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Fibroblast Growth Factor 19 Increases Metabolic Rate and Reverses Dietary and Leptin-Deficient Diabetes

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Hormonal control of metabolic rate can be important in regulating the imbalance between energy intake and expenditure that underlies the development of obesity. In mice fed a high-fat diet, human fibroblast growth factor 19 (FGF19) increased metabolic rate [1.53 ± 0.06 liters O_2/h·kg$^{0.75}$ (vehicle) *vs.* 1.93 ± 0.05 liters O_2/h·kg$^{0.75}$ (FGF19); $P < 0.001$] and decreased respiratory quotient [0.82 ± 0.01 (vehicle) *vs.* 0.80 ± 0.01 (FGF19); $P < 0.05$]. In contrast to the vehicle-treated mice that gained weight (0.14 ± 0.05 g/mouse·d), FGF19-treated mice lost weight (−0.13 ± 0.03 g/mouse·d; $P < 0.001$) without a significant change in food intake. Furthermore, in addition to a reduction in weight gain, treatment with FGF19 prevented or reversed the diabetes that develops in mice made obese by genetic ablation of brown adipose tissue or genetic absence of leptin. To explore the mechanisms underlying the FGF19-mediated increase in metabolic rate, we profiled the FGF19-induced gene expression changes in the liver and brown fat. In brown adipose tissue, chronic exposure to FGF19 led to a gene expression profile that is consistent with activation of this tissue. We also found that FGF19 acutely increased liver expression of the leptin receptor (1.8-fold; $P < 0.05$) and decreased the expression of acetyl coenzyme A carboxylase 2 (0.6-fold; $P < 0.05$). The gene expression changes were consistent with the experimentally determined increase in fat oxidation and decrease in liver triglycerides. Thus, FGF19 is able to increase metabolic rate concurrently with an increase in fatty acid oxidation. (*Endocrinology* 145: 2594–2603, 2004)

IN THE LAST 20 yr, there has been a significant increase in the incidence of obesity and diabetes (1, 2). Whereas obesity *per se* is a significant health concern, a larger health problem arises from the predisposition to type II diabetes in obese patients and the morbidity and mortality associated with type II diabetes. The relationship between obesity and type II diabetes has been demonstrated by epidemiological studies (3) and experiments demonstrating that the increased tissue triglycerides that accompany obesity can cause insulin resistance (4). Obesity arises as a consequence of an imbalance between energy intake and energy expenditure, with the energy difference being stored, primarily as fat. Although there is an increase in the basal metabolic rate component of energy expenditure in response to increased food intake (5, 6), this homeostatic mechanism may not fully compensate for chronic changes in energy intake, and weight gain can occur. Thus, there is an increasing interest in a better understanding of how metabolic rate is controlled.

Several pharmacological agents have been found to increase metabolic rate in humans and experimental animals. These include noradrenaline reuptake inhibitors (*e.g.* sibutramine) (7), selective adrenergic receptor agonists (8), and drugs that increase serotonin levels, [*e.g.* dexfenfluramine (9)]. Although some of these appear to have clinical efficacy (*e.g.* Ref. 10), modest efficacy and significant side effects have limited their general utility. There are also several naturally occurring hormones (*e.g.* epinephrine, thyroid hormone, insulin) that can increase metabolic rate. Because leptin increases metabolic rate (11) as well as suppresses food intake [both effects mediated primarily through the hypothalamus (12, 13)], there was considerable optimism that this hormone could be an important antiobesity drug. However, most obese humans have elevated serum leptin (14), and, in standard overweight humans, the weight loss in response to exogenous leptin is quite small (15). Another hormone that may contribute to metabolic control is the recently described fibroblast growth factor (FGF), FGF19. We recently reported that transgenic mice expressing FGF19 have increased metabolic rate and are resistant to diet-induced obesity and diabetes (16).

The FGFs are a family of more than 20 small (~17–26 kDa) secreted peptides. The initial characterization of these proteins focused on their ability to stimulate fibroblast proliferation. This mitogenic activity was mediated through FGF receptors (FGFRs) 1, 2, or 3. A fourth closely related tyrosine kinase receptor (FGFR4) was able to bind the FGFs but did not lead to a mitogenic response (17). Although the importance of the FGFs as differentiation factors is widely appreciated (*e.g.* Refs. 18 and 19), it has become apparent that the

Abbreviations: ACC2, Acetyl CoA carboxylase 2; BAT, brown adipose tissue; CNS, central nervous system; CoA, coenzyme A; FFA, free fatty acid; FGF, fibroblast growth factor; FGFR, FGF receptor; FXR, farnesoid X receptor; GTT, glucose tolerance test; HFD, high-fat diet; icv, intracerebroventricular; rh, recombinant human; RQ, respiratory quotient; SCD, stearoyl CoA desaturase; SE, squalene epoxidase; UCP-DTA, uncoupling protein-diphtheria toxin A.

