Brief Communication

MOLECULAR METABOLISM

# The anti-obesity effect of FGF19 does not require UCP1-dependent thermogenesis

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## ABSTRACT

**Objective:** Fibroblast growth factor 19 (FGF19) is a postprandial hormone which plays diverse roles in the regulation of bile acid, glucose, and lipid metabolism. Administration of FGF19 to obese/diabetic mice lowers body weight, improves insulin sensitivity, and enhances glycemic control. The primary target organ of FGF19 is the liver, where it regulates bile acid homeostasis in response to nutrient absorption. In contrast, the broader pharmacologic actions of FGF19 are proposed to be driven, in part, by the recruitment of the thermogenic protein uncoupling protein 1 (UCP1) in white and brown adipose tissue. However, the precise contribution of UCP1-dependent thermogenesis to the therapeutic actions of FGF19 has not been critically evaluated.

**Methods:** Using WT and germline UCP1 knockout mice, the primary objective of the current investigation was to determine the in vivo pharmacology of FGF19, focusing on its thermogenic and anti-obesity activity.

**Results:** We report that FGF19 induced mRNA expression of UCP1 in adipose tissue and show that this effect is required for FGF19 to increase caloric expenditure. However, we demonstrate that neither UCP1 induction nor an elevation in caloric expenditure are necessary for FGF19 to induce weight loss in obese mice. In contrast, the anti-obesity action of FGF19 appeared to be associated with its known physiological role. In mice treated with FGF19, there was a significant reduction in the mRNA expression of genes associated with hepatic bile acid synthesis enzymes, lowered levels of hepatic bile acid species, and a significant increase in fecal energy content, all indicative of reduced lipid absorption in animals treated with FGF19.

**Conclusion:** Taken together, we report that the anti-obesity effect of FGF19 occurs in the absence of UCP1. Our data suggest that the primary way in which exogenous FGF19 lowers body weight in mice may be through the inhibition of bile acid synthesis and subsequently a reduction of dietary lipid absorption.



**Keywords** FGF19; Thermogenesis; UCP1; Metabolic; BAT; CYP7A1

## 1. INTRODUCTION

There has been a significant increase in the prevalence of individuals clinically diagnosed as overweight or obese in the United States [1]. The relevance of increased adiposity is exemplified by the relationship between obesity and multiple chronic metabolic diseases including type 2 diabetes, non-alcoholic steatohepatitis, and cardiovascular disease [2–5]. As such, there remains a need to identify therapeutics with the capability of treating this key underlying driver of metabolic disease [6]. In line with this, the endocrine hormone fibroblast growth factor 19 (FGF19) has emerged as a potential therapy for the treatment of obesity and its associated comorbidities [7–10].

FGF15, the mouse ortholog of FGF19, is an ileum derived metabolic factor that functions in the postprandial period to regulate bile acid homeostasis [11]. Promoting lipid absorption, bile acids are made in the liver, stored in the gall bladder, and subsequently released into the small intestine [12]. Deletion or overexpression of FGF15 causes defects in bile acid production [11,13]. Furthermore, regulation of bile acids by FGF19/15 is dependent upon its action in the liver, where it binds to FGF receptor 4 (FGFR4) and inhibits the expression of cholesterol 7 alpha-hydroxylase (CYP7A1), the rate limiting enzyme in bile acid production [14,15].

Both FGF19 and its endocrine FGF family member FGF21, appear to have broadly similar effects on whole-body metabolism. Animals overexpressing FGF19 or FGF21 as well as those treated with recombinant protein, exhibit elevated metabolic rates and decreased fat mass in addition to improvements in glucose utilization, insulin sensitivity, and lipid profiles [16–20]. We and others have previously shown that induction of UCP1 by FGF21 is required for its thermogenic effects. However, the majority of the metabolic benefits characteristic of FGF21 action remain in the absence of UCP1 [21]. Similar to FGF21, the therapeutic benefits associated with FGF19 administration have also been proposed to be linked to the recruitment of UCP1 and other key thermogenic pathways in white and brown adipose tissue [14,16,17]. However, the role of UCP1 in mediating the metabolic effects observed with FGF19 has not been fully investigated. To determine the specific contribution of UCP1 to the known pharmacology of FGF19, we treated wild-type (WT) and UCP1 null (UCP1KO)

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mice chronically with FGF19. We show that chronic FGF19 administration reduced body weight but did not increase energy expenditure in UCP1KO mice. UCP1 null animals treated with FGF19 exhibited a down regulation of hepatic genes linked to bile acid production, lowered levels of certain hepatic bile acid species, and a significant increase in fecal energy content. Taken together, these data suggest that the anti-obesity action of FGF19 may be driven by its classical role in the regulation of hepatic bile acid synthesis rather than the upregulation of thermogenic pathways in adipose tissue.

