., Khanna, L., Khoshbin, K., Prokop, L.J., Davitkov, P., et al., 2021. Association of Glucagon-like Peptide 1 Analogs and Agonists Administered for Obesity with Weight Loss and Adverse Events: A Systematic Review and Network Meta-analysis. EClinicalMedicine 42: 101213.

[10] Secher, A., Jelsing, J., Baquero, A.F., Hecksher-Sørensen, J., Cowley, M.A., Dalbøge, L.S., et al., 2014. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. Journal of Clinical Investigation 124(10): 4473–88, Doi: 10.1172/JCI75276.

[11] Sisley, S., Gutierrez-Aguilar, R., Scott, M., D'Alessio, D.A., Sandoval, D.A., Seeley, R.J., 2014. Neuronal GLP1R mediates liraglutide's anorectic but not glucose-lowering effect. Journal of Clinical Investigation 124(6): 2456–63, Doi: 10.1172/JCI72434.

[12] Burmeister, M.A., Ayala, J.E., Smouse, H., Landivar-Rocha, A., Brown, J.D., Drucker, D.J., et al., 2017. The hypothalamic glucagon-like peptide 1 receptor is sufficient but not necessary for the regulation of energy balance and glucose homeostasis in mice. Diabetes 66(2): 372–84, Doi: 10.2337/db16-1102.

[13] Adams, J.M., Pei, H., Sandoval, D.A., Seeley, R.J., Chang, R.B., Liberles, S.D., et al., 2018. Liraglutide modulates appetite and body weight through glucagon-like peptide 1 receptor-expressing glutamatergic neurons. Diabetes, vol. 67. American Diabetes Association Inc. p. 1538–48.

[14] Varin, E.M., Mulvihill, E.E., Baggio, L.L., Koehler, J.A., Cao, X., Seeley, R.J., et al., 2019. Distinct Neural Sites of GLP-1R Expression Mediate Physiological versus Pharmacological Control of Incretin Action. Cell Reports 27(11): 3371-3384.e3, Doi: 10.1016/J.CELREP.2019.05.055.
[15] Potthoff, M.J., Kliewer, S.A., Mangelsdorf, D.J., 2012. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. Genes & Development 26(4): 312–24, Doi: 10.1101/GAD.184788.111.
[16] Fisher, F.M., Maratos-Flier, E., 2016. Understanding the Physiology of FGF21. Annual Review of Physiology 78: 223–41, Doi: 10.1146/ANNUREV-PHYSIOL-021115-105339.
[17] Kharitonenkov, A., DiMarchi, R., 2017. Fibroblast growth factor 21 night watch: advances and uncertainties in the field. Journal of Internal Medicine 281(3): 233–46.
[18] BonDurant, L.D., Potthoff, M.J., 2018. Fibroblast Growth Factor 21: A Versatile Regulator of Metabolic Homeostasis. Annual Review of Nutrition 38(1): annurev-nutr-071816-064800, Doi: 10.1146/annurev-nutr-071816.
[19] Kliewer, S.A., Mangelsdorf, D.J., 2019. A Dozen Years of Discovery: Insights into the Physiology and Pharmacology of FGF21. Cell Metabolism 29(2): 246–53, Doi: 10.1016/J.CMET.2019.01.004.
[20] Hill, C.M., Qualls-Creekmore, E., Berthoud, H.R., Soto, P., Yu, S., McDougal, D.H., et al., 2020. FGF21 and the Physiological Regulation of Macronutrient Preference. Endocrinology 161(3), Doi: 10.1210/ENDOCR/BQAA019.
[21] Xu, J., Lloyd, D.J., Hale, C., Stanislaus, S., Chen, M., Sivits, G., et al., 2009. Fibroblast Growth Factor 21 Reverses Hepatic Steatosis, Increases Energy Expenditure, and Improves Insulin Sensitivity in Diet-Induced Obese Mice. Diabetes 58(1): 250–9, Doi: 10.2337/db08-0392.
[22] Kharitonenkov, A., Shiyanova, T.L., Koester, A., Ford, A.M., Micanovic, R., Galbreath, E.J., et al., 2005. FGF-21 as a novel metabolic regulator. The Journal of Clinical Investigation 115(6): 1627–35, Doi: 10.1172/JCI23606.
[23] Davies, M.J., Bergenstal, R., Bode, B., Kushner, R.F., Lewin, A., Skjøth, T.V., et al., 2015. Efficacy of Liraglutide for Weight Loss Among Patients With Type 2 Diabetes: The SCALE Diabetes Randomized Clinical Trial. JAMA 314(7): 687–99, Doi: 10.1001/JAMA.2015.9676.
[24] Kaufman, A., Abuqayyas, L., Denney, W.S., Tillman, E.J., Rolph, T., 2020. AKR-001, an Fc-FGF21 Analog, Showed Sustained Pharmacodynamic Effects on Insulin Sensitivity and Lipid Metabolism in Type 2 Diabetes Patients. Cell Reports Medicine 1(4): 100057, Doi: 10.1016/J.XCRM.2020.100057.
[25] Coskun, T., Bina, H.A., Schneider, M.A., Dunbar, J.D., Hu, C.C., Chen, Y., et al., 2008. Fibroblast Growth Factor 21 Corrects Obesity in Mice. Endocrinology 149(12): 6018–27, Doi: 10.1210/en.2008-0816.
[26] Gaich, G., Chien, J.Y., Fu, H., Glass, L.C., Deeg, M.A., Holland, W.L., et al., 2013. The Effects of LY2405319, an FGF21 Analog, in Obese Human Subjects with Type 2 Diabetes. Cell Metabolism 18(3): 333–40, Doi: 10.1016/j.cmet.2013.08.005.
[27] Talukdar, S., Zhou, Y., Li, D., Rossulek, M., Dong, J., Somayaji, V., et al., 2016. A Long-Acting FGF21 Molecule, PF-05231023, Decreases Body Weight and Improves Lipid Profile in Non-human Primates and Type 2 Diabetic Subjects. Cell Metabolism 23(3): 427–40, Doi: 10.1016/J.CMET.2016.02.001.
[28] Talukdar, S., Owen, B.M., Mangelsdorf, D.J., Goodwin, B., Correspondence, S.A.K., Song, P., et al., 2016. FGF21 Regulates Sweet and Alcohol Preference Cell Metabolism FGF21 Regulates Sweet and Alcohol Preference. Cell Metabolism 23: 344–9, Doi: 10.1016/j.cmet.2015.12.008.
[29] Von Holstein-Rathlou, S., Bondurant, L.D., Peltekian, L., Naber, M.C., Yin, T.C., Claflin, K.E., et al., 2016. FGF21 mediates endocrine control of simple sugar intake and sweet

taste preference by the liver. Cell Metabolism 23(2): 335–43, Doi: 10.1016/j.cmet.2015.12.003.
[30] Søberg, S., Sandholt, C.H., Jespersen, N.Z., Toft, U., Madsen, A.L., von Holstein-Rathlou, S., et al., 2017. FGF21 Is a Sugar-Induced Hormone Associated with Sweet Intake and Preference in Humans. Cell Metabolism 25(5): 1045-1053.e6, Doi: 10.1016/J.CMET.2017.04.009.
[31] Yang, M., Zhang, L., Wang, C., Liu, H., Boden, G., Yang, G., et al., 2012. Liraglutide Increases FGF-21 Activity and Insulin Sensitivity in High Fat Diet and Adiponectin Knockdown Induced Insulin Resistance. PLoS ONE 7(11): e48392, Doi: 10.1371/journal.pone.0048392.
[32] Nonogaki, K., Hazama, M., Satoh, N., 2014. Liraglutide Suppresses Obesity and Hyperglycemia Associated with Increases in Hepatic Fibroblast Growth Factor 21 Production in KKA y Mice. BioMed Research International 2014: 1–8, Doi: 10.1155/2014/751930.
[33] Lee, J., Hong, S.W., Park, S.E., Rhee, E.J., Park, C.Y., Oh, K.W., et al., 2014. Exendin-4 regulates lipid metabolism and fibroblast growth factor 21 in hepatic steatosis. Metabolism: Clinical and Experimental 63(8): 1041–8, Doi: 10.1016/J.METABOL.2014.04.011.
[34] Lynch, L., Hogan, A.E., Duquette, D., Lester, C., Banks, A., LeClair, K., et al., 2016. iNKT Cells Induce FGF21 for Thermogenesis and Are Required for Maximal Weight Loss in GLP1 Therapy. Cell Metabolism 24(3): 510–9, Doi: 10.1016/J.CMET.2016.08.003.
[35] Liu, J., Yang, K., Yang, J., Xiao, W., Le, Y., Yu, F., et al., 2019. Liver-derived fibroblast growth factor 21 mediates effects of glucagon-like peptide-1 in attenuating hepatic glucose output. EBioMedicine 41: 73–84, Doi: 10.1016/J.EBIOM.2019.02.037.
[36] Liu, D., Pang, J., Shao, W., Gu, J., Zeng, Y., He, H.H., et al., 2021. Hepatic fibroblast growth factor 21 is involved in mediating functions of liraglutide in mice with dietary challenge. Hepatology 74(4): 2154–69, Doi: 10.1002/hep.31856.
[37] Badman, M.K., Pissios, P., Kennedy, A.R., Koukos, G., Flier, J.S., Maratos-Flier, E., 2007. Hepatic Fibroblast Growth Factor 21 Is Regulated by PPARα and Is a Key Mediator of Hepatic Lipid Metabolism in Ketotic States. Cell Metabolism 5(6): 426–37, Doi: 10.1016/j.cmet.2007.05.002.
[38] Inagaki, T., Dutchak, P., Zhao, G., Ding, X., Gautron, L., Parameswara, V., et al., 2007. Endocrine Regulation of the Fasting Response by PPARα-Mediated Induction of Fibroblast Growth Factor 21. Cell Metabolism 5(6): 415–25, Doi: 10.1016/j.cmet.2007.05.003.
[39] Markan, K.R., Naber, M.C., Ameka, M.K., Anderegg, M.D., Mangelsdorf, D.J., Kliewer, S.A., et al., 2014. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes 63(12): 4057–63, Doi: 10.2337/db14-0595.
[40] Capozzi, M.E., Wait, J.B., Koech, J., Gordon, A.N., Coch, R.W., Svendsen, B., et al., 2019. Glucagon lowers glycemia when β-cells are active. JCI Insight 5(16), Doi: 10.1172/JCI.INSIGHT.129954.
[41] Owen, B.M., Ding, X., Morgan, D.A., Coate, K.C., Bookout, A.L., Rahmouni, K., et al., 2014. FGF21 acts centrally to induce sympathetic nerve activity, energy expenditure, and weight loss. Cell Metabolism 20(4): 670–7, Doi: 10.1016/j.cmet.2014.07.012.
[42] Hill, C.M., Laeger, T., Dehner, M., Albarado, D.C., Clarke, B., Wanders, D., et al., 2019. FGF21 Signals Protein Status to the Brain and Adaptively Regulates Food Choice and Metabolism. Cell Reports 27(10): 2934-2947.e3, Doi: 10.1016/J.CELREP.2019.05.022.
[43] Panjwani, N., Mulvihill, E.E., Longuet, C., Yusta, B., Campbell, J.E., Brown, T.J., et al., 2013. GLP-1 Receptor Activation Indirectly Reduces Hepatic Lipid Accumulation But Does Not Attenuate Development of Atherosclerosis in Diabetic Male ApoE−/− Mice. Endocrinology 154(1): 127–39, Doi: 10.1210/en.2012-1937.