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biology of the FGFs is more complex and participates in the maintenance of physiological homeostasis. Thus, mutations in the coding region for FGF23 lead to autosomal dominant hypophosphatemic rickets and injection of recombinant FGF23 into mice reduces serum phosphate (20). In addition, mice with a genetic absence of FGFR4 have increased expression of the cholesterol catabolic enzyme cyp7a.

FGF19 (21, 22) is unusual in that, in cell-free assays, it appears to bind to only one of the FGF receptors (FGFR4), and, as noted above, this receptor appears not to lead to a mitogenic response. The normal function of FGF19 has not been resolved, although a role for inner ear development has been suggested (23). Holt *et al.* (24) also found that hepatocyte expression of FGF19 is induced by the transcription factor, farnesoid X receptor (FXR). FXR is a key regulator of cholesterol metabolism through suppression of the catabolic enzyme cyp7a (24). As noted above, FGF19 transgenic mice have a significant increase in metabolic rate and are resistant to diet-induced obesity (16). We have followed up on our initial report to investigate whether similar responses could be obtained with recombinant FGF19 and to explore further the mechanism(s) for these effects.

Here we report that recombinant FGF19 increased metabolic rate, reduced body weight, and reversed the diabetes in both high-fat-fed mice and leptin-deficient mice. Injection of FGF19 into the lateral ventricle of the brain also increased metabolic rate. A hypothesis for explaining these changes is suggested by the observation that FGF19 increased lipid oxidation and decreased expression of ACC2 [acetyl coenzyme A (CoA) carboxylase 2], the rate-limiting enzyme for fatty acid entry into the mitochondria.

Materials and Methods

An Institutional Use and Care Committee approved all protocols. Mice were maintained in a temperature- and humidity-controlled environment. A 12-h (1800–0600 h) light cycle was used.

Mice and FGF19 treatment

The MLC-FGF19 transgenic mice have been described (16). UCP-DTA (uncoupling protein-diphtheria toxin A) mice on an FVB background (25) were obtained from the Jackson Laboratories (Bar Harbor, ME) and identified by the dominant small eye phenotype (as recommended by Jackson Laboratories). C57BL/6 lepob/lepob mice (ob/ob) (Jackson Laboratories) were genotyped using a Jackson Laboratories protocol. FVB mice were obtained from Taconic Farms and placed on either standard mouse chow (Purina 5010, Harlen Teklab, Madison, WI) or a high-fat diet (26) purchased from Research Diets (New Brunswick, NJ). The energy content of this diet (catalog no. D12330N) is provided by fat (~58%), carbohydrates (~26%), and protein (~16%). Unless otherwise stated, we used male FVB mice that had been maintained on a high-fat diet for 4–6 wk.

Unless otherwise stated, FGF19 (or vehicle control) was injected iv daily for 7 d at 1 mg/kg in a total volume of 100 μl. The FGF19 was purified (see below) into a 0.3 M arginine/phosphate (pH 6.0) buffer. This buffer was also used as the vehicle control. The acute response to FGF19 described in Fig. 5 was examined by injecting FVB mice on the high-fat diet (HFD) with FGF19 (iv 1 mg/kg each injection) at time 0, 2, and 4 h (three injections). The mice were killed at time 6 h (2 h after the last injection).

Male FVB mice with indwelling intracerebroventricular (icv) catheters were purchased from Charles River Laboratories (Wilmington, MA) and maintained on a standard chow diet. Cannula placements were (from bregma) anterior posterior, −0.22 mm; medial lateral, +1 mm; dorsal ventral, −3 mm; depth 3 mm. Proper cannula placement was verified by confirming that leptin (2 μg) reduced food intake in fasted/refed mice. The leptin test was carried out 5 d before initiating FGF19 injections. FGF19 was diluted appropriately into artificial cerebrospinal fluid (Harvard Apparatus, Holliston, MA) before injections. The control mice received the FGF19 vehicle similarly diluted with the artificial cerebrospinal fluid. Intracerebroventricular injections (5 μl) were carried out just before the dark cycle, with the mice under light isoflurane anesthesia.