## 2. MATERIALS AND METHODS

### 2.1. Animals

All animal studies were approved by the Eli Lilly and Company Institutional Animal Care and Use Committee. Obese male wild-type C57Bl/6NTac mice were obtained from Taconic Farms, male UCP1KO mice (B6.129-ucp1tmkz/J) and their WT siblings were obtained from the Jackson Laboratory. All animals were individually housed in a temperature-controlled (24°C–27 °C) environment with 12 h/12 h light/dark cycle. Wild-type and UCP1KO mice were fed a calorie-rich diet consisting of 40% fat, 39% carbohydrate, and 21% protein caloric content (TD95217; Envigo) for a minimum of 16 weeks prior to the start of treatment and had free access to food and water. During the study period, mice received a daily intraperitoneal injection with either vehicle (PBS) or recombinant human FGF19 (0.2 or 2 mg/kg). Recombinant human FGF19 (FGF19) was generated in house at Eli Lilly and Company. Food intake and body weights were recorded daily. Body composition was determined by quantitative nuclear magnetic resonance (QNMR) using an Echo System instrument (ECHO MRI, 3-1 Composition Analyzer; Echo Medical Systems, Houston, TX).

### 2.2. Indirect calorimetry

Changes in metabolic rate associated with chronic dosing of FGF19 were assessed over an 8 day period using an open respirometer system (LabMaster System; TSE Systems, Bad Homburg, Germany). Briefly, oxygen consumption ($VO_2$, mL/kg/h) and carbon dioxide production ($VCO_2$, mL/kg/h) were measured throughout the 8 day dosing period. $VO_2$ (mL/kg/h) and $VCO_2$ (mL/kg/h) were used to calculate energy expenditure and respiratory exchange ratio (RER = $VCO_2/VO_2$). Animals had *ad libitum* access to water and high-fat diet when in metabolic cages. All measurements were made at approximately 24 °C. The effects of FGF19 on metabolic rate in UCP1KO mice and their WT siblings was determined during the final 24 h of dosing using the CLAMS™ animal monitoring system (Columbus instruments, Columbus, OH USA). Animals had *ad libitum* access to water and high-fat diet when in the CLAMS. All measurements were made at approximately 24 °C.

### 2.3. Tissue collection

Animals were euthanized by $CO_2$ asphyxiation followed by exsanguination via cardiac puncture. Blood was collected in EDTA coated tubes; plasma was separated by centrifugation, aliquoted, and frozen for future analysis. Adipose tissues (epididymal white (eWAT), inguinal white (iWAT) and interscapular brown (iBAT)) and liver were removed and flash frozen in liquid nitrogen.

### 2.4. Determination of insulin sensitivity

Following 7 days of FGF19 treatment, insulin sensitivity was determined in wild-type and UCP1KO mice. Briefly, on the morning of the procedure, animals were fasted for four hours. The animals were anesthetized with isoflurane throughout the entire procedure. A blood sample was collected by tail clip method. Each animal received 10 μCi of [$^3$H] 2-Deoxyglucose (Perkin–Elmer) and 0.5 U/kg of insulin (Humilin R, Eli Lilly and Company, Indianapolis, IN) by retro-orbital injection. Additional blood samples were taken at 2, 5, 10, 15, 20, and 30 min after injection. The blood samples were treated with Barium Hydroxide and then precipitated with Zinc Sulfate. The samples were centrifuged, the supernatant was collected, and the radioactivity was measured by liquid scintillation. After the final blood collection, the animals were euthanized, and tissues were collected. The tissue samples were clamp frozen in liquid nitrogen. For in vivo glucose uptake, tissue samples were weighed and homogenized in 0.1% perchloric acid. The homogenates were combined with either water to determine total 2-deoxyglucose or barium hydroxide/zinc sulfate to determine free 2-deoxyglucose. Radioactivity was measured by liquid scintillation. Data are presented as μmol/100 g/min.

### 2.5. Lipid tolerance test

Following 7 days of FGF19 treatment, wild-type and UCP1KO mice were fasted overnight (14–16 h) in standard cages with *ad libitum* access to water. Mice were gavaged with 0.5 mL of olive oil and blood was collected for measurement of triglyceride content. Blood samples were collected via tail bleed using a Microvette® CB 300 K2E (Sarstedt) at 0, 1, 2, 3, and 5 h of the lipid challenge. Serum levels of triglycerides were quantified using a triglyceride assay kit (Liquicolor (Mono®)).

### 2.6. Hepatic bile acids and fecal energy content

To determine fecal energy content, feces were collected from WT and UCP1KO animals dosed with vehicle or FGF19 over the last 3 days of treatment. Fecal energy content was measured via bomb calorimetry (Covance Laboratories, Madison). Hepatic bile acids were analyzed as previously described [22].

### 2.7. Analysis of metabolites and circulating factors

Plasma triglycerides, cholesterol and free fatty acids were measured using a Hitachi 912 Clinical Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN). Insulin (Crystal Chem Inc., Downers Grove, IL, USA), total Adiponectin (BioVendor Inc., Asheville, NC, USA) and FGF21 (R&D Systems, Minneapolis, MN, USA) were measured by ELISA.

### 2.8. RNA isolation, RT and real-time quantitative PCR

Total RNA was isolated from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) and RNeasy lipid mini kit (Qiagen, Venlo, Netherlands). RNA concentration was determined with a Nanodrop 1000 spectrophotometer (ThermoFisher Scientific). One μg of RNA was used to synthesize cDNA using a High-Capacity cDNA RT Kit (PE Applied Biosystems). Expression of mRNA was determined on a QuantStudio 7 system using Universal PCR Master Mix and TaqMan primers (Applied Biosystems). Data were normalized to β-actin and fold-change calculated using $2^{-\Delta\Delta CT}$.