[44] McLean, B.A., Wong, C.K., Kaur, K.D., Seeley, R.J., Drucker, D.J., 2021. Differential importance of endothelial and hematopoietic cell GLP-1Rs for cardiometabolic versus hepatic actions of semaglutide. JCI Insight 6(22), Doi: 10.1172/JCI.INSIGHT.153732.
[45] McLean, B.A., Wong, C.K., Campbell, J.E., Hodson, D.J., Trapp, S., Drucker, D.J., 2021. Revisiting the Complexity of GLP-1 Action from Sites of Synthesis to Receptor Activation. Endocrine Reviews 42(2): 101–32, Doi: 10.1210/endrev/bnaa032.
[46] Zhang, Y., Seid, K., Obermayr, F., Just, L., Neckel, P.H., 2017. Activation of Wnt Signaling Increases Numbers of Enteric Neurons Derived From Neonatal Mouse and Human Progenitor Cells. Gastroenterology 153(1): 154-165.e9, Doi: 10.1053/j.gastro.2017.03.019.
[47] Chu, A.Y., Workalemahu, T., Paynter, N.P., Rose, L.M., Giulianini, F., Tanaka, T., et al., 2013. Novel locus including FGF21 is associated with dietary macronutrient intake. Human Molecular Genetics 22(9): 1895–902, Doi: 10.1093/HMG/DDT032.
[48] Tanaka, T., Ngwa, J.S., Van Rooij, F.J.A., Zillikens, M.C., Wojczynski, M.K., Frazier-Wood, A.C., et al., 2013. Genome-wide meta-analysis of observational studies shows common genetic variants associated with macronutrient intake. The American Journal of Clinical Nutrition 97(6): 1395–402, Doi: 10.3945/AJCN.112.052183.
[49] Frayling, T.M., Beaumont, R.N., Jones, S.E., Yaghootkar, H., Tuke, M.A., Ruth, K.S., et al., 2018. A Common Allele in FGF21 Associated with Sugar Intake Is Associated with Body Shape, Lower Total Body-Fat Percentage, and Higher Blood Pressure. Cell Reports 23(2): 327–36, Doi: 10.1016/J.CELREP.2018.03.070.
[50] Merino, J., Dashti, H.S., Li, S.X., Sarnowski, C., Justice, A.E., Graff, M., et al., 2019. Genome-wide meta-analysis of macronutrient intake of 91,114 European ancestry participants from the cohorts for heart and aging research in genomic epidemiology consortium. Molecular Psychiatry 24(12): 1920–32, Doi: 10.1038/S41380-018-0079-4.
[51] Meddens, S.F.W., de Vlaming, R., Bowers, P., Burik, C.A.P., Linnér, R.K., Lee, C., et al., 2020. Genomic analysis of diet composition finds novel loci and associations with health and lifestyle. Molecular Psychiatry 26(6): 2056–69, Doi: 10.1038/s41380-020-0697-5.
[52] Janzi, S., González-Padilla, E., Sonestedt, E., Najafi, K., Ramne, S., Ahlqvist, E., et al., 2021. Single Nucleotide Polymorphisms in Close Proximity to the Fibroblast Growth Factor 21 (FGF21) Gene Found to Be Associated with Sugar Intake in a Swedish Population. Nutrients 13(11), Doi: 10.3390/NU13113954.
[53] Ogawa, Y., Kurosu, H., Yamamoto, M., Nandi, A., Rosenblatt, K.P., Goetz, R., et al., 2007. βKlotho is required for metabolic activity of fibroblast growth factor 21. Proceedings of the National Academy of Sciences 104(18): 7432–7, Doi: 10.1073/PNAS.0701600104.
[54] Adams, A.C., Cheng, C.C., Coskun, T., Kharitonenkov, A., 2012. FGF21 Requires βklotho to Act In Vivo. PLOS ONE 7(11): e49977, Doi: 10.1371/JOURNAL.PONE.0049977.
[55] Ding, X., Boney-Montoya, J., Owen, B.M., Bookout, A.L., Coate, K.C., Mangelsdorf, D.J., et al., 2012. βKslotho is required for fibroblast growth factor 21 effects on growth and metabolism. Cell Metabolism 16(3): 387–93, Doi: 10.1016/j.cmet.2012.08.002.
[56] Tacer, K.F., Bookout, A.L., Ding, X., Kurosu, H., John, G.B., Wang, L., et al., 2010. Research Resource: Comprehensive Expression Atlas of the Fibroblast Growth Factor System in Adult Mouse. Molecular Endocrinology 24(10): 2050–64, Doi: 10.1210/ME.2010-0142.
[57] Jensen-Cody, S.O., Flippo, K.H., Claflin, K.E., Yavuz, Y., Sapouckey, S.A., Walters, G.C., et al., 2020. FGF21 Signals to Glutamatergic Neurons in the Ventromedial Hypothalamus to Suppress Carbohydrate Intake. Cell Metabolism 32(2): 273-286.e6, Doi: 10.1016/j.cmet.2020.06.008.
[58] O'Neil, P.M., Birkenfeld, A.L., McGowan, B., Mosenzon, O., Pedersen, S.D., Wharton, S., et al., 2018. Efficacy and safety of semaglutide compared with liraglutide and placebo for weight loss in patients with obesity: a randomised, double-blind, placebo and active

controlled, dose-ranging, phase 2 trial. Lancet (London, England) 392(10148): 637–49, Doi: 10.1016/S0140-6736(18)31773-2.

[59] Capehorn, M.S., Catarig, A.-M., Furberg, J.K., Janez, A., Price, H.C., Tadayon, S., et al., 2020. Efficacy and safety of once-weekly semaglutide 1.0mg vs once-daily liraglutide 1.2mg as add-on to 1-3 oral antidiabetic drugs in subjects with type 2 diabetes (SUSTAIN 10). Diabetes & Metabolism 46(2): 100–9, Doi: 10.1016/J.DIABET.2019.101117.

[60] Garvey, W.T., Batterham, R.L., Bhatta, M., Buscemi, S., Christensen, L.N., Frias, J.P., et al., 2022. Two-year effects of semaglutide in adults with overweight or obesity: the STEP 5 trial. Nature Medicine 28(10): 2083–91, Doi: 10.1038/S41591-022-02026-4.

[61] Salinas, C.B.G., Lu, T.T.H., Gabery, S., Marstal, K., Alanentalo, T., Mercer, A.J., et al., 2018. Integrated Brain Atlas for Unbiased Mapping of Nervous System Effects Following Liraglutide Treatment. Scientific Reports 8(1): 1–12, Doi: 10.1038/s41598-018-28496-6.

[62] He, Z., Gao, Y., Lieu, L., Afrin, S., Cao, J., Michael, N.J., et al., 2019. Direct and indirect effects of liraglutide on hypothalamic POMC and NPY/AgRP neurons – Implications for energy balance and glucose control. Molecular Metabolism 28: 120–34, Doi: 10.1016/j.molmet.2019.07.008.