Indirect calorimetry

Oxygen consumption was measured in an open circuit calorimeter (Columbus Instruments Oxymax, Columbus, OH). Mice were housed individually in oxygen consumption chambers with *ad libitum* access to food and water and acclimated to the chambers for 48 h before the initiation of the experiment. The chambers were maintained in a temperature- (23 C) and light-controlled (12-h light, 12-h dark cycle, lights on at 0600 h) environment. The system was calibrated against standard gas mixtures before every experiment. Each chamber received ambient air at a rate of 0.6 liters/min. Computer-controlled measurements of oxygen consumption (metabolic rate) and CO_2 production from each chamber were determined by paramagnetic and spectrophotometric sensors, respectively. Data were excluded if water bottles leaked and flooded the chambers. Measurements were made at approximately 42-min intervals throughout the study, and mean light- or dark-cycle values calculated every day for each individual using within-animal data collected between 0600 and 1759 h and 1800 and 0559 h, respectively. Within-animal baseline dark- or light-cycle values were defined as the mean values observed during those cycles in the 24 h preceding the first FGF19 injections. Oxygen consumption values are normalized by body weight$^{0.75}$ (measurements taken before FGF19/vehicle treatment). Note that the calculated oxygen consumption values assume a constant body weight. Therefore, any increase in metabolic rate due to FGF19 treatment (Fig. 1, A and C) underestimates the true metabolic rate because there is also a concurrent decrease in body weight.

Assays

Insulin, leptin (Crystal Chem, Chicago, IL), and adiponectin (B-Bridge International, San Jose, CA) were assayed by ELISA kits. Glucose was assayed by either Fast Take glucose meter (Lifescan, Milpitas, CA) or the glucose oxidase method. Tissue fat content in the muscle and liver was assayed using the extraction procedures of Folch *et al.* (27) and an enzymatic triglyceride reagent kit (Sigma, St. Louis, MO).

Glucose and free fatty acid (FFA) metabolism

Glucose tolerance tests (GTTs) were performed in nonfasted mice by injecting each mouse with 1 g/kg glucose ip for the FVB (HFD) mice and 0.75 g/kg glucose ip for the ob/ob mice. Unless otherwise stated, the GTT was initiated 4 h after the last of seven daily injections of FGF19. Whole blood glucose was measured at the indicated times using a Lifescan Fast Take glucose meter. The response to lipid injections was performed by injecting each mouse iv with 30 μl Intralipid (Baxter Healthcare, Deerfield, IL) and measuring serum FFA levels at the indicated times.

Expression and purification of recombinant human (rh) FGF19

FGF19 was expressed in *Escherichia coli* by inserting the coding sequence for amino acids 26–216 downstream of the *phoA* promoter and upstream of the lambda t_0 transcriptional terminator (28, 29). Silent codon changes were introduced to reduce the likelihood of potential secondary structure formation in the translation initiation region (30). Human FGF19 was purified via anion exchange chromatography, size exclusion chromatography, and preparative reverse-phase chromatography. Sequence analysis and analysis by mass spectrometry indicated that purified recombinant FGF19 had the expected mass and N-terminal sequence. From an examination of sodium dodecyl sulfate polyacrylamide gels, the purity of the material was estimated to be greater than 95%. Binding of the human recombinant FGF19 to recombinant recep-