### 2.9. Statistical analysis

All data were graphed and analyzed using GraphPad Prism, version 7.03. Data are presented as mean ± SEM. Statistical analyses performed included student unpaired t-test, one-way ANOVA or two-way ANOVA, followed by Dunnett's multiple comparisons test where appropriate. Differences were considered significant when $p < 0.05$*.

## 3. RESULTS

### 3.1. FGF19 lowers body weight in obese mice

The anti-obesity action of FGF19 is suggested to be linked, in part, with its ability to induce a negative energy balance via an increase in whole-

 

body caloric expenditure [16,17]. However, the kinetics of the onset of weight loss and the increase in caloric expenditure following FGF19 administration has not been shown. To address this, we evaluated the effect of daily subcutaneous administration of FGF19 (0.2 or 2 mg/kg) in obese mice over an 8 day period. As previously reported [16,18], obese animals treated with FGF19 lost a significant amount of body weight, an effect which appeared to be saturated at a dose of 0.2 mg/kg of FGF19 per day (Figure 1A). This loss of body mass was a result of reductions in both fat mass and fat-free mass (Figure 1B) and was independent of changes in daily food intake (Figure 1C). Additionally, while there was no effect on circulating levels of triglycerides or free fatty acids (FFA), FGF19 treatment led to reductions in liver triglyceride content after 8 days of treatment (Figure 1D).

### 3.2. FGF19 enhances insulin stimulated glucose disposal

Next, we characterized the effect of FGF19 on glucose metabolism. While there was no effect of FGF19 on fed blood glucose, 8 days of FGF19 treatment did cause a significant reduction in fed plasma insulin levels (Figure 1E,F). Tissue insulin sensitivity was assessed in vivo by quantifying radiolabeled glucose uptake in response to a submaximal insulin dose (0.5 U/kg). Glucose clearance in response to insulin was significantly increased in FGF19 treated animals (Figure 1F). Tissue glucose uptake was determined in muscle (red and white quad) and adipose tissue (subcutaneous inguinal white (iWAT) and interscapular brown (iBAT)), to determine which tissues contributed to the enhanced glucose disposal. Basal glucose uptake was not different between vehicle and FGF19 treated mice however, insulin stimulated glucose disposal was significantly enhanced in iBAT of FGF19 treated animals (Figure 1G,H).

### 3.3. FGF19 rapidly increase caloric expenditure in obese mice

To determine the impact of FGF19 on whole body metabolism, animals were housed in a TSE indirect calorimetry system for 7 days. FGF19 treated animals exhibited a time-dependent and dose-dependent increase in energy expenditure, which was statistically significant 48 h after study onset, and remained elevated throughout the treatment period (Figure 2A). There was no effect of FGF19 on carbohydrate and lipid oxidation rates (respiratory exchange ratio (RER)) when compared to vehicle treated animals (Figure 2B).

### 3.4. FGF19 induces thermogenic gene expression in adipose tissue

In support of FGF19 recruiting adipose tissue thermogenic pathways, gene expression analyses indicated a clear impact of FGF19 on gene transcripts associated with enhanced thermogenic activity at the high dose of FGF19 (2 mg/kg/day). In both white and brown adipose tissue, there was a significant increase in the mRNA expression of key regulators of mitochondrial capacity as well as thermogenic activity. Specifically, in subcutaneous white adipose tissue (iWAT) there was a significant increase in mRNA expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1$\alpha$) as well as type II iodothyronine deiodinase (DIO2) (Figure 2D). Furthermore, in interscapular brown adipose tissue (iBAT) there was a significance increase in PGC1$\alpha$ and bone morphogenic protein 8b (BMP8b) (Figure 2E). Importantly, mRNA expression of UCP1 was significantly elevated in both iWAT and iBAT following 7 days of FGF19 treatment (Figure 2D, E). There was no effect of FGF19 on these transcripts, in visceral, epididymal (eWAT) white adipose tissue (Figure 2C).

### 3.5. FGF19 does not require UCP1 to lower body weight in mice

To determine which facets of FGF19's effects require the activity of UCP1, we compared the pharmacological effects of FGF19 in mice with whole body deletion of UCP1 to their wild-type littermate controls [23]. Surprisingly, chronic administration of FGF19 (2 mg/kg/d for 7 days) was equally efficacious at reducing body weight in obese WT (Figure 3A) and UCP1 null mice (Figure 3B). Furthermore, in an important contrast to our earlier work on FGF21, there was no effect of FGF19 on daily caloric intake in WT (Figure 3C) or UCP1KO (Figure 3D) mice. There was a significant reduction in fed glucose concentration, as well as a significant decrease in plasma insulin levels in WT (Figure 3E) treated with FGF19. However, in UCP1KO animals this effect of FGF19 on fed glucose was absent and there was only a trend ($p = 0.06$) towards significantly lowered insulin concentrations (Figure 3F). Of note, FGF19's therapeutic impact on insulin stimulated glucose disposal in iBAT of WT animals was not observed in UCP1 null animals (Figure 3G,H). To determine if the thermogenic effects of FGF19 depend on the presence of UCP1, whole body energy expenditure was evaluated at the end of the 7 day treatment period in both WT and UCP1KO animals treated with FGF19. Once again, we found that FGF19 treatment led to elevated energy expenditure in both the light and dark phase without affecting substrate utilization rates in WT mice (Figure 4A). Conversely, the effect of FGF19 treatment on energy expenditure was entirely absent in UCP1KO animals (Figure 4B).