[63] Knudsen, L.B., Secher, A., Hecksher-Sørensen, J., Pyke, C., 2016. Long-acting glucagon-like peptide-1 receptor agonists have direct access to and effects on pro-opiomelanocortin/cocaine- and amphetamine-stimulated transcript neurons in the mouse hypothalamus. Journal of Diabetes Investigation 7(S1): 56–63, Doi: 10.1111/jdi.12463.

[64] Lockie, S.H., Heppner, K.M., Chaudhary, N., Chabenne, J.R., Morgan, D.A., Veyrat-Durebex, C., et al., 2012. Direct Control of Brown Adipose Tissue Thermogenesis by Central Nervous System Glucagon-Like Peptide-1 Receptor Signaling. Diabetes 61(11): 2753–62, Doi: 10.2337/DB11-1556.

[65] Beiroa, D., Imbernon, M., Gallego, R., Senra, A., Herranz, D., Villarroya, F., et al., 2014. GLP-1 Agonism Stimulates Brown Adipose Tissue Thermogenesis and Browning Through Hypothalamic AMPK. Diabetes 63(10): 3346–58, Doi: 10.2337/DB14-0302.

[66] Kooijman, S., Wang, Y., Parlevliet, E.T., Boon, M.R., Edelschaap, D., Snaterse, G., et al., 2015. Central GLP-1 receptor signalling accelerates plasma clearance of triacylglycerol and glucose by activating brown adipose tissue in mice. Diabetologia 58(11).

[67] Lee, S.J., Sanchez-Watts, G., Krieger, J.P., Pignalosa, A., Norell, P.N., Cortella, A., et al., 2018. Loss of dorsomedial hypothalamic GLP-1 signaling reduces BAT thermogenesis and increases adiposity. Molecular Metabolism 11: 33–46, Doi: 10.1016/J.MOLMET.2018.03.008.

[68] Larsen, P.J., Tang-Christensen, M., Jessop, D.S., 1997. Central Administration of Glucagon-Like Peptide-1 Activates Hypothalamic Neuroendocrine Neurons in the Rat. Endocrinology 138(10): 4445–55, Doi: 10.1210/endo.138.10.5270.

[69] Kinzig, K.P., D'Alessio, D.A., Herman, J.P., Sakai, R.R., Vahl, T.P., Figueiredo, H.F., et al., 2003. CNS glucagon-like peptide-1 receptors mediate endocrine and anxiety responses to interoceptive and psychogenic stressors. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience 23(15): 6163–70, Doi: 10.1523/JNEUROSCI.23-15-06163.2003.

[70] Gil-Lozano, M., Pérez-Tilve, D., Alvarez-Crespo, M., Martís, A., Fernandez, A.M., Catalina, P.A.F., et al., 2010. GLP-1(7-36)-amide and Exendin-4 Stimulate the HPA Axis in Rodents and Humans. Endocrinology 151(6): 2629–40, Doi: 10.1210/en.2009-0915.

[71] Patel, R., Bookout, A.L., Magomedova, L., Owen, B.M., Consiglio, G.P., Shimizu, M., et al., 2015. Glucocorticoids Regulate the Metabolic Hormone FGF21 in a Feed-Forward Loop. Molecular Endocrinology 29(2): 213–23, Doi: 10.1210/me.2014-1259.

[72] Fisher, ffolliott M., Kim, M.S., Doridot, L., Cunniff, J.C., Parker, T.S., Levine, D.M., et al., 2017. A critical role for ChREBP-mediated FGF21 secretion in hepatic fructose metabolism. Molecular Metabolism 6(1): 14–21.

[73] Lundsgaard, A.M., Fritzen, A.M., Sjøberg, K.A., Myrmel, L.S., Madsen, L., Wojtaszewski, J.F.P., et al., 2017. Circulating FGF21 in humans is potently induced by short term overfeeding of carbohydrates. Molecular Metabolism 6(1): 22–9, Doi: 10.1016/J.MOLMET.2016.11.001.
[74] Drucker, D.J., 2018. Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. vol. 27, Cell Press, pp. 740–56.
[75] Oost, L.J., Kustermann, M., Armani, A., Blaauw, B., Romanello, V., 2019. Fibroblast growth factor 21 controls mitophagy and muscle mass. Journal of Cachexia, Sarcopenia and Muscle 10(3): 630–42, Doi: 10.1002/jcsm.12409.
[76] Flippo, K.H., Jensen-Cody, S.O., Claflin, K.E., Potthoff, M.J., 2020. FGF21 signaling in glutamatergic neurons is required for weight loss associated with dietary protein dilution. Scientific Reports 10(1): 19521, Doi: 10.1038/s41598-020-76593-2.
[77] Claflin, K.E., Sullivan, A.I., Naber, M.C., Flippo, K.H., Morgan, D.A., Neff, T.J., et al., 2022. Pharmacological FGF21 signals to glutamatergic neurons to enhance leptin action and lower body weight during obesity. Molecular Metabolism 64: 101564, Doi: 10.1016/j.molmet.2022.101564.
[78] Dickson, S.L., Shirazi, R.H., Hansson, C., Bergquist, F., Nissbrandt, H., Skibicka, K.P., 2012. The Glucagon-Like Peptide 1 (GLP-1) Analogue, Exendin-4, Decreases the Rewarding Value of Food: A New Role for Mesolimbic GLP-1 Receptors. Journal of Neuroscience 32(14): 4812–20, Doi: 10.1523/JNEUROSCI.6326-11.2012.
[79] Alhadeff, A.L., Grill, H.J., 2014. Hindbrain nucleus tractus solitarius glucagon-like peptide-1 receptor signaling reduces appetitive and motivational aspects of feeding. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 307(4): R465–70, Doi: 10.1152/ajpregu.00179.2014.
[80] Farr, O.M., Sofopoulos, M., Tsoukas, M.A., Dincer, F., Thakkar, B., Sahin-Efe, A., et al., 2016. GLP-1 receptors exist in the parietal cortex, hypothalamus and medulla of human brains and the GLP-1 analogue liraglutide alters brain activity related to highly desirable food cues in individuals with diabetes: a crossover, randomised, placebo-controlled trial. Diabetologia 59(5): 954–65, Doi: 10.1007/s00125-016-3874-y.
[81] Treesukosol, Y., Moran, T.H., 2022. Administration of Exendin-4 but not CCK alters lick responses and trial initiation to sucrose and intralipid during brief-access tests. Chemical Senses 47: bjac004, Doi: 10.1093/chemse/bjac004.
[82] Downer, S., Berkowitz, S.A., Berkowitz, S.A., Harlan, T.S., Olstad, D.L., Mozaffarian, D., 2020. Food is medicine: actions to integrate food and nutrition into healthcare. BMJ 369, Doi: 10.1136/BMJ.M2482.
[83] Hager, K., Cudhea, F.P., Wong, J.B., Berkowitz, S.A., Downer, S., Lauren, B.N., et al., 2022. Association of National Expansion of Insurance Coverage of Medically Tailored Meals With Estimated Hospitalizations and Health Care Expenditures in the US. JAMA Network Open 5(10): e2236898–e2236898, Doi: 10.1001/JAMANETWORKOPEN.2022.36898.
[84] Baggio, L.L., Drucker, D.J., 2021. Glucagon-like peptide-1 receptor co-agonists for treating metabolic disease. Molecular Metabolism 46: 101090, Doi: 10.1016/J.MOLMET.2020.101090.
[85] Bethel, M.A., Patel, R.A., Merrill, P., Lokhnygina, Y., Buse, J.B., Mentz, R.J., et al., 2018. Cardiovascular outcomes with glucagon-like peptide-1 receptor agonists in patients with type 2 diabetes: a meta-analysis. The Lancet Diabetes & Endocrinology 6(2): 105–13, Doi: 10.1016/S2213-8587(17)30412-6.
[86] Pan, X., Shao, Y., Wu, F., Wang, Y., Xiong, R., Zheng, J., et al., 2018. FGF21 Prevents Angiotensin II-Induced Hypertension and Vascular Dysfunction by Activation of ACE2/Angiotensin-(1–7) Axis in Mice. Cell Metabolism 27(6): 1323-1337.e5, Doi: 10.1016/J.CMET.2018.04.002.

