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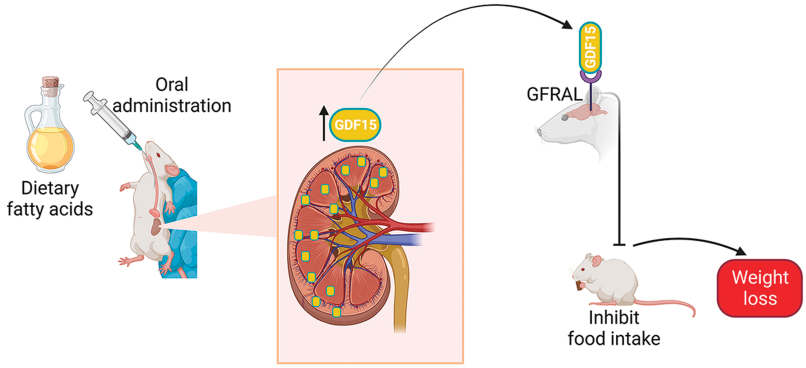

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In contrast to the well-defined biological feedback loops controlling glucose, the mechanisms by which the body responds to changes in fatty acid availability are less clearly defined. Growth differentiating factor 15 (GDF15) suppresses the consumption of diets high in fat but is paradoxically increased in obese mice fed a high-fat diet. Given this interrelationship, we investigated whether diets high in fat could directly increase GDF15 independently of obesity. We found that fatty acids increase GDF15 levels dose dependently, with the greatest response observed with linolenic acid. GDF15 mRNA expression was modestly increased in the gastrointestinal tract; however, kidney GDF15 mRNA was ∼1,000-fold higher and was increased by more than threefold, with subsequent RNAscope analysis showing elevated expression within the cortex and outer medulla. Treatment of wild-type mice with linolenic acid reduced food intake and body mass; however, this effect disappeared in mice lacking the GDF15 receptor GFRAL. An equal caloric load of glucose did not suppress food intake or reduce body mass in either wild-type or GFRAL-knockout mice. These data indicate that fatty acids such as linolenic acid increase GDF15 and suppress food intake through a mechanism requiring GFRAL. These data suggest that a primary physiological function of GDF15 may be as a fatty acid sensor designed to protect cells from fatty acid overload.

ARTICLE HIGHLIGHTS

- The mechanisms by which the body responds to changes in fatty acid availability are less clearly defined.
- We investigated whether diets high in fat could directly increase growth differentiating factor 15 (GDF15) independently of obesity.
- Fatty acids increase GDF15 and reduce food intake through a GFRAL signaling axis.
- GDF15 is a sensor of fatty acids that may have important implications for explaining increased satiety after consumption of diets high in fat.

Obesity is a major health disorder that affects >2 billion people and has severe health consequences, including increasing the risk of type 2 diabetes, cardiovascular disease, and nonalcoholic steatohepatitis (1,2). The causes of obesity are complex and involve an imbalance in which energy intake exceeds energy expenditure, ultimately leading to expansion of adipose tissue mass (3). The regulation of energy balance involves many overlapping homeostatic mechanisms, which may differ based on the primary macronutrients consumed (4). For carbohydrates, this involves the gastrointestinal tract, liver, pancreas, and brain signaling axis, which collectively coordinate the actions of glucagon-like peptide 1 (GLP-1), insulin, and glucagon to maintain blood glucose (5). In addition, as highlighted by recent approvals for weight loss, this signaling axis is also critical for controlling food intake and body mass (6,7). In

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contrast to the well-defined biological feedback loops controlling glucose, the mechanisms by which the body responds to changes in fatty acid availability are less clearly defined.