The most well described biological function of FGF19 is its role in regulation of bile acid homeostasis [11]. We found that FGF19 significantly reduced the mRNA expression of CYP7A and CYP8B in the liver of UCP1KO mice and although it did not reach significance, CYP7A was also decreased (approx. 50%) in WT animals when compared to vehicle (Figure 4C,D). To further characterize the effect of FGF19 on bile acid homeostasis, we measured hepatic bile acid content. FGF19 significantly lowered the amount of certain bile acid species in the liver of both WT and UCP1KO animals (Figure 4E,F). To determine if the reduction in body weight was associated with a reduction in energy absorption, we measured fecal energy content via bomb calorimetry in feces collected during the last 3 days of treatment in both WT and UCP1KO mice. There was a significant increase in fecal energy content in both WT (10% increase, Figure 4G) and UCP1KO (17% increase, Figure 4H) animals treated with FGF19 when compared to vehicle. The effect of FGF19 treatment on fecal energy content was significantly greater in UCP1KO animals compared to WT animals ($p = 0.035$).

## 4. DISCUSSION

Administration or overexpression of FGF19 in preclinical models of obesity and T2DM provides protection from weight gain and enhances glycemic control. The metabolic efficacy of FGF19 appears to be linked to its ability to increase whole-body caloric expenditure, an effect in rodents underpinned by the action of the thermogenic protein UCP1, in white and brown adipose tissue [16,17]. The primary objective of the current study was to determine the contribution of UCP1-dependent thermogenesis to the pharmacology of FGF19.

Firstly, we attempted to delineate in wild type mice, the impact of FGF19 treatment on caloric expenditure and weight loss. To do this, we conducted a time-course study monitoring both body weight and metabolic rate throughout FGF19 treatment. We found that the effect of FGF19 on body weight was significant by day 7 with no significant difference between the high and low treatment groups. FGF19 treated animals exhibited reductions in both fat mass and fat-free mass, furthermore, these effects were independent of changes in food intake. Since the liver is a major site of FGF19 action, we sought to determine the effect of FGF19 treatment on hepatic lipid content. While we found hepatic triglyceride content was lower in animals treated with FGF19, circulating triglycerides and FFAs were unchanged.

  