[87] Armstrong, M.J., Gaunt, P., Aithal, G.P., Barton, D., Hull, D., Parker, R., et al., 2016. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. The Lancet 387(10019): 679–90, Doi: 10.1016/S0140-6736(15)00803-X.
[88] Sanyal, A., Charles, E.D., Neuschwander-Tetri, B.A., Loomba, R., Harrison, S.A., Abdelmalek, M.F., et al., 2018. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. The Lancet 392(10165): 2705–17, Doi: 10.1016/S0140-6736(18)31785-9.
[89] Athauda, D., Maclagan, K., Skene, S.S., Bajwa-Joseph, M., Letchford, D., Chowdhury, K., et al., 2017. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. The Lancet 390(10103): 1664–75, Doi: 10.1016/S0140-6736(17)31585-4.
[90] Kuroda, M., Muramatsu, R., Maedera, N., Koyama, Y., Hamaguchi, M., Fujimura, H., et al., 2017. Peripherally derived FGF21 promotes remyelination in the central nervous system. The Journal of Clinical Investigation 127(9): 3496–509, Doi: 10.1172/JCI94337.
[91] Jiang, Y., Liu, N., Wang, Q., Yu, Z., Lin, L., Yuan, J., et al., 2018. Endocrine Regulator rFGF21 (Recombinant Human Fibroblast Growth Factor 21) Improves Neurological Outcomes Following Focal Ischemic Stroke of Type 2 Diabetes Mellitus Male Mice. Stroke 49(12): 3039–49, Doi: 10.1161/STROKEAHA.118.022119.
[92] Sørensen, G., Caine, S.B., Thomsen, M., 2016. Effects of the GLP-1 Agonist Exendin-4 on Intravenous Ethanol Self-Administration in Mice. Alcoholism, Clinical and Experimental Research 40(10): 2247–52, Doi: 10.1111/ACER.13199.
[93] Vallöf, D., MacCioni, P., Colombo, G., Mandrapa, M., Jörnulf, J.W., Egecioglu, E., et al., 2016. The glucagon-like peptide 1 receptor agonist liraglutide attenuates the reinforcing properties of alcohol in rodents. Addiction Biology 21(2): 422–37, Doi: 10.1111/ADB.12295.
[94] Flippo, K.H., Trammell, S.A.J., Gillum, M.P., Aklan, I., Perez, M.B., Yavuz, Y., et al., 2022. FGF21 suppresses alcohol consumption through an amygdalo-striatal circuit. Cell Metabolism 34(2): 317-328.e6, Doi: 10.1016/J.CMET.2021.12.024.

| Mouse Line | Source | MGI ID |
|---|---|---|
| *vGlut2*-Cre | Jax Labs #028863 | MGI:5141263 |
| *Wnt1*-Cre | Jax Labs #022501 | MGI:5485027 |
| MIP-CreERT | Jax Labs #024709 | MGI:4410453 |
| Glp1r$^{flox/flox}$ | Dr. RJ Seeley U. Michigan | MGI:5637837 |
| Alb-Cre | Jax Labs #003574 | MGI:2176228 |
| PPAR alpha$^{flox/flox}$ | Dr. DP Kelly, UPenn | MGI:6273327 |
| Fgf21$^{flox/flox}$ | Jax Labs #022361 | MGI:5486224 |
| *Camk2a*-Cre | Jax Labs #005359 | MGI:2177650 |
| Klb$^{flox/flox}$ | Jax Labs #026883 | MGI:5446140 |

**Table 1. Source and MGI identification numbers for mice used.**

**Figure 1. GLP-1RA stimulate FGF21 independent of their effect on food intake.** (**A**) Study outline for B-C. (**B**) Plasma FGF21 levels at 0 and 7 h following treatment in fasted C57BL6/J male mice treated with vehicle or liraglutide (400 µg/kg) (Mixed-effects analysis: Interaction, F (1, 12) = 6.62, P = 0.0244; N = 6-8). (**C**) Relative change in liver *Fgf21* gene expression in fasted C57BL6/J male mice 7 h following treatment with vehicle or liraglutide (400 µg/kg) (Unpaired t-test: P = 0.0152; N = 6-8). (**D**) Study outline for E-F. (**E-F**) Changes in body weight (E; One-way ANOVA: Interaction, F (2, 13) = 12.02, P = 0.0011; N = 5-6) ) and plasma FGF21 levels (F; One-way ANOVA: Interaction, F (2, 13) = 4.111, P = 0.0414; N = 5-6) in C57BL6/J male mice *ad libitum*-fed and treated with vehicle or liraglutide (200 µg/kg, *b.i.d*), or pair-fed to weight match the liraglutide-treated group for 48 hours. (**G-H**) Plasma FGF21 levels in C57BL/6J mice following 7 day treatment with (**G**) vehicle or exendin-4 (10 µg/kg, *b.i.d*) (Unpaired t-test: P = 0.0425; N = 4-5) and (**H**) vehicle or liraglutide (200 µg/kg, *b.i.d*) (Unpaired t-test: P = 0.0053, N = 8). Data are shown as mean ± SEM, ns not significant, * P < 0.05, ** < 0.01.

**Figure 2**. **Central GLP-1R and liver PPARα are required for liraglutide to stimulate plasma FGF21.** Absolute (A, C, E) and relative change (B, D, F) in FGF21 levels at 0 and 7 h post treatment with vehicle or liraglutide (400 µg/kg) in fasted control and respective knockout mice. (**A-B**) Control and vGLUT2+ neuron-*Glp1r* knockout (Vglut2$^{Glp1r-/-}$) mice (A, Mixed-effects analysis: Interaction, F (1, 23) = 2.021, P = 0.1686; Genotype Effect, F (1, 23) = 5.965, P = 0.0227; B, Mixed-effects analysis: Interaction, F (1, 23) = 4.571, P = 0.0434; N = 5-6). (**C-D**) Control and β cell-*Glp1r* knockout (β cell$^{Glp1r-/-}$) mice (C, Mixed-effects analysis: Interaction, F (1, 56) = 0.05070, P = 0.8227; Genotype Effect, F (1, 56) = 11.28, P = 0.0014; D, Mixed-effects analysis: Interaction, F (1, 28) = 0.01663, P = 0.8983; Genotype Effect, F (1, 28) = 10.41, P = 0.0032; N = 6-10). (**E-F**) Control and liver *Ppara* knockout (Liv$^{Ppara-/-}$) mice (E, Mixed-effects analysis: Interaction, F (1, 66) = 9.627, P=0.0039; liraglutide-treated control vs. Liv$^{Ppara-/-}$ at 7 h, P < 0.0001; F, Mixed-effects

812 analysis: Interaction, F (1, 33) = 8.775, P = 0.0056; N = 6-13). Data are shown as mean ± SEM,
813 ns not significant, * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001.
814
815 **Figure 3**. **Liver FGF21 mediates the appetite- and weight-lowering actions of liraglutide.**
816 Chow-fed control and liver *Fgf21* knockout (Liv$^{Fgf21-/-}$) mice housed in metabolic cages and treated
817 with vehicle or liraglutide (200 µg/kg, *b.i.d*) for 11 days (N = 7-9). (**A-B**) Relative weight loss over
818 time (A; Mixed-effects analysis: Interaction, F (11, 156) = 1.920, P = 0.0405) and relative weight
819 loss on the last day of treatment (B; Mixed-effects analysis: Interaction, F (1, 27) = 13.11 P =
820 0.0012). (**C**) Time course of food intake (Mixed-effects analysis: Interaction, F (36, 324) = 2.154,
821 P = 0.0003; Genotype Effect, F (3, 27) = 1.271, P = 0.3042). (**D**) Total food intake during the
822 treatment period (One-way ANOVA: F (3,27) = 4.728, P = 0.0089). (**E**) Time course of energy
823 expenditure (EE) (Mixed-effects analysis: Interaction, F (36, 324) = 1.151, P = 0.2606; Genotype
824 Effect, F (3, 27) = 5.035, P = 0.0067). (**F**) Total EE during the treatment period (One-way ANOVA:
825 F (3,27) = 2.946, P = 0.0507). (**G-I**) Total body mass (G; Mixed-effects analysis: Interaction, F (1,
826 27) = 10.60, P = 0.0030), fat-free (or lean) mass (H; Mixed-effects analysis: Interaction, F (1, 27)
827 = 1.383, P = 0.2499; Genotype Effect, F (1, 27) = 1.983, P = 0.1705), and fat mass (I; Mixed-
828 effects analysis: Interaction, F (1, 27) = 0.2012, P = 0.6573; Genotype Effect, F (1, 27) = 0.4077,
829 P = 0.5285) at the end of treatment. Data are shown as mean ± SEM, ns not significant, * P <
830 0.05, ** P < 0.01, and **** P < 0.0001 for comparisons between liraglutide-treated control vs.
831 Liv$^{Fgf21-/-}$ mice (A, C, E) and those delineated by lines (B, F, G-I).
832
833 **Figure 4**. **Liver FGF21 contributes to liraglutide-induced weight loss in the context of high-**
834 **carbohydrate diets.** Control and liver *Fgf21* knockout (Liv$^{Fgf21-/-}$) mice fed a low fat, low or high
835 carbohydrate diet for 4 weeks (A-D), a high fat, high sugar diet for 4 weeks of 1 week (E-H), or a
836 high fat, low carbohydrate diet for 4 weeks (I-J) followed by treatment with vehicle or liraglutide
837 (200 µg/kg, *b.i.d*) for 14 days. (**A-B**) Relative weight loss over time (A; Mixed-effects analysis:

Interactions, F (14, 168) = 0.5293, P = 0.9134; Genotype Effect, F (1, 12) = 0.02792, P = 0.8701; N = 7-8) and on the last day of treatment (B; Mixed-effects analysis: Interactions, F (1, 25) = 0.5317, P = 0.4727; N = 7-8) of mice fed a low fat, low carbohydrate diet for 4 weeks. (**C-D**) Relative weight loss over time (C; Mixed-effects analysis: Interactions, F (14, 266) = 1.865, P = 0.0303; N =7-14) and on the last day of treatment (D; Mixed-effects analysis: Interactions, F (1, 37) = 10.35, P = 0.0027; N = 7-14) of mice fed a low fat, high carbohydrate diet for 4 weeks. (**E-F**) Relative weight loss over time (E; Mixed-effects analysis: Interactions, F (14, 434) = 7.323, P < 0.0001; N = 14-19) and on the last day of treatment (F; Mixed-effects analysis: Interactions, F (1, 62) = 13.12, P = 0.0006; N = 14-19) of mice fed a high fat, high sugar diet for 4 weeks. (**G-H**) Relative weight loss over time (G; Mixed-effects analysis: Interactions, F (14, 210) = 4.004, P < 0.0001; N = 7-9) and on the last day of treatment (H; Mixed-effects analysis: Interactions, F (1, 29) = 2.291, P = 0.1410; Genotype Effect, F (1, 29) = 2.303, P = 0.1400; N = 7-9) of mice fed a high fat, high sugar diet for 1 week. (**I-J**) Relative weight loss over time (I; Mixed-effects analysis: Interactions, F (42, 322) = 44.58, P < 0.0001; N = 7) and on the last day of treatment (H; Mixed-effects analysis: Interactions, F (3, 23) = 65.53, P < 0.0001; N = 7) of mice fed a high fat, low carbohydrate diet for 4 weeks. Data are shown as mean ± SEM, ns not significant, * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001 for comparisons between liraglutide-treated control vs. Liv$^{Fgf21-/-}$ mice (A, C, E, G, I) and those delineated by lines (B, D, F, H, J).

**Figure 5**. **Liraglutide promotes weight loss in the context of high-carbohydrate diets via brain FGF21 action.** Control and Camk2a *Klb* knockout (Camk2a$^{Klb-/-}$) mice fed a low fat, high carbohydrate diet for 4 weeks (A-B) and control and Vglut2 *Klb* knockout (Vglut2$^{Klb-/-}$) mice fed a high fat, high sugar diet for 1 week (C-D) followed by treatment with vehicle or liraglutide (200 µg/kg, *b.i.d*) for 14 days. (**A-B**) Relative weight loss over time (A; Mixed-effects analysis: Interactions, F (14, 294) = 3.552, P <0.0001; N=9-15) and on the last day of treatment (B; Mixed-effects analysis: Interactions, F (1, 38) = 7.686, P = 0.009; N = 9-15). (**C-D**) Relative weight loss

over time (C; Mixed-effects analysis: Interactions, F (14, 252) = 5.031, P <0.0001; N = 9-11) and on the last day of treatment (D; Mixed-effects analysis: Interactions, F (1, 35) = 7.296, P = 0.0106; N = 9-11). Data are shown as mean ± SEM, ns not significant, * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001 for comparisons between liraglutide-treated control vs. Camk2a$^{Klb-/-}$ mice (A), liraglutide-treated control vs. Vglut2$^{Klb-/-}$ mice (C) and those delineated by lines (B, D).

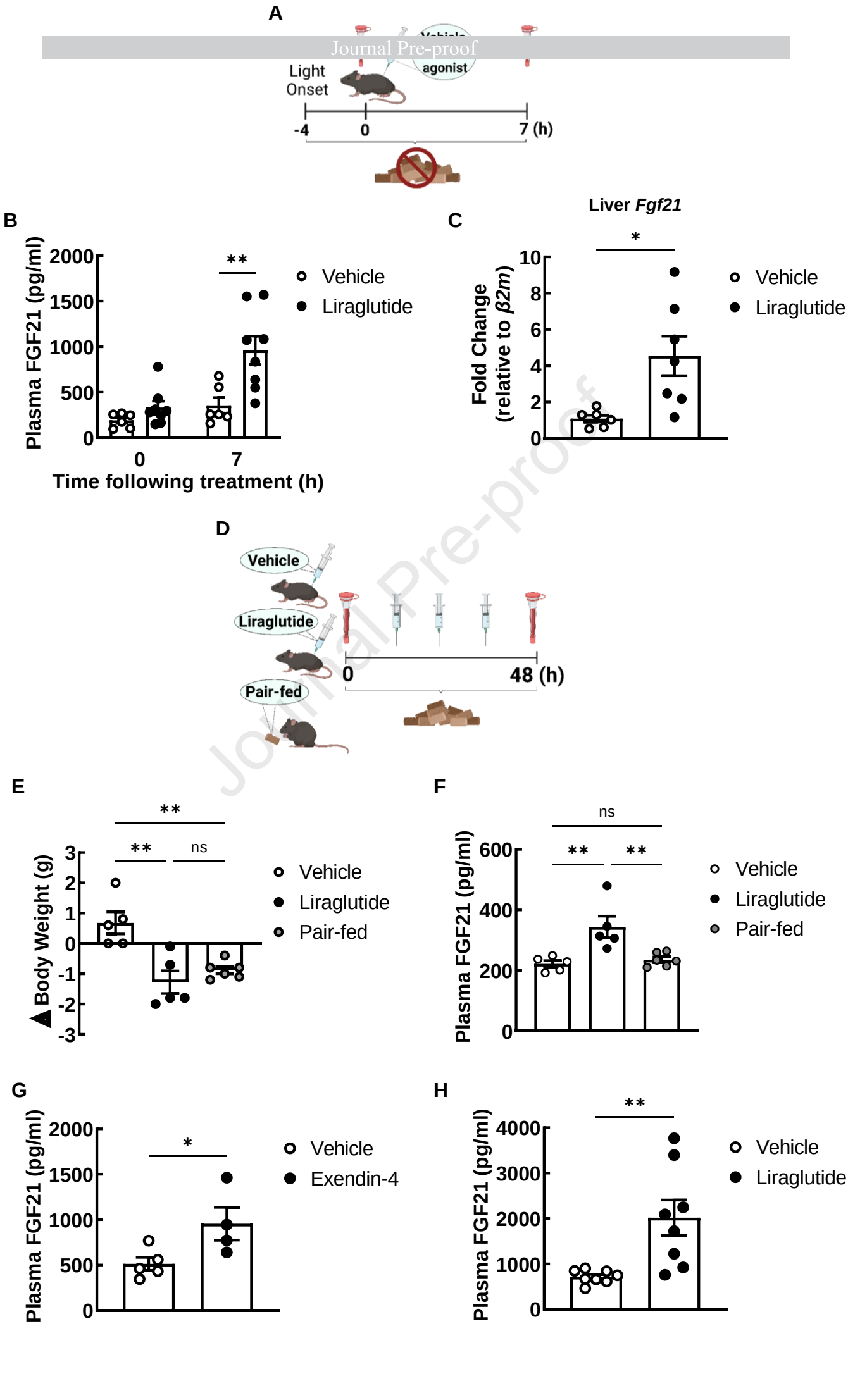
**Supplemental Figure 1. Exendin-4 and semaglutide stimulate FGF21 in fasted mice.** Plasma FGF21 levels in mice treated with (**A**) exendin-4 (10 µg/kg) (Mixed-effects analysis: Interaction, F (1, 26) = 2.93, p = 0.0987; Treatment Effect, F (1, 26) = 4.30, P = 0.0481; N = 8) or (**B**) semaglutide (120 µg/kg) (Mixed-effects analysis: Interaction, F (1, 10) = 10.90, p = 0.0080; Treatment Effect, F (1, 10) = 10.50, P = 0.0089; N = 6) following the study protocol shown in **Figure 1A**. Data are shown as mean ± SEM, * P < 0.05, *** P < 0.001.

**Supplemental Figure 2. The GLP-1R in Wnt1+ cells is required for the increase in plasma FGF21 levels following liraglutide treatment.** Absolute (**A**) and relative change (**B**) in FGF21 levels at 0 and 7 h post treatment with vehicle or liraglutide (400 µg/kg) in fasted control mice and mice lacking the GLP-1R in Wnt1+ neurons ($Wnt1^{Glp1r-/-}$) (A, Mixed-effects analysis: Interaction, F (1, 28) = 4.131, P = 0.0517; Genotype Effect, F (1, 28) = 0.6444, P = 0.4289; B, Mixed-effects analysis: Interaction, F (1, 28) = 7.458, P = 0.0108; N = 7-9). Data are shown as mean ± SEM, ns not significant, ** P < 0.01, *** P < 0.001, **** P < 0.0001.