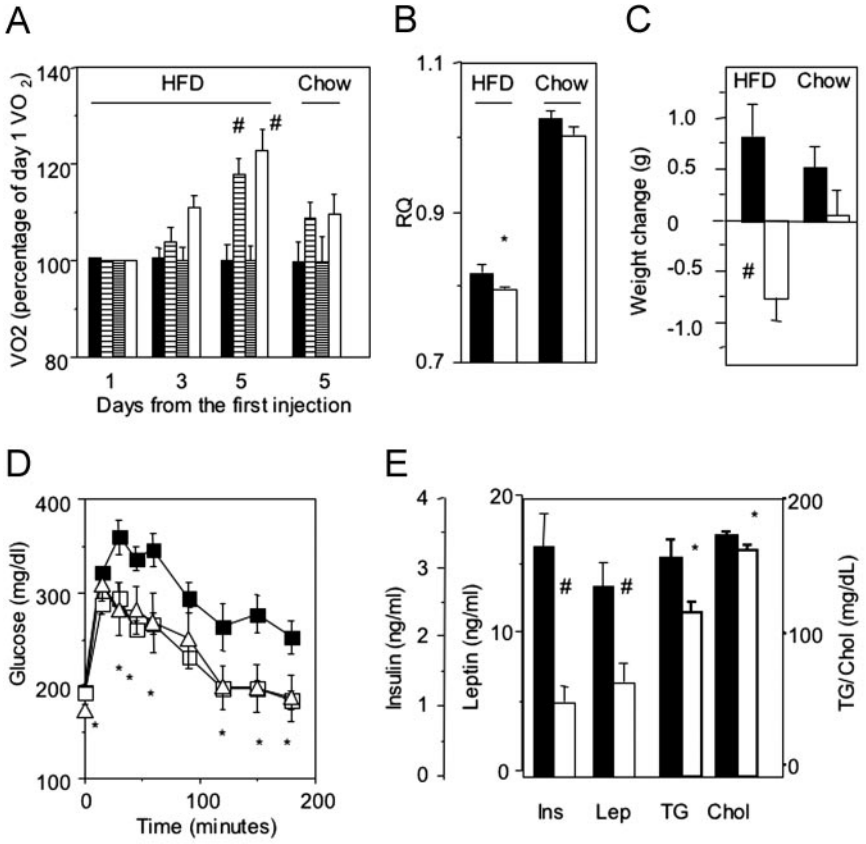

FIG. 1. Recombinant FGF19 increases metabolic rate, decreases body weight, and improves glucose homeostasis. A, Metabolic rate (VO_2, oxygen consumption) as a function of time from the first injection of either FGF19 (1 mg/kg·d) or vehicle. The data are presented as a percentage of the metabolic rate measured on the day/night before injection. ■, Vehicle, day; ▤, FGF19, day; ▤, vehicle, night; □, FGF19, night. $P < 0.001$ (HFD, d 5 and night 5) for effect of FGF19 on VO_2 (Dunnett's test; control group = d 1 or night 1, as appropriate). B, FGF19 reduced the RQ in HFD mice. The RQ shown is the mean of the RQ measured over nights 3–5 of the experiment shown in A. ■, Vehicle; □ FGF19. C, FGF19 treatment led to weight loss in the FVB (HFD) mice. The weight change is from d 1 through d 7. Symbols as for B. D, FGF19 lowered glucose excursion during a GTT performed 7 d after the first FGF19 injection. ■, Vehicle; □, daily FGF19; △, FGF19 every second day. $P < 0.05$ for effect of FGF19 on glucose at 0, 30, 45, 60, 120, 150, and 180 min (ANOVA). E, FGF19 reduced insulin, leptin, triglycerides, and cholesterol. Serum taken from nonfasted FVB (HFD) mice 4 h after the last of seven daily injections of vehicle or FGF19. Symbols as in B. A–C, and E, n = 8 per group. B, C, and E, *, $P < 0.05$; #, $P < 0.01$. D, n = 7/group.

tor-4 was measured using I^{125}-FGF19 and IgG tagged FGFr4 as described (21). Endotoxin contamination was less than 0.05 EU/mg.

Gene expression

RNA was extracted from liver or brown adipose tissue (BAT) of 8-wk-old mice using Biotecx reagents and protocols. Five micrograms of total RNA from each sample was used to generate biotinylated cRNA [as per the standard Affymetrix (Santa Clara, CA) GeneChip labeling protocol]. Ten micrograms of cRNA were added to murine Mu11K A and B Genechips. Hybridization, staining, and scanning of the Genechips was performed using the standard Affymetrix protocols. Gene expression data were generated using MicroArray Suite version 4. Gene expression differences were determined using a Mann-Whitney approach (31). Sixteen comparisons among the four transgenic and the four control samples were performed, and a difference is reported if at least 62.5% of the comparisons were in agreement and the mean difference in expression was greater than 2.5-fold. RT-PCR primers and probe sequences are available on request. Differences in expression were determined assuming that one cycle threshold value difference corresponds to a 2-fold change in mRNA level.

Analysis

Unless otherwise noted, all data are presented as the means ± SE. Statistical comparisons were carried out using either the two-tailed Student's unpaired *t* test or, among more than two groups, by one-way ANOVA. Post-ANOVA comparisons were made using the Dunnett or Tukey-Kramer methods, as appropriate, when one-way ANOVA tests yielded $P < 0.05$.

Results

rhFGF19 increased metabolic rate

The rhFGF19 increased the metabolic rate of HFD mice (Fig. 1A and Table 1). The increase in metabolic rate became statistically significant on the fifth day and night after the first injection of FGF19. The change in metabolic rate in the chow-fed mice did not reach statistical significance. HFD mice have a lower respiratory quotient (RQ), reflecting a proportionally higher lipid oxidation rate (Fig. 1B). FGF19 further reduced this value in the HFD mice (Fig. 1B). FGF19 did not increase food intake on either diet (data not shown but see below). Vehicle-treated mice gained weight over the 7 d of treatment, whereas the FGF19-treated mice lost weight (Fig. 1C).