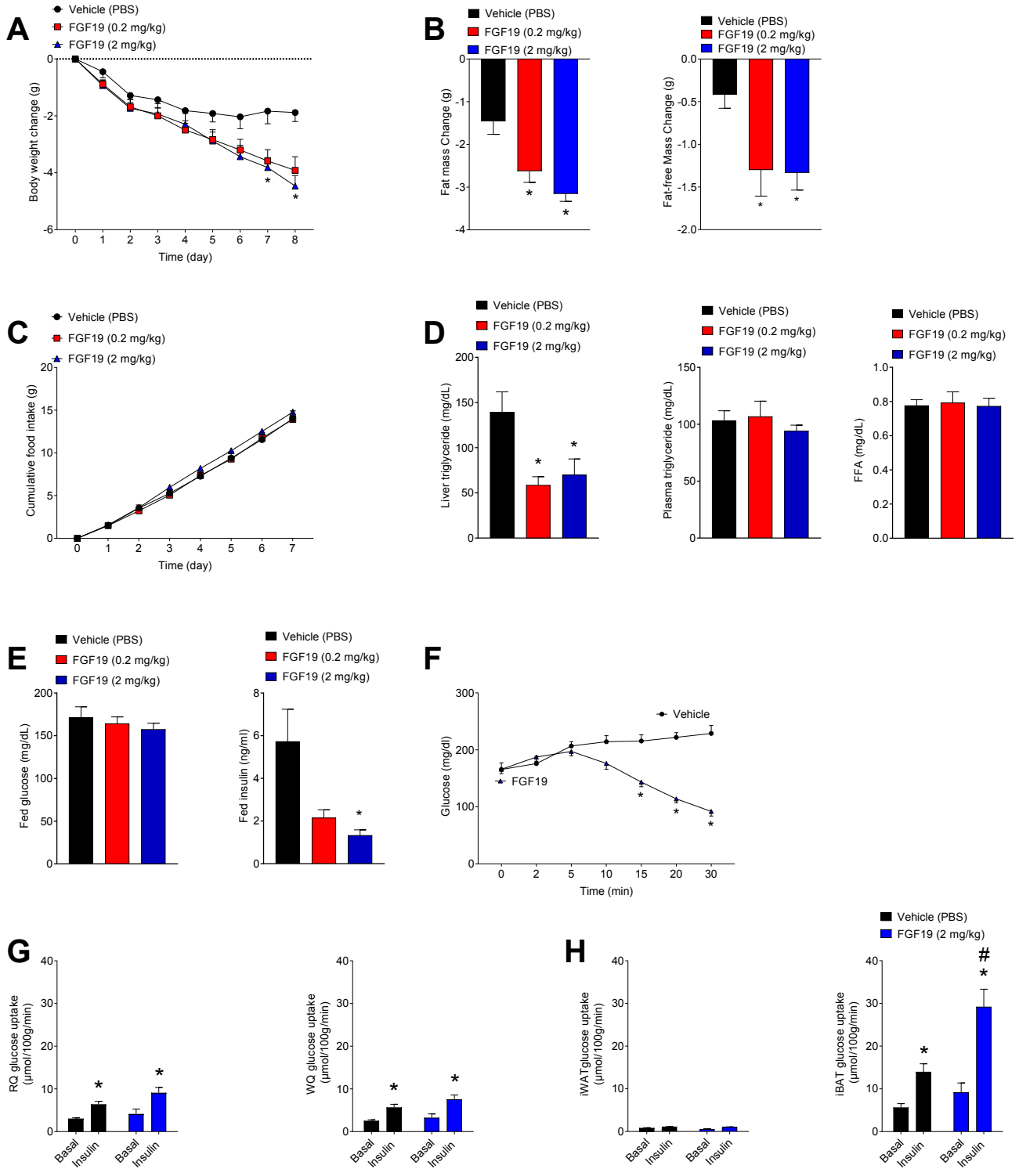


**Figure 1: FGF19 lowers body weight in obese mice.** Obese mice (C57Bl/6NTac) were treated once a day with either vehicle (saline, n = 6) or FGF19 (n = 6 per dose group). Eight days of FGF19 treatment significantly lowered body weight when compared to control mice (A). Weight loss was associated with a reduction in fat and fat free mass (B). There was no effect of FGF19 treatment on daily food intake (C). FGF19 significantly reduced liver triglyceride content but had no impact on circulating triglycerides or free fatty acids (FFAs) at the end of the 8 day dosing period (D). While there was no effect of FGF19 on fed glucose levels, there was a significant reduction in fed plasma insulin in animals treated with FGF19 (E and F). In support of improved glycemic control, obese animals treated with FGF19 (n = 6) exhibited lower glucose levels in response to an insulin challenge (n = 6, F). FGF19 treatment significantly enhanced insulin stimulated glucose disposal in interscapular brown adipose tissue (iBAT), but not muscle (red quadriceps [RQ], white quadriceps [WQ]) or white adipose tissue (iWAT) (G and H). Data are presented as mean ± SEM. $P < 0.05^{*}$ compared to vehicle and $p < 0.05^{\#}$ compared to treatment.

 

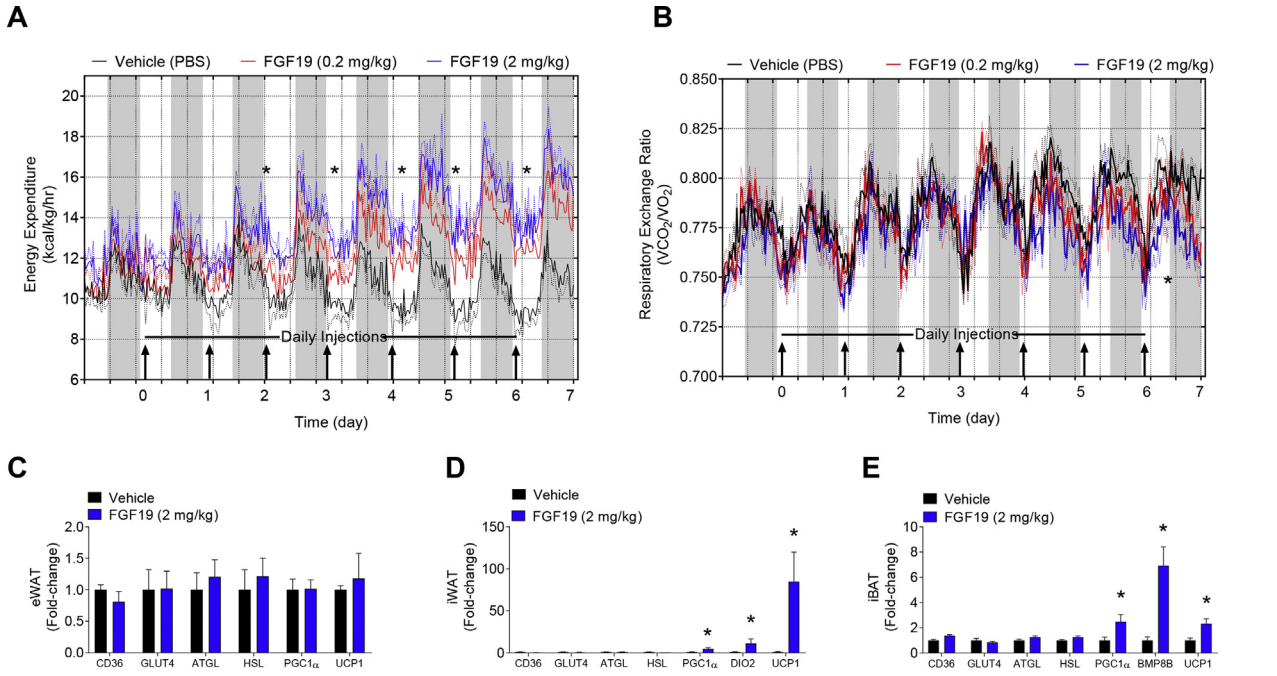


**Figure 2: FGF19 increases caloric expenditure and thermogenic gene expression.** Energy expenditure was measured for 7 days in obese wild-type (C57Bl/6NTac) animals treated with FGF19 daily. FGF19 treated (n = 6) mice exhibited a time and dose dependent increase in caloric expenditure (kcal/kg/hr) when compared to vehicle (n = 6, A). There was no effect of FGF19 on carbohydrate or lipid oxidation rates (respiratory exchange ratio (RER), $VCO_2/VO_2$, B). Data are presented mean ± SEM (dotted line in graphs). The expression of genes associated with thermogenic capacity was assessed in epididymal white (eWAT, C), inguinal white (iWAT, D) and interscapular brown (iBAT, E) adipose tissue. Thermogenic gene expression was elevated in both iWAT and iBAT, but not eWAT of animals treated with FGF19. Data are presented as mean ± SEM. $P < 0.05$* compared to vehicle.