Growth differentiating factor 15 (GDF15) is a member of the transforming growth factor-β superfamily, which can be induced in all cell types in response to mitochondrial stress and the unfolded protein response through transcription factor ATF4 (8). Increases in GDF15 suppress appetite (9–12) and promote energy expenditure during weight loss (13). These effects are mediated through the GDF15 receptor GFRAL, which is localized to the hindbrain (9–13). Interestingly, these effects of GDF15 to suppress appetite are highly conserved from rodents to humans and seem to involve primarily aversion away from diets high in fat compared with carbohydrates (14). We (13) and others (9,15,16) have demonstrated that in addition to the suppression of appetite, GDF15 also promotes increases in fatty acid oxidation. These linkages with fatty acid intake and oxidation suggest that GDF15 may play a critical role in fatty acid sensing. Therefore, the purpose of this study was to directly evaluate the acute effects of fatty acids on serum GDF15, the potential tissues involved, and whether this is important for controlling energy balance.

RESEARCH DESIGN AND METHODS

Details about research design and methods are available in the Supplementary Material.

Data and Resource Availability

All data sets generated and/or analyzed in this study are available from the corresponding author upon request.

RESULTS

Fatty Acids Increase Serum GDF15

When delivered to male C57BL/6J mice (age 8–16 weeks) via oral gavage, both palm oil and soybean oil increased serum GDF15 (Fig. 1*A*). Because palm oil and soybean oil are primarily composed of the saturated fatty acid palmitic acid (C16:0), monounsaturated fatty acid oleic acid (C18:1), and polyunsaturated fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) (17,18), we treated mice with each of these fatty acids to establish which were primarily contributing to this effect. At a dose of 4 g/kg, serum GDF15 was elevated by oleic acid (+33%), linoleic acid (+45%), and linolenic acid (+76%) (Fig. 1*B*) after 4 h. Surprisingly, palmitic acid had no effect at this dose and time point (Fig. 1*B*) but did lead to increases in GDF15 after 8 h (Fig. 1*C*). A subsequent experiment conducted at a higher dose of oleic, linoleic, and linolenic acids (8 g/kg) increased GDF15 after 4 h (oleic acid +72%; linoleic acid +98%; linolenic +126%) or 8 h (oleic acid +115%; linoleic acid +83%; linolenic +269%), with effects declining after 13 h (oleic acid no change; linolenic acid +176%) (Fig. 1*D–F*). These data indicate that the oral gavage of fatty acids increases serum GDF15; this effect was sustained and seemed maximal after 8 h, and the greatest increase was observed with linolenic acid.

Linolenic Acid Increases Kidney GDF15 Expression

To determine the primary tissues contributing to increases in serum GDF15, we analyzed GDF15 mRNA expression in different tissues from male mice at 8 h after oral gavage with linolenic acid. We found that GDF15 mRNA expression was surprisingly not elevated in the liver or heart but was increased on a relative basis by approximately two- to threefold in the small intestine, colon, cecum, spleen, and epididymal white adipose tissue. However, the most dramatic absolute increase in GDF15 was observed in the kidney, where GDF15 expression was ∼10- to 1,000-fold higher than in most other tissues, with the exception of the liver, suggesting the kidney may be the primary source for increases in serum GDF15 (Fig. 2*A*). RNAscope analysis of the kidneys of mice treated with linolenic acid showed increased expression within the cortex and outer stripe of the outer medulla (Fig. 2*B* and *C*). Consistent with the known effects of the unfolded protein response to increase GDF15 (19,20), we found that *Chop*, *Atf4*, and *Xbp1* mRNA were increased in the kidneys of mice treated with linolenic acid (Fig. 2*D*). ATF4 protein expression was also elevated in the kidney after treatment with linolenic acid (Fig. 2*E*). These data indicate that linolenic acid acutely increases serum GDF15, and this is associated with increases in the unfolded protein response and *Gdf15* mRNA in the cortex and outer medulla of the kidney.

Linolenic Acid but Not an Equal Caloric Load of Glucose Reduces Food Intake and Body Mass Through GFRAL