The FVB HFD mice diet became diabetic as defined by glucose levels greater than 200 mg/dl, measured 2 h after a standard GTT (Fig. 1D). The glucose excursion (Fig. 1D) in the FGF19-treated mice was reduced and not distinguishable from vehicle-treated chow-fed mice (data not shown). Delivery of FGF19 every second day produced an effect on glucose comparable with that of daily injections (Fig. 1D). FGF19 reduced leptin (consistent with reduced adiposity), insulin (consistent with increased insulin sensitivity), and serum triglycerides and cholesterol (Fig. 1E).

FGF19 reduced body weight and reversed diabetes in leptin-deficient mice

We crossed the FGF19 transgene onto the C57BL/6 lepob/lepob (ob/ob) background and monitored body weight and fed glucose levels. The transgenic ob/ob mice weighed significantly less than the nontransgenic ob/ob mice but were still heavier than the lean controls (Fig. 2A). At 20 wk of age, the fat pads (Fig. 2B) of the transgenic ob/ob mice were

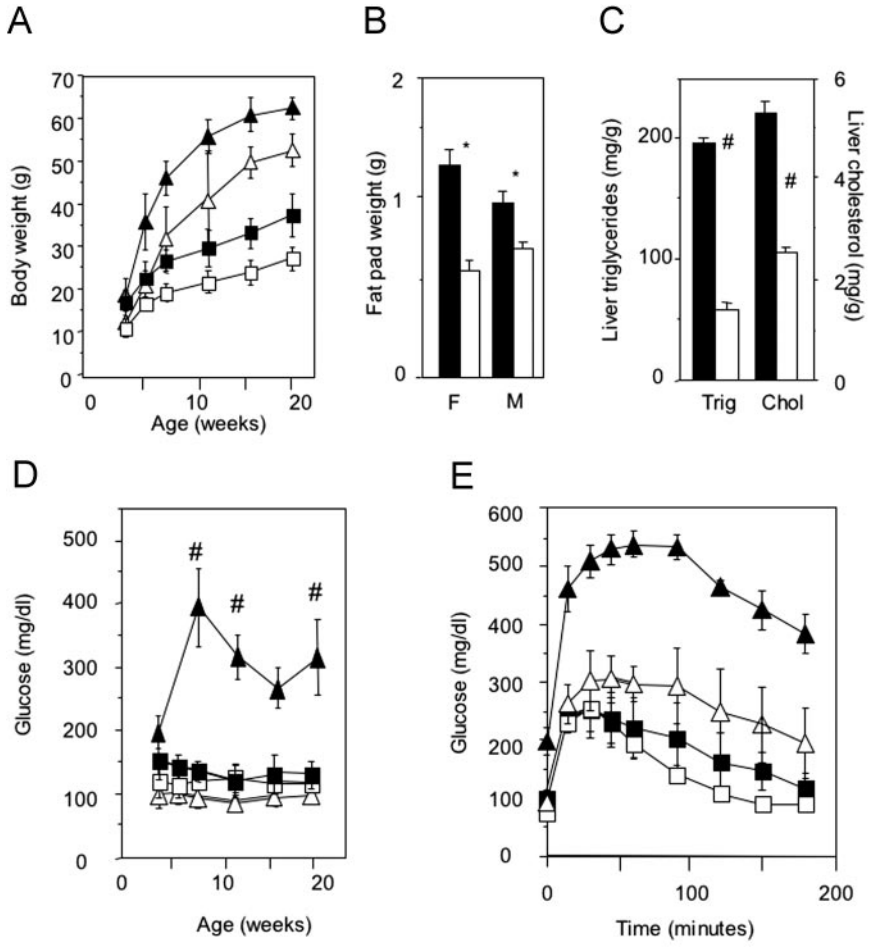

FIG. 2. Transgenic expression of FGF19 in ob/ob mice decreases body weight and fat content and improves glucose homeostasis. A, Transgenic expression of FGF19 reduced weight gain in ob/ob mice. ■, Control lean (Co, ?/+); □, transgenic FGF19 lean (FGF19, ?/+); ▲, control obese (Co, ob/ob); △, transgenic FGF19 obese (FGF19, ob/ob). Males, n = 6–42/group. Significant differences exist among all groups, $P < 0.05$, at wk 8, 12, 16, and 20 (Tukey-Kramer test). B, Transgenic expression of FGF19 reduced perirenal fat pad weights in male (M) and female (F) obese (ob/ob) mice. ■, (Co, ob/ob); □, (FGF19, ob/ob); n = 7–10/group. *, $P < 0.05$. C, FGF19 reduced liver triglycerides (Trig) and cholesterol (Chol). Males, n = 6/group. Symbols as in B. #, $P < 0.01$. D, Transgenic expression of FGF19 prevented the development of diabetes in obese mice. Males, n = 6–42/group. The control obese mice possess significantly higher glucose levels than all other groups ($P < 0.05$, Tukey-Kramer test) at 6, 8, 12, and 20 wk. The other three groups are not significantly different from each other. Symbols as in A. E, Transgenic expression of FGF19 reduced the glucose excursion after a GTT in ob/ob mice (12 wk of age). Males, n = 5–7/group. Glucose in the control obese group is significantly different from the other three groups at all time points ($P < 0.05$, Tukey-Kramer test). The other three groups are not significantly different from each other. Symbols as in A.

significantly smaller than those of the nontransgenic ob/ob mice. Liver cholesterol and triglycerides were also decreased (Fig. 2C).