Next, we assessed the effects of FGF19 on glucose metabolism. While there was no effect on fed glucose levels, FGF19 treatment led to a reductions in fed insulin as well as improved insulin sensitivity. Importantly, we found that FGF19 treatment led to improved insulin stimulated glucose disposal in brown adipose tissue. Taken together, these data may indicate that FGF19 is an insulin sensitizer and that adipose tissue is an important mediator of its anti-diabetic action.

In agreement with previously published data [16,17], whole-body calorimetry showed that FGF19 treatment in WT animals does indeed cause an elevation in whole-body caloric expenditure, an effect that reached significance 48 h following the onset of treatment. Concordant with this finding, we also show that FGF19 treatment leads to increased expression of genes involved in thermogenesis, most notably UCP1, in both white and brown adipose tissue. These data show that an elevation of energy expenditure precedes weight loss, supporting the contention that the anti-obesity activity of FGF19 may be linked to its action on UCP1.

To directly test whether the pharmacologic effects we saw in our time-course study are dependent on UCP1-mediated thermogenesis, we treated both WT and UCP1KO animals chronically with FGF19. The primary novel finding of the current investigation is that FGF19 is able to induce weight loss in the absence of UCP1 and an elevation of whole-body caloric expenditure. In fact, in contrast to prior hypotheses, we show here that the activity of UCP1 in adipose tissue is dispensable for FGF19's anti-obesity action, illustrated by the fact that UCP1KO animals lost equivalent weight when compared to their WT counterparts. These results are in agreement with our previously published findings that activation of the predominant FGF receptor in adipose, FGFR1, is not required for FGF19-induced weight loss [24]. However, in contrast to its anti-obesity activity, our data suggest that the action of UCP1 is required for FGF19 to enhance tissue specific insulin sensitivity. Specifically, in UCP1KO animals, FGF19 is no longer able to induce whole-body thermogenesis or insulin stimulated glucose disposal in brown adipose tissue. These findings support the contention that the pharmacologic effects of FGF19 treatment on caloric expenditure and glucose uptake in brown adipose tissue require the action of UCP1, potentially through FGF receptor 1 (FGFR1). In support of this, it has been demonstrated that an FGF19 variant unable to bind and activate FGFR1 was unable to improve blood glucose levels in *ob/ob* mice [25]. Conversely, FGF19 retained its ability to improve glucose tolerance in FGFR4 null animals [14].

We have previously shown that weight loss in UCP1KO animals in response to FGF21 is associated with a reduction in caloric intake when energy expenditure is not increased [21]. Interestingly, FGF19-induced body weight lowering in UCP1KO mice was not associated with a change in food intake. While neither FGF19 nor FGF21 are expressed in the brain, both their receptors and their requisite co-receptor (β-Klotho) are expressed centrally [26,27]. Indeed, central infusion of FGF19 or FGF21 is able to modulate feeding behavior and glucose homeostasis in rodents [16,28–31]. The importance of central signaling is highlighted by studies showing that many of the beneficial metabolic effects of both FGF19 and FGF21 are abrogated in animals deficient in central β-Klotho [32,33]. However, it is important to note that FGF21 crosses the blood-brain barrier more readily than FGF15/19 [34,35]; therefore, it may be the pharmacodynamic properties of FGF19 and FGF21, rather than differential FGFR activation, that delineates the differences observed between the current study and our previous work.

Next, we investigated whether the ability of FGF19 to induce weight loss independently of UCP1-mediated thermogenesis is tied to its

 