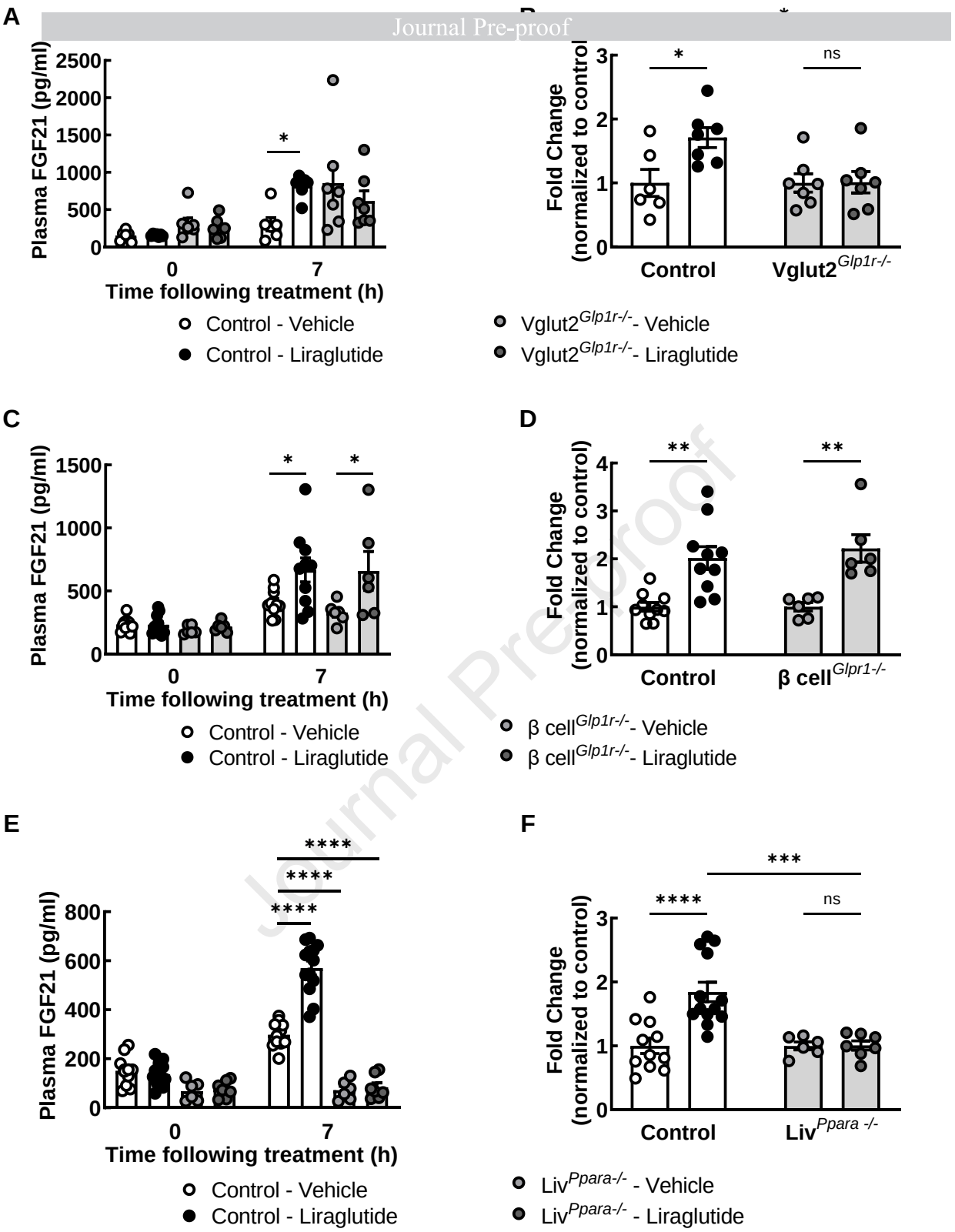
**Supplemental Figure 3. Loss of liver FGF21 expression attenuates the anorectic effect of liraglutide.** (**A**) Pre-treatment body weight in control and $Liv^{Fgf21-/-}$ mice (One-way ANOVA: F (3, 27) = 0.1226, P = 0.9460). (**B**) Change in EE relative to baseline (Day 0) (Mixed-effects analysis: Interaction, F (1, 27) = 2.086, P = 0.1601; Genotype Effect, F (1, 27) = 5.068, P=0.0327; N = 7-9. (**C**) Plasma FGF21 levels in control and $Liv^{Fgf21-/-}$ mice following treatment with vehicle or liraglutide (200 µg/kg, *b.i.d*) for 12 days (Mixed-effects analysis: Interaction, F (1, 15) = 31.45, P < 0.0001; Genotype Effect, F (1, 15) = 92.74, P < 0.0001; N = 5-6). Data are shown as mean ± SEM, * P < 0.05, ** P < 0.001 for comparisons between vehicle- and liraglutide-treated $Liv^{Fgf21-/-}$ mice (B) and those delineated by the lines (C).

**Supplemental Figure 4. Plasma FGF21 in LC and HC-fed mice.** Plasma FGF21 levels in (**A**) LC- and (**B**) HC-fed control and Liv$^{Fgf21-/-}$ mice following treatment with vehicle or liraglutide (200 µg/kg, *b.i.d*) for 12 days (A; Mixed-effects analysis: Interaction, F (1, 14) = 3.111, P = 0.0996; Genotype Effect, F (1, 14) = 21.25, P=0.0004; N = 3-6 and B; Mixed-effects analysis: Interaction, F (1, 17) = 1.353, P = 0.2609; Genotype Effect, F (1, 17) = 7.268, P=0.0153; N = 4-7). Data are shown as mean ± SEM, * P < 0.05.