Fed ob/ob mice are diabetic (nonfasting glucose in excess of 200 mg/dl), although if they are deprived of food, there will be a rapid fall in serum glucose. Under both fed and fasted regimens, ob/ob mice will be glucose intolerant as measured by a GTT. In contrast to the nontransgenic ob/ob mice, the FGF19 transgenic ob/ob mice did not become diabetic up to the termination of the experiment at 20 wk (Fig. 2D). The glucose excursion curve after a GTT performed at 12 wk was significantly ($P < 0.05$) reduced in the FGF19 transgenic ob/ob mice, compared with nontransgenic ob/ob mice at all time points (Fig. 2E) and was not statistically different from the lean (?/+) mice.

Treatment of obese, diabetic, leptin-deficient mice with recombinant FGF19 for 7 d reduced weight gain to a level that was not significantly different from that seen in untreated lean ?/+ mice (Fig. 3A). Glucose levels were reduced in the obese FGF19-treated mice and became indistinguishable from the vehicle-treated lean mice (Fig. 3B). After 7 d, the FGF19-treated mice had a marked reduction in the glucose excursion during a GTT and, as for the random fed glucose values, this was not measurably different from the vehicle-treated lean mice.

Physiological and molecular mechanisms of FGF19 action

FGF19 leads to weight loss and a reduction in glucose in obese, diabetic mice. The weight loss could result from a reduction in food intake. We have previously shown that FGF19 transgenic mice actually have the opposite response: they eat more than their transgenic littermates [although this may not be a direct effect of FGF19 (16)]. In mice treated with recombinant FGF19 the difference in food intake was not statistically different between the two groups [3.56 ± 0.16 g/d (vehicle) *vs.* 4.02 ± 0.23 g/d (FGF19)]. Because we have shown directly that FGF19 increased metabolic rate and, at least in the mice on HFD, a decreased RQ, it appears that these may be the primary mechanisms underlying the physiological effects. We have also begun an exploration of how FGF19 might alter metabolic rate and RQ.

Adiponectin is a recently described hormone that increases with increasing fat mass (32) and that markedly increases insulin sensitivity in diabetic mice, perhaps by decreasing glucose production (33, 34). Accordingly, we measured changes in the expression of adiponectin. Treatment of FVB mice with FGF19 did not significantly change serum adiponectin [10.10 ± 0.52 μg/ml (vehicle); 9.64 ± 0.3 μg/ml (FGF19)]. We did measure a significant increase in adiponectin levels in the FGF19 transgenic mice (12.07 ± 0.54 μg/ml, $P = 0.02$) although this may a long-term compen-