Conjugated linolenic acid has been tested as a dietary supplement to promote weight loss, with modest efficacy (21). GFRAL is critical for mediating the effects of GDF15 on food intake; therefore, to explore whether linolenic acid could influence food intake and body mass through the GDF15–GFRAL axis, male wild-type (WT) and GFRAL-knockout (KO) mice were placed on a high-fat, high-fructose diet (HFD; 40 kcal% fat (mostly palm oil), 20 kcal% fructose with 0.02% cholesterol) starting at age 12 to 16 weeks for 12 weeks before being singly housed and treated with vehicle or linolenic acid via oral gavage for 7 days (Fig. 3*A*). A daily gavage of linolenic acid reduced food intake and body mass in WT mice but not in GFRAL-KO mice (Fig. 3*B–D*). These genotypic differences were not due to differences in serum GDF15, which was elevated in both WT and GFRAL-KO mice to a similar degree (Fig. 3*E*). Other regulators of food intake, including GLP-1, insulin, and ghrelin, were not significantly different between genotypes basally or after treatment with linolenic acid (Supplementary Fig. 1*A–C*). Taken together, these data indicate that linolenic acid suppresses food intake through a mechanism requiring GFRAL.

To examine whether this effect of linolenic acid to suppress food intake via GFRAL was potentially due to