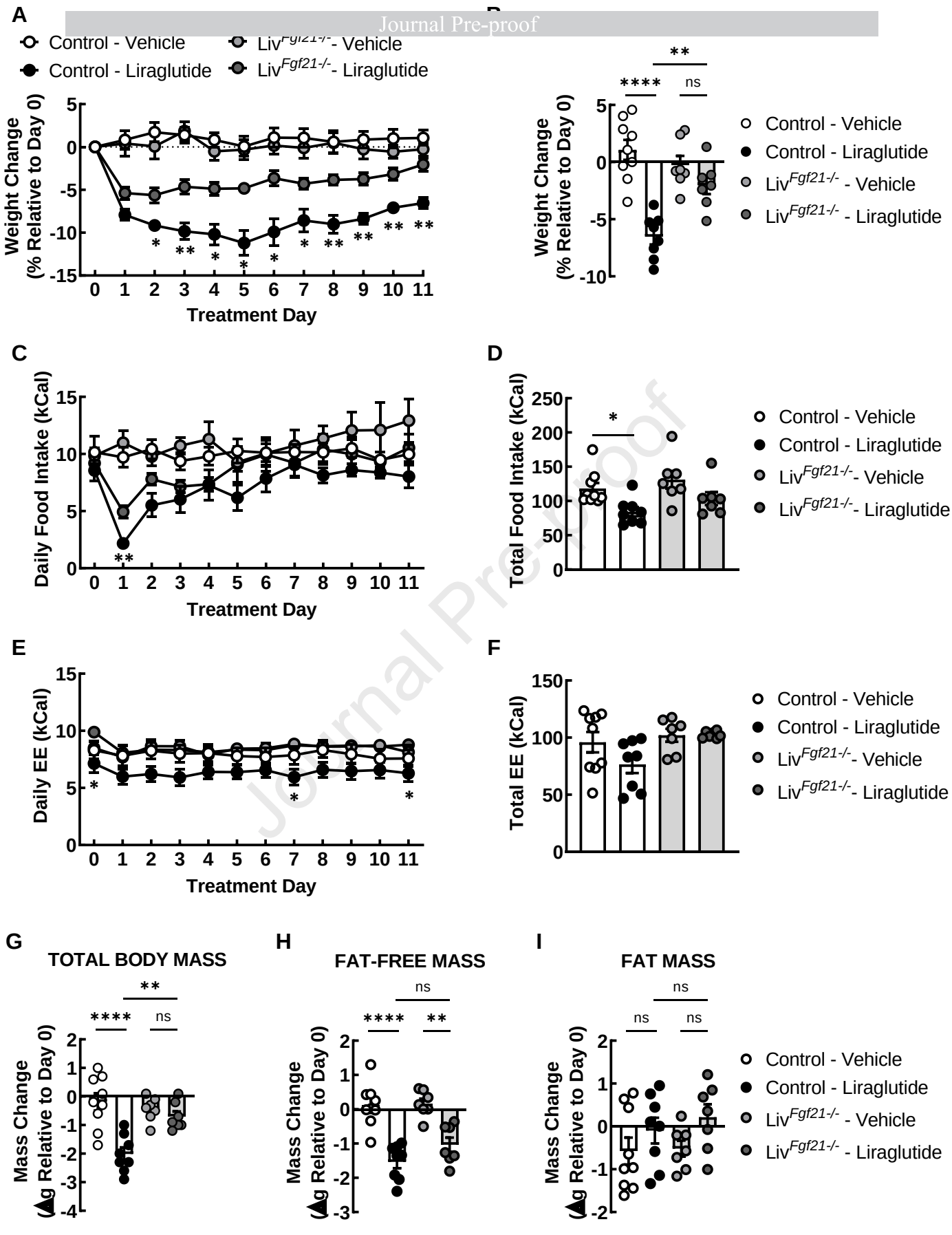
# Figure 1



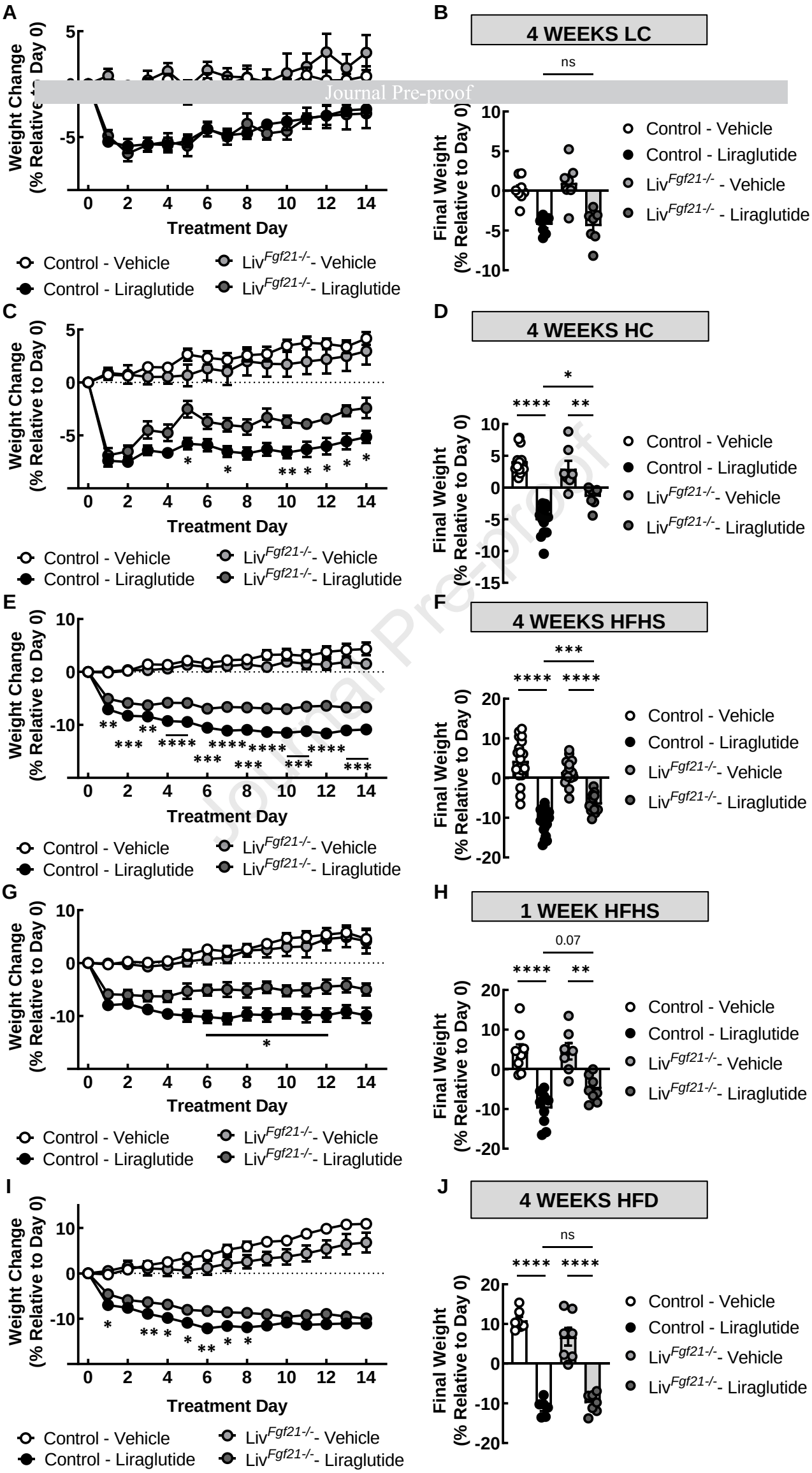
# Figure 2



# Figure 3

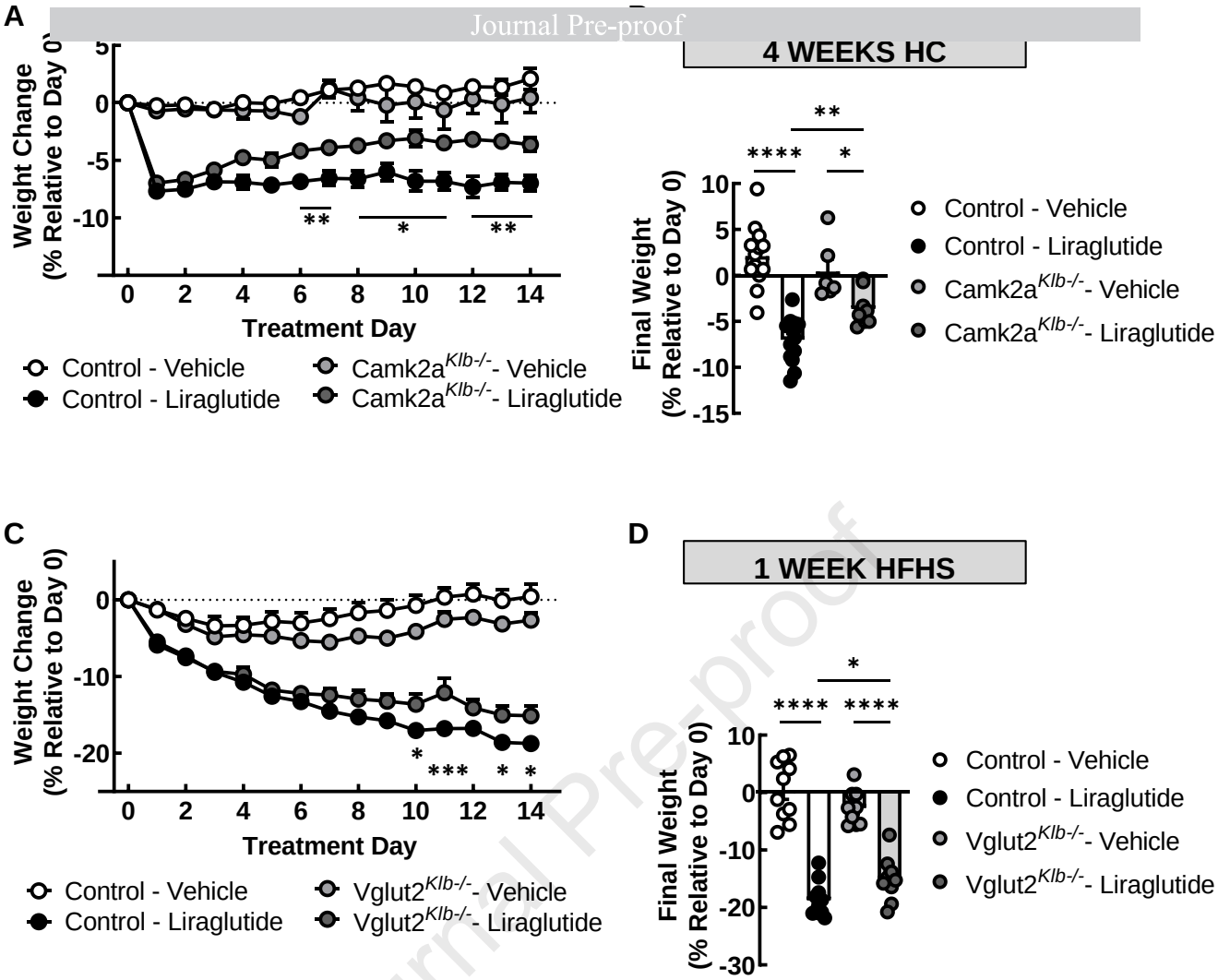


# Figure 4



# Figure 5

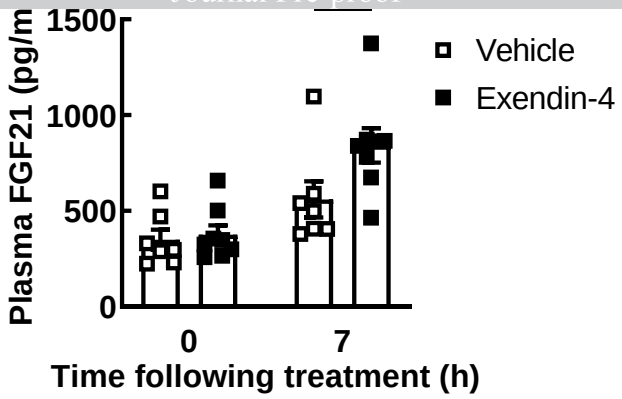




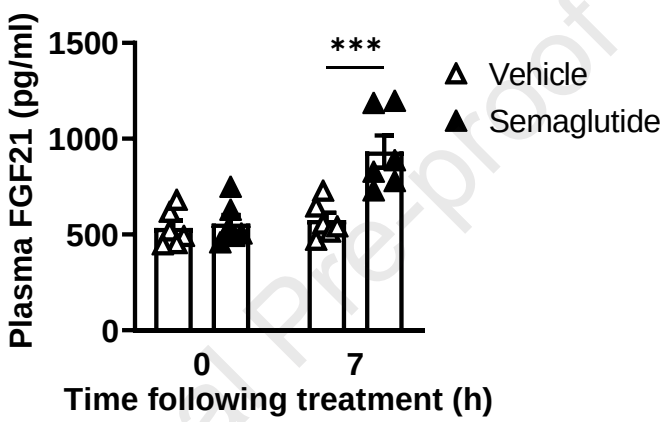
# Supplemental Figure 1



A

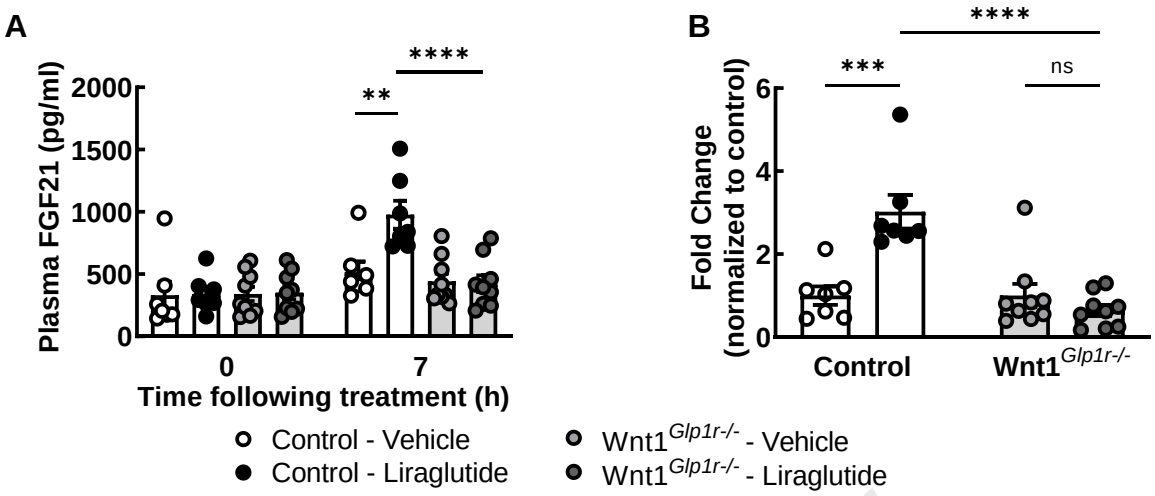



B



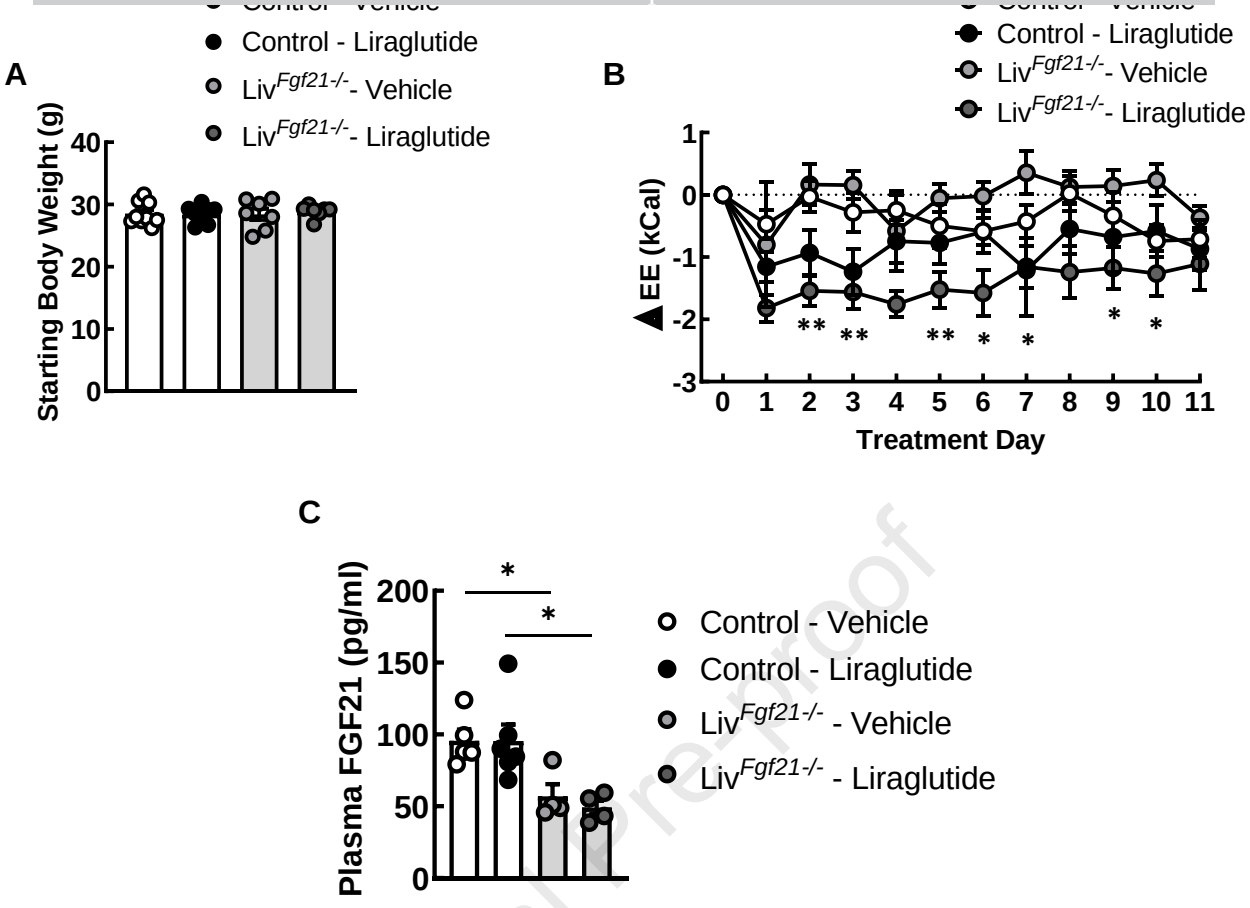
# Supplemental Figure 2





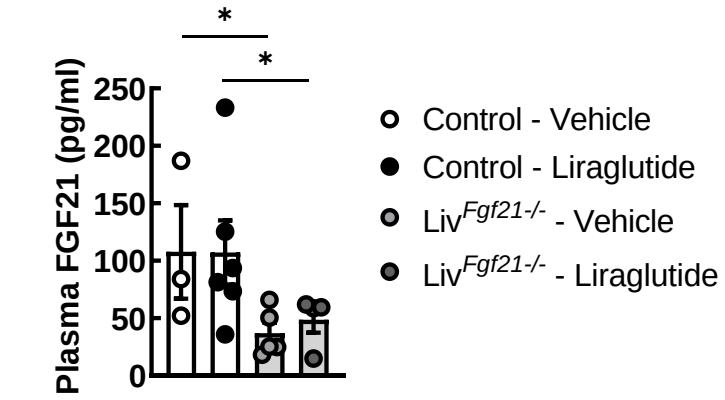
# Supplemental Figure 3



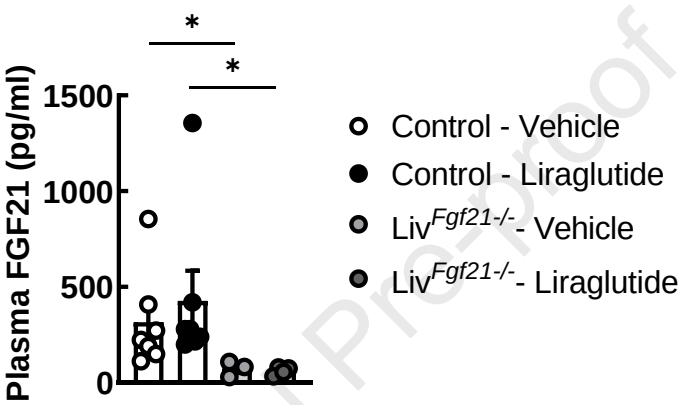


# Supplemental Figure 4






**B**



**Highlights:**

- The GLP-1 receptor agonist liraglutide increases circulating FGF21 levels.
- Liraglutide-induced FGF21 reduces weight only in mice fed high carbohydrate diets.
- Weight lowering by liraglutide-induced FGF21 is via neuronal FGF21 action.

**Declaration of interests**

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

☐x The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Daniel Drucker has served as a consultant or speaker within the past 12 months to Altimmune, Amgen, Kallyope, Merck Research Laboratories, novo Nordisk, Inc., and Pfizer, Inc. Investigator-initiated research in the Drucker lab is supported in part by funding from Novo Nordisk, Inc. and Pfizer, Inc. to Mt. Sinai Hospital. Neither Dr. Drucker nor his family members hold issued stock directly or