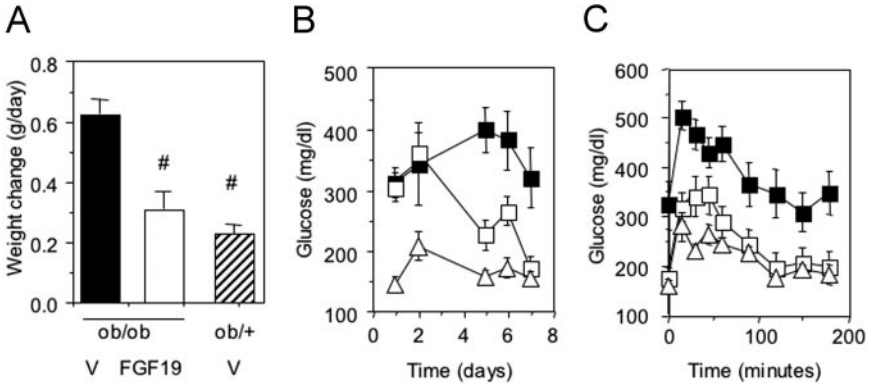

FIG. 3. Recombinant FGF19 decreased body weight and improved glucose homeostasis in obese, leptin-deficient mice. A, Recombinant FGF19 reduced body weight gain in obese (ob/ob) mice. ■, Obese (ob/ob), vehicle; □, obese (ob/ob), FGF19 (1 mg/kg·d); the cross-hatched bar represents vehicle-treated lean (?/+) mice. The ob/ob vehicle group is significantly different from the other two groups ($P < 0.01$, Dunnett's test). B, Recombinant FGF19 normalized glucose levels in the obese diabetic mice. ■, Obese (ob/ob), vehicle; □, obese (ob/ob), FGF19; △, lean (ob/+), vehicle. The ob/ob vehicle group is significantly different from ob/ob FGF19 on d 5–7 ($P < 0.05$. Dunnett's test). C, FGF19 normalized glucose excursion curves after a GTT. Symbols as in B. A–C, Males, n = 5–14/group. The ob/ob vehicle group is significantly different from ob/ob FGF19 at all time points except 90 and 150 min ($P < 0.05$, Tukey-Kramer test). Ob/ob FGF19 is not different from ob/? vehicle except at 15 and 30 min.

satory change produced by the marked reduction in adipose tissue in the transgenic mice.

Leptin also increases metabolic rate. This hormone acts primarily through the central nervous system (CNS) (35) and activates the BAT (36). Although leptin decreases with FGF19 treatment (Fig. 1), we investigated whether FGF19 might impact leptin-related thermogenic pathways. Thus, we asked whether FGF19 delivered centrally would increase metabolic rate and whether the BAT is implicated in the effects of FGF19. FGF19 injected into the lateral ventricle (intracerebroventricular (icv injections) produced an increase in metabolic rate comparable, in terms of time of onset and magnitude, with the mice in which FGF19 was delivered systemically (Fig. 4A). In these experiments, the amount of FGF19 injected was 6-fold less (5 *vs.* 30 μg) than the amount injected in the mice receiving iv injections of FGF19. At lower doses (0.5 μg/mouse per day) that have no measurable response when given systemically (data not shown), the nighttime metabolic rate on d 5 was 118 ± 3.6% of baseline, compared with 99 ± 4.2% for the vehicle-injected mice ($P < 0.05$).

The time required for a detectable effect of FGF19 (Figs. 1A and 4A) is consistent with FGF19 acting through gene expression changes rather than a rapid hormonal effect. Thus, we first determined whether FGF19 led to gene expression changes in the BAT (Table 2). Transgenic expression of FGF19 led to significant increases in the expression of genes that control glycolysis (*e.g.* phosphofructokinase), fatty acid metabolism (*e.g.* citrate lyase, stearoyl CoA desaturase 2), and mitochondrial function (*e.g.* PGC1). This pattern of gene expression changes is similar to that seen in cold acclimated mice (37) and indicated that FGF19 increased BAT activity.

To determine whether BAT activation is required for the effects of FGF19, the FGF19 transgenic mice were backcrossed to UCP-DTA transgenic mice (25). The UCP-DTA mice have a marked reduction in BAT and become obese and hyperglycemic. In contrast to the control UCP-DTA mice, the doubly transgenic mice (FGF19 and UCP-DTA) did not become obese (Fig. 4B) and had normal-fed glucose levels (Fig. 4C). Thus, it appears that BAT is not essential for FGF19 to produce decreased adiposity and improved glucose homeostasis. We also examined whether acute exposure to FGF19 changes BAT gene expression. Although the expression of both citrate lyase and squalene epoxidase (SE) was elevated in the transgenic mice (Table 2 and Fig. 4D), the expression of these two genes did not change in response to five daily injections of FGF19 (Fig. 4D), and there was no significant change in BAT weight (data not shown).