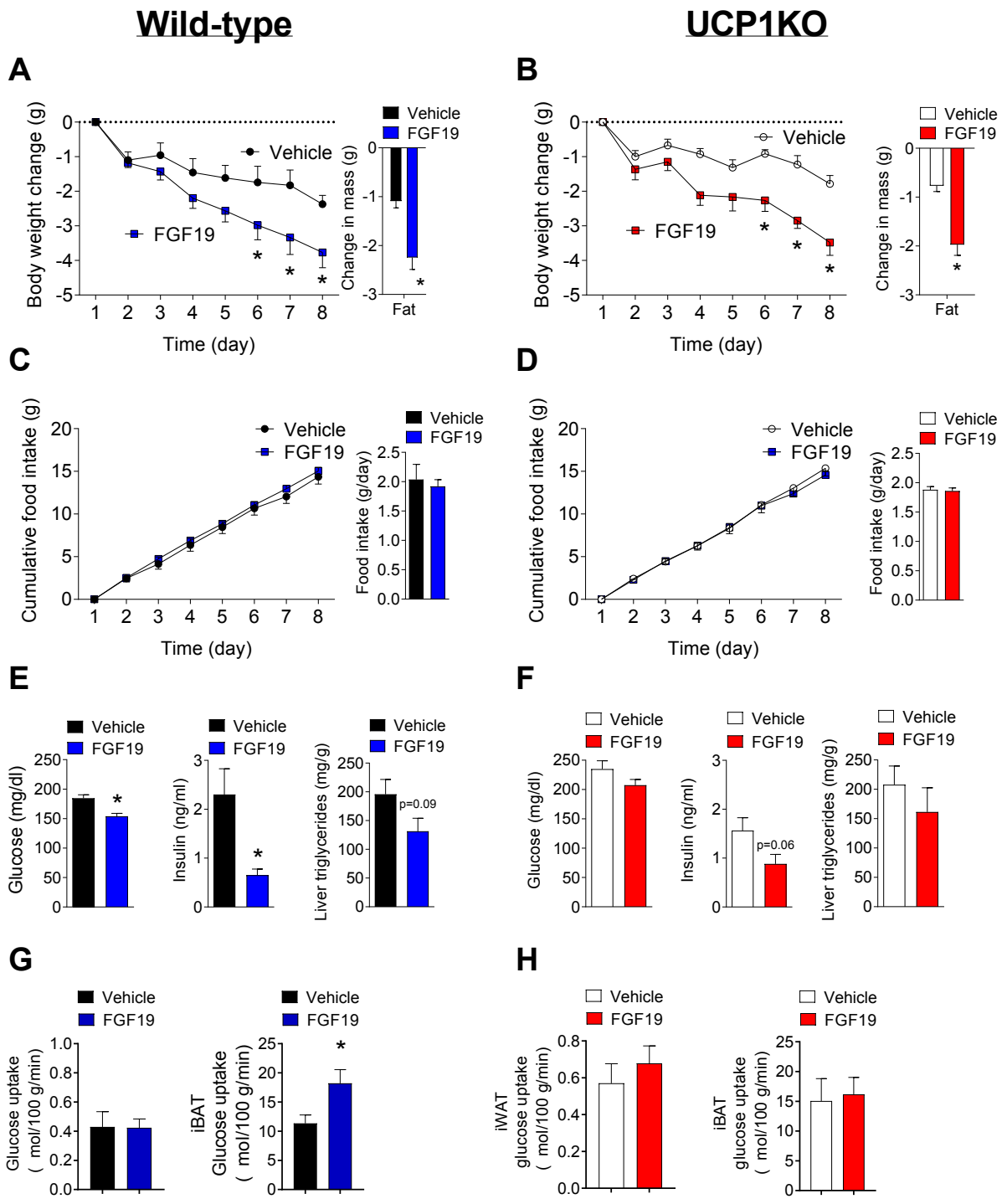


**Figure 3: UCP1 is not required for the antiobesity effects of FGF19.** Both wild-type sibling controls (WT, B6.129-ucp1tmkz/J) and uncoupling protein 1 knockout (UCP1KO, B6.129-ucp1tmkz/J) mice were treated once daily with either vehicle (n = 6) or FGF19 (n = 6, 2 mg/kg). Chronic administration of FGF19 was equally efficacious at reducing body weight in obese WT and UCP1 null mice (A and B) without effecting food intake (C and D). While there was not a significant reduction in fed glucose, FGF19 treatment lead to reduced insulin levels in WT and UCP1KO animals (E and F). While insulin stimulated glucose uptake was increased in interscapular brown adipose tissue (iBAT) of WT animals, this effect was absent in UCP1KO treated animals (G and H). Data are presented as mean ± SEM. $p < 0.05$* compared to vehicle.

known role in bile acid and lipid homeostasis. We found that FGF19 robustly inhibited hepatic transcription of CYP7A1 and CYP8B, proteins required for bile acid synthesis, in UCP1 null mice. While in this experiment there was only a trend towards FGF19 lowering CYP7A a key enzyme associated with bile acid production in WT animals, this action of FGF19 has been shown previously [14]. However, it should be noted that this is effect is potentially due to the elevated hepatic expression of both CYP7A1 and CYP8b in UCP1KO animals compared to WT animals. When individual hepatic bile acid species were measured, we found that FGF19 treatment did indeed lead to

 