indirectly in any of these companies. Dr. Drucker holds non-exercised options in Kallyope. Randy Seeley has served as a consultant or on the Scientific Advisory Board for Novo Nordisk, Inc., Scohia, CinRx, Fractyl, and Structure Therapeutics and has equity in Calibrate and Rewind. The Seeley lab is supported in part by funding from Novo Nordisk, Inc., Astra Zeneca, Fractyl, and Eli Lilly. The Campbell lab is supported in part by funding from Eli Lilly, Merck, and Novo Nordisk, Inc.

**Declarations of Interest:** Daniel Drucker has served as a consultant or speaker within the past 12 months to Altimmune, Amgen, Kallyope, Merck Research Laboratories, Novo Nordisk Inc., and Pfizer Inc. Investigator-initiated research in the Drucker lab is supported in part by funding from Novo Nordisk and Pfizer Inc to Mt. Sinai Hospital. Neither Dr. Drucker or his family members hold issued stock directly or indirectly in any of these companies. Dr. Drucker holds non-exercised options in Kallyope. Randy Seeley has served as a consultant or on the Scientific Advisory Board for Novo Nordisk, Scohia, CinRx, Fractyl, and Structure Therapeutics and has equity in Calibrate and Rewind. The Seeley lab is supported in part by funding from Novo Nordisk, Astra Zeneca, Fractyl, and Eli Lilly. The Campbell lab is supported in part by funding from Eli Lilly, Merck, and Novo Nordisk.