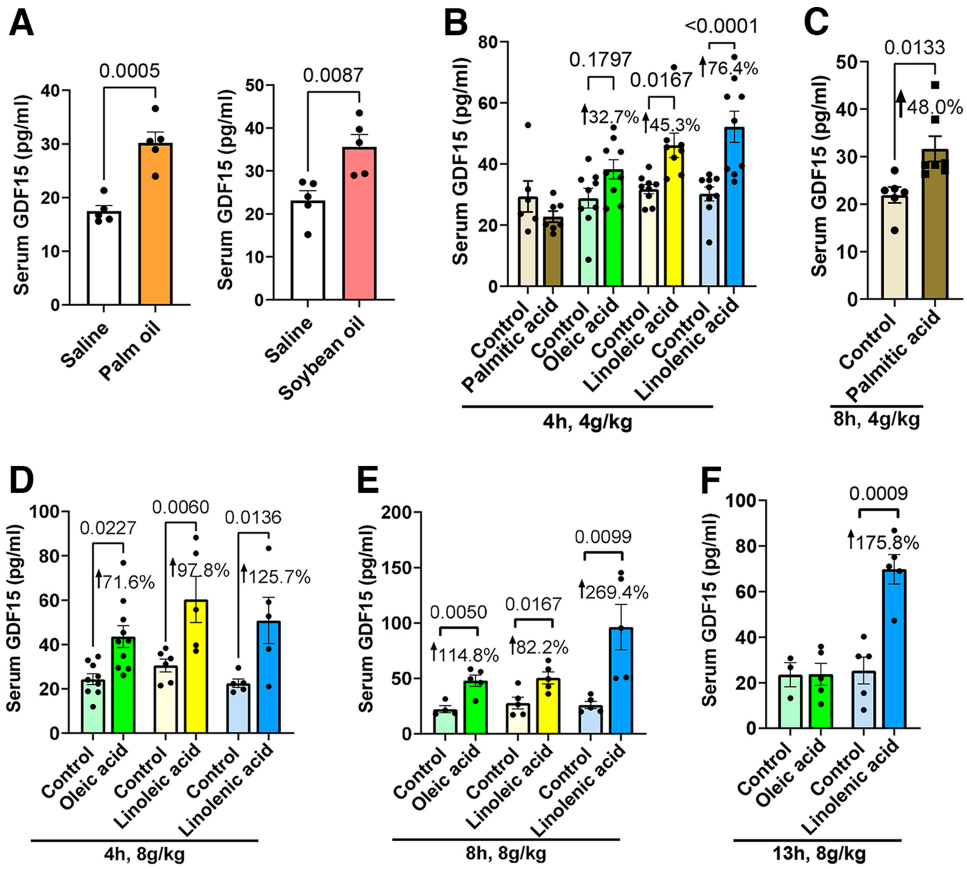

Figure 1—Fatty acids increase serum GDF15. *A*: Serum GDF15 levels at 4 h after oral gavage with palm oil (10 mL/kg) or soybean oil (10 mL/kg). Data are mean ± SEM; $n = 5$ mice per group. *P* values were calculated using two-sided unpaired *t* test. *B*: Serum GDF15 levels at 4 h after oral gavage with palmitic acid, oleic acid, linoleic acid, and linolenic acid at dose of 4 g/kg. Data are mean ± SEM; $n = 6–9$ mice per group. *P* values were calculated using one-way ANOVA with Šídák multiple comparisons test. *C*: Serum GDF15 levels at 8 h after oral gavage with palmitic acid at dose of 4 g/kg. Data are mean ± SEM; $n = 6–8$ mice per group. *P* values were calculated using two-sided unpaired *t* test. *D*: Serum GDF15 levels at 4 h after oral gavage with oleic acid, linoleic acid, and linolenic acid at dose of 8 g/kg. Data are mean ± SEM; $n = 5–10$ mice per group. *P* values were calculated using one-way ANOVA with Šídák multiple comparisons test. *E*: Serum GDF15 levels at 8 h after oral gavage with oleic acid and linolenic acid at dose of 8 g/kg. Data are mean ± SEM; $n = 3–5$ mice per group. *P* values were calculated using one-way ANOVA with Šídák multiple comparisons test.

increased energy intake, we repeated the experiment with the same number of calories from glucose. In contrast to the observations with linolenic acid, daily oral gavage of glucose did not change food intake or body mass over 7 days (Supplementary Fig. 2).

DISCUSSION

Given the known connections between recombinant GDF15 treatment and the avoidance of foods high in fat (10,22), we hypothesized there may be a feedback loop between fatty acids and GDF15 synthesis. To this end, we examined the acute effects of fatty acids on serum GDF15 and found that oral gavage of mice with long-chain fatty acids of different chain lengths and saturation indexes increased serum GDF15 within 8 h and that the greatest response was observed with linolenic acid. Collection of tissues after oral gavage showed that *Gdf15* mRNA expression was increased by approximately twofold in the small intestine, colon, cecum, spleen, and epididymal white adipose tissue. However, the absolute increase in *Gdf15* mRNA expression in these tissues was minimal compared with in the kidney, with subsequent experiments indicating that the primary portions of the kidney responding to fatty acids were in the cortex and outer medulla. Interestingly, these data are consistent with recent findings showing that metformin also stimulates GDF15 in the kidney (23). Collectively, these data indicate that fatty acids stimulate GDF15 expression in the kidney, and this may be an important source for increased serum GDF15. Future studies in which GDF15 floxed mice are crossed with Ksp-Cre mice will be required to definitively confirm this hypothesis. Our study suggests that kidney-derived cytokines or kidneykines may be a relatively unexplored source for food intake–regulating endocrine factors.

To examine the physiological significance of increases in GDF15, we delivered linolenic acid via oral gavage for 7 days into WT and GFRAL-KO mice. We completed this experiment in mice fed an HFD because we previously