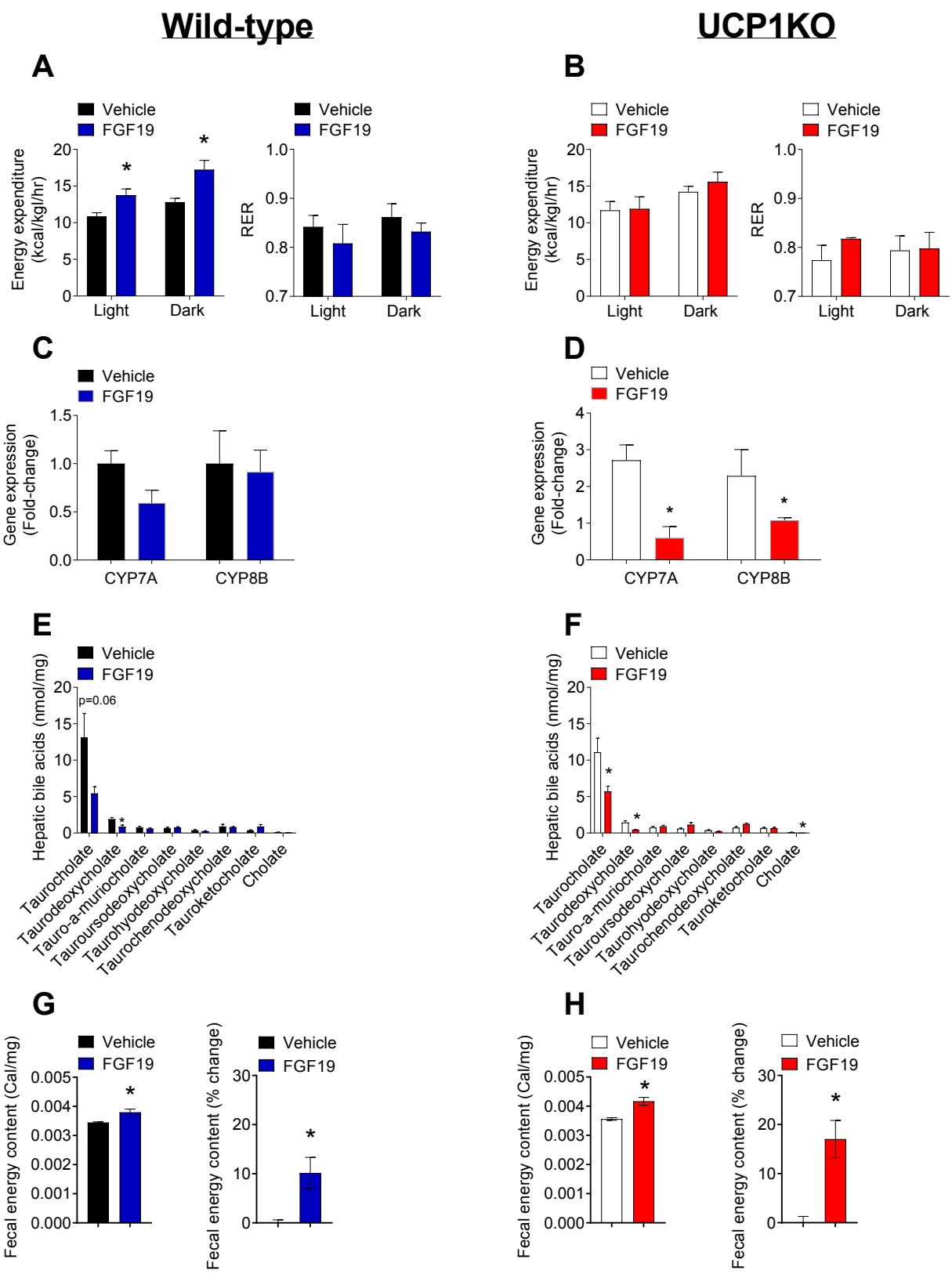


**Figure 4: UCP1 is required for the thermogenic effect of FGF19.** Whole body energy expenditure was measured in wild-type sibling controls (WT, B6.129-ucp1tmkz/J) and uncoupling protein 1 knockout (UCP1KO, B6.129-ucp1tmkz/J) animals dosed once daily with vehicle (saline) or FGF19 (2 mg/kg). FGF19 significantly increased caloric expenditure in WT mice during the light and dark phase (A) of their daily cycle, this effect was absent in UCP1 null animals (B). There was no effect of FGF19 treatment on substrate utilization rates in both genotypes (A and B). Hepatic expression of CYP7A and CYP8B mRNA was measured in both WT and UCP1KO animals (C and D). Bile acid species were measured from livers of animals treated with FGF19 or vehicle (E and F). While both WT and UCP1KO animals showed a significant increase in fecal energy content (G and H) this effect was significantly greater in UCP1KO animals compared to WT animals ($p = 0.035$). Data are presented as mean ± SEM. $p < 0.05$* compared to vehicle.

 

significant reduction of certain bile acids in both WT and UCP1KO animals. As bile acids are crucial for absorption of lipophilic nutrients, we assessed fecal energy content in WT and UCP1KO animals treated with FGF19. Importantly, we found that FGF19 increased fecal energy content in WT mice and that this effect was greater in UCP1 null animals ($p = 0.035$). These data suggest that the primary way in which pharmacologic administration of FGF19 reduces body weight may be through the inhibition of bile acid synthesis and subsequent impairment of lipid uptake in the gut.

## 5. CONCLUSIONS

Taken together, we report here for the first time that the anti-obesity effect of FGF19 occurs in the absence of the key thermogenic protein UCP1. Our data suggest that the primary way in which pharmacologic administration of FGF19 reduces body weight may be through the inhibition of bile acid synthesis and a subsequent reduction of dietary lipid absorption.

## AUTHOR CONTRIBUTIONS

**P.J.A.** was involved in experimental design of all experiments, performed all experiments except those listed below, data interpretation and wrote the manuscript.
**T.E.C.** performed hepatic bile acid analysis.
**B.A.D.** contributed to data interpretation and writing the manuscript.
**R.C.** contributed to data interpretation and writing the manuscript.
**L.S.F and T.C** were involved in designing and performing metabolic cage studies.
**J.W.P.** was involved in experimental design, performing lipid tolerance tests, data interpretation and contributed to writing of the manuscript.
**S.M.B and M.R.W.** were involved in experimental design, data interpretation and performing 2-DG and C-14 tissue uptake experiments.
**J.T.B** was involved in experimental design, performing 2-DG and C-14 experiments, data interpretation, in addition to writing of the manuscript.
**A.C.A.** was involved in the experimental design of all experiments, data interpretation and contributed to writing of the manuscript.
**R.J.S.** was involved in the experimental design of all experiments, data analysis, data interpretation, in addition to writing of the manuscript.

## CONFLICT OF INTEREST

None declared.

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