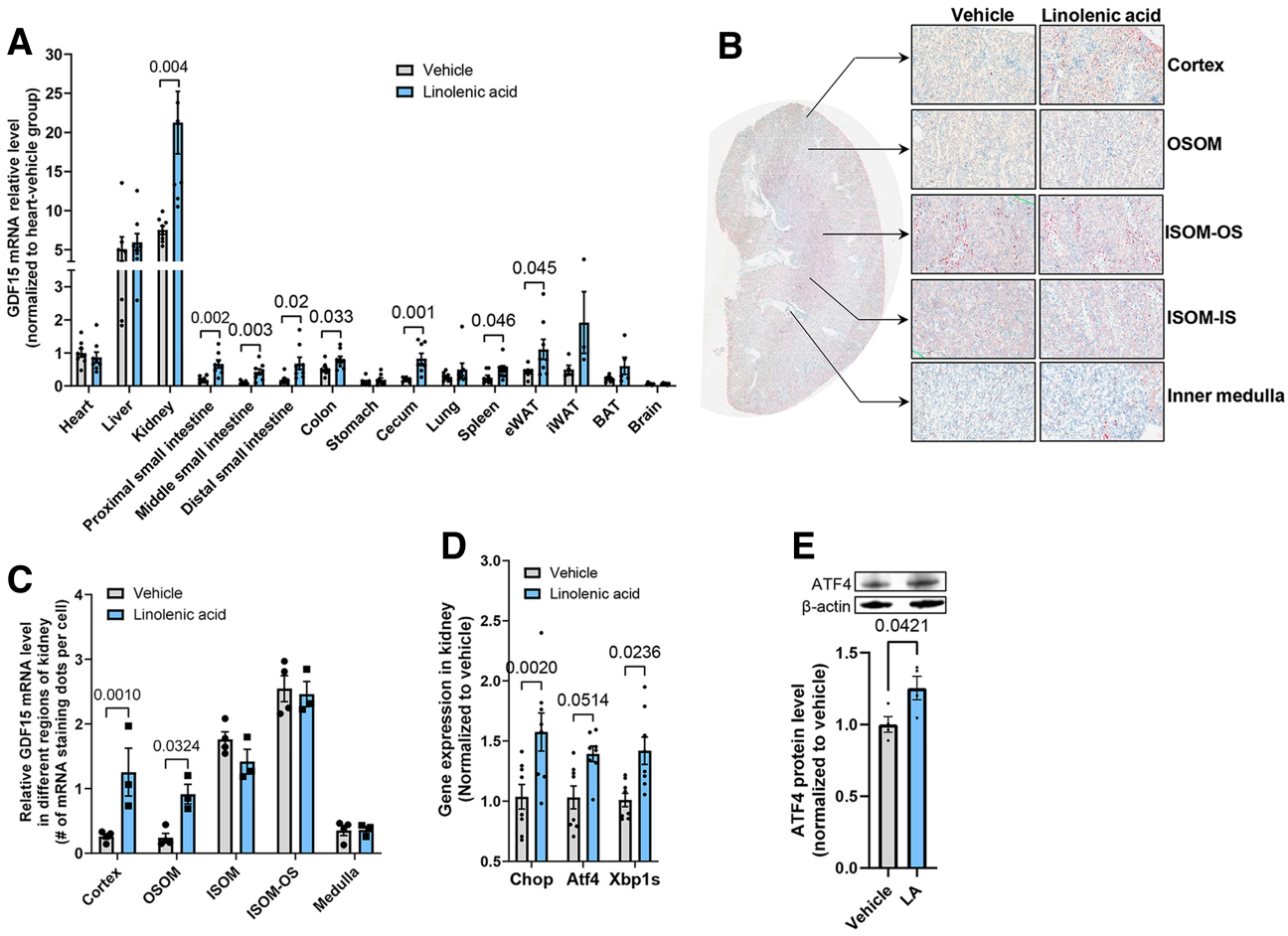

Figure 2—Linolenic acid (LA) increases kidney GDF15 expression. *A*: *Gdf15* mRNA expression in different tissues at 8 h after oral gavage with LA (8 g/kg). Data are mean ± SEM; *n* = 3–8 samples per group. *P* values were calculated using two-sided multiple unpaired *t* test. *B*: Representative images of paraffin-embedded kidney sections stained with *Gdf15* mRNA probe. *C*: RNAscope analysis for *Gdf15* mRNA expression in different areas of kidney after oral gavage with LA (8 g/kg). Data are mean ± SEM; *n* = 3–4 mice per group. *P* values were calculated using two-sided multiple unpaired *t* test. *D*: *Chop*, *Atf4*, and *Xbp1s* mRNA expression in the kidney after oral gavage with LA (8 g/kg). Data are mean ± SEM; *n* = 7–8 mice per group. *P* values were calculated using two-sided multiple unpaired *t* test. *E*: LA at dose of 8 g/kg increases ATF4 protein levels in the kidney. Data are mean ± SEM; *n* = 4 mice per group. *P* values were calculated using two-sided unpaired *t* test. BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; ISOM, inner stripe of the outer medulla; ISOM-IS, inner stripe of the outer medulla (inner stripe); ISOM-OS, inner stripe of the outer medulla (outer stripe); iWAT, inguinal white adipose tissue; OSOM, outer stripe of the outer medulla.

showed (24) that metformin-induced increases in GDF15 suppress food intake in mice fed an HFD but not a high-carbohydrate chow diet, a finding consistent with other studies showing that GDF15 elicits taste aversion to fat but not carbohydrates (10,22). Remarkably, we found that linolenic acid reduced food intake and body mass in WT but not GFRAL-KO mice. Serum insulin, GLP-1, and ghrelin were not different between genotypes, suggesting that the reductions in food intake in WT but not GFRAL-KO mice are not secondary to differences in these known effectors of food intake. Taken together, these data support the concept that the GDF15–GFRAL signaling axis directly regulates food intake in response to linolenic acid. Future studies investigating whether there are acute changes in food intake in response to other fatty acids or mixed meals of fatty acids and carbohydrates will be important.

A critical control for our experiments is that we found that the suppression of food intake dependent on linolenic acid, GDF15, and GFRAL was not observed after the gavage of an equal caloric load of glucose. These data indicate that GDF15 is not just a sensor of caloric intake but rather is acutely responding to fatty acid availability. Mechanistically, linolenic acid increased the expression of transcription factors ATF4 and CHOP in the kidney, which are known to regulate GDF15 expression. Future studies examining whether ATF4- and CHOP-KO mice have reduced GDF15 and a blunted response to linolenic acid will be important for further characterizing this pathway. However, it should be noted that consistent with our findings, ATF4-KO mice have increased obesity associated with increased food consumption when fed an HFD (8). Because impairments in mitochondrial function are known to stimulate GDF15 secretion through ATF4 (16), these data

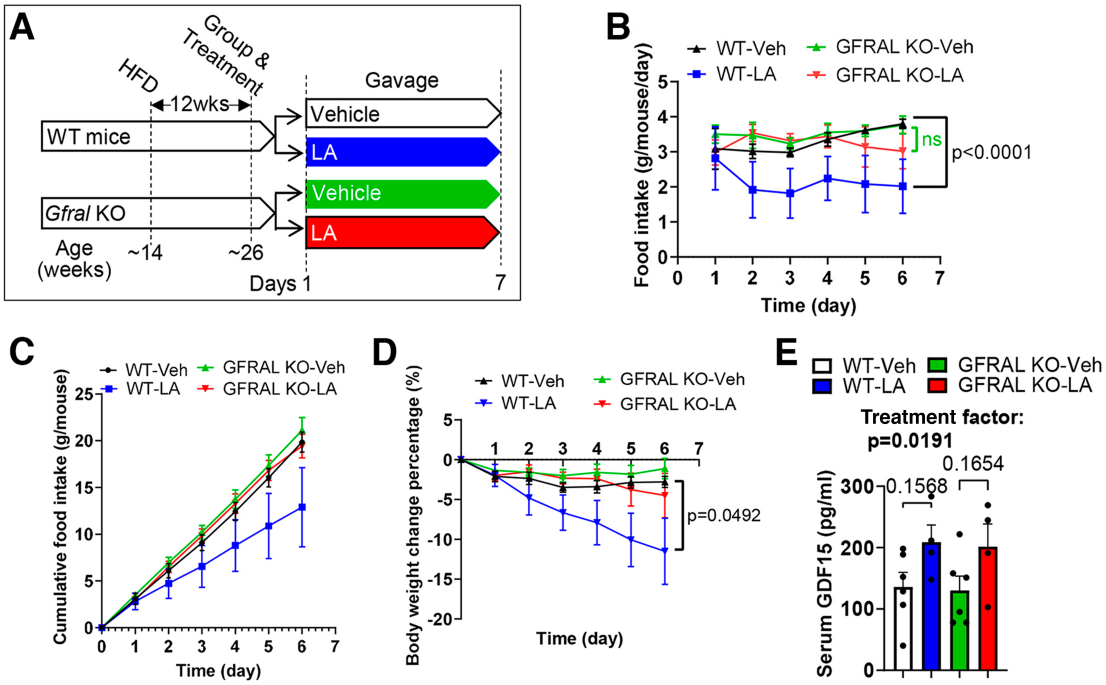

Figure 3—Linolenic acid (LA) reduces appetite and body mass through GDF15–GFRAL. *A*: Experimental scheme testing the effects of LA (1 g/kg/day) on food intake and body mass in WT and GFRAL-KO mice. *B*: Daily food intake. Data are mean ± SEM; *n* = 5–6 mice per group. *P* values were calculated using one-way ANOVA with Šídák multiple comparisons test. *C*: Cumulative food intake. Data are mean ± SEM; *n* = 5–6 mice per group. *D*: Body weight change. Data are mean ± SEM; *n* = 5–6 mice per group. *P* values were calculated using one-way ANOVA with Šídák multiple comparisons test. *E*: GDF15 levels in serum from mice after treatment with LA for 7 days. Data are mean ± SEM; *n* = 4–6 mice per group. *P* values were calculated using two-way ANOVA with Šídák multiple comparisons test or not.

suggest that an important evolutionary role of GDF15 may be to protect cells with impaired mitochondrial function from fatty acid overload.

Our study had several limitations. First, we tested the effects of fatty acids in male mice only; therefore, it is unknown whether a similar effect of fatty acids on GDF15 and food intake would be observed in female mice. Second, we observed that linolenic acid induced weight loss in obese WT but not GFRAL-KO mice, but it was not established whether this was due to reductions in adipose tissue or lean mass. We previously (13,24) found that treating obese mice with metformin or recombinant GDF15 leads to GDF15/GFRAL-dependent reductions in adipose tissue mass without altering lean mass; however, future studies investigating whether this occurs with linolenic acid treatment are warranted. Our study found that the kidney expressed high levels of GDF15, and this was increased after exposure to fatty acids. However, we also observed elevations in GDF15 in several other tissues, including the gastrointestinal tract. These findings showing increases in gastrointestinal GDF15 are consistent with a recent report (25) that suggested the gastrointestinal tract and liver may be the primary tissues for the suppressive effects of medium-chain fatty acids on appetite. Future studies using mice with targeted deletion of GDF15 in specific cell types will be important to establish the primary tissues contributing to reductions in food intake after exposure to fatty acids. We also did not assess whether chronic treatment with fatty acids had differential effects on glucose homeostasis or insulin sensitivity, and given recent findings showing that GDF15 can acutely improve insulin sensitivity (26), this may be important.

Although speculative, we believe our findings may have important implications for the control of energy balance. As detailed in the energy balance model (EBM), it is now clear that there are multiple complex interactions between genetics and environmental factors, including reduced activity levels and increased consumption of highly palatable foods, that contribute to obesity (27). A derivation of the EBM proposes that the primary driver of this imbalance is that increased carbohydrate consumption drives an increase in insulin, which promotes appetite and de novo lipogenesis (28). This model, known as the carbohydrate insulin model (CIM), proposes that insulin acts as a feed-forward pull on the system that propagates increased caloric intake and a positive energy balance (29). A core tenant of the CIM is that reducing dietary carbohydrate consumption through substitution with fatty acids will lead to weight loss; however, the mechanisms mediating these acute effects are currently not understood. Our data indicating that increases in the intake of fatty acids stimulate GDF15 and that this is important for suppressing food intake compared with an equal number of calories from glucose may have important implications

in explaining potential weight loss associated with HFDs (ketogenic diets), thus providing a potential mechanistic underpinning to explain both the EBM and CIM. Future studies investigating whether consuming HFDs stimulates GDF15 in humans and whether this correlates with food intake and weight loss will be important.

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Duality of Interest. G.R.S. is a cofounder and shareholder of Espervita Therapeutics, a company developing new medications for liver cancer. McMaster University has received funding from Cambrian Biosciences, Espervita Therapeutics, Esperion Therapeutics, Poxel Pharmaceuticals, Merck, Novo Nordisk, and Nestle for research conducted in the laboratory of G.R.S. G.R.S. has received consulting/speaking fees from AstraZeneca, Eli Lilly, Esperion Therapeutics, Merck, Novo Nordisk, Poxel Pharmaceuticals, and Cambrian Biosciences. G.R.S. is one of the inventors on a patent identifying GDF15 as a biomarker for metformin. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. D.W. and M.J.T.J. conducted experiments and analyzed data. D.W. and G.R.S. designed experiments and wrote the manuscript. J.L., L.K.T., C.M.V., J.G., B.B., E.E.T., and J.S.V.L. conducted experiments and edited the manuscript. D.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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