Because BAT appeared to be dispensable for the FGF19-induced increase in metabolic rate, we investigated the liver as a target organ. Although we recently reported that transgenic mice (exposed to high levels of FGF19 throughout development and adult life) develop hepatic adenocarcinomas (38), the livers of FGF19 transgenic mice are normal in size: 4.6 ± 0.07 g (vehicle) *vs.* 4.6 ± 1.6 g (FGF19). Affymetrix gene expression analysis of the livers of transgenic mice demonstrated that the expression of several classes of genes was altered (Table 2). First, genes involved in cholesterol metabolism were either decreased (cyp7a, cyb7b1, CD36, Ileal Na-dependent bile acid transporter, SREBP1) or increased (stearoyl CoA desaturase 2, SE). A full description of the impact of FGF19 on cholesterol metabolism is being prepared. There are also multiple changes involving genes that encode steroid hormone-modifying enzymes. The possibility that these changes could contribute to the metabolic changes elicited by FGF19 will be discussed below.

The array analysis revealed increased liver expression of the leptin receptor (39). This result was confirmed using RT-PCR in FVB (HFD) mice that had been injected with recombinant FGF19 (Fig. 5C). RT-PCR, using primers and probes that detect a region of the mRNA that is common to the short and long forms of the leptin receptor (40), demonstrated that it is an acute response (Fig. 5C). Use of alternative primers and probes also demonstrated a specific induction of the long form of the receptor (Fig. 5C). We also found that FGF19 suppressed stearoyl CoA desaturase (SCD)1 expression (Table 2). SCD1 is a target gene for leptin and mediates, by an unknown mechanism, some of the effects of leptin (41). SCD1 expression does not

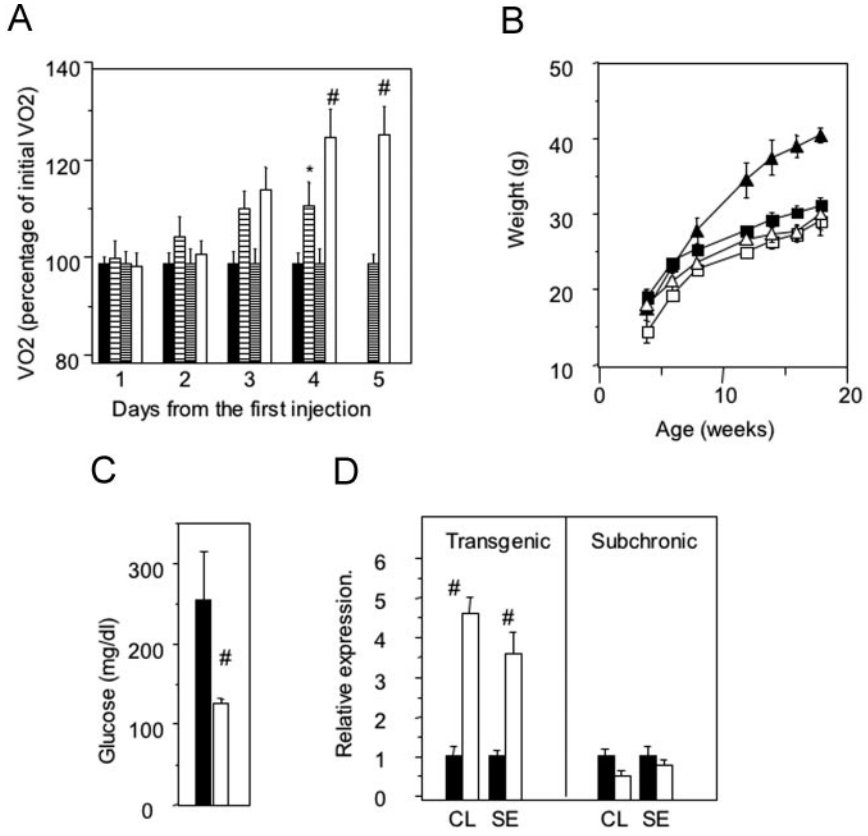

FIG. 4. CNS-delivered FGF19 can increase metabolic rate; BAT is not essential for the effects of FGF19. A, FGF19 (5 μg) injected via cannula into the lateral ventricle increased metabolic rate (VO_2, oxygen consumption). The data are presented as a percentage of the metabolic rate determined on the day (night) before the first injection. ■, Vehicle, day; ▤, FGF19, day; ▤, vehicle, night; □ FGF19, night. Males, n = 8/group. VO_2 in the FGF19 group is significantly higher than for vehicle-injected mice during the fourth and fifth dark periods after the first injection [($P < 0.001$) and the fourth light period after the first injection ($P < 0.05$)]. B, Transgenic expression of FGF19 reduced body weight in mice with diminished BAT (UCP-DTA). Mice are either transgenic or nontransgenic for FGF19, and either lean (nontransgenic for UCP-DTA) or obese, BAT deficient (transgenic for UCP-DTA). ■, Nontransgenic/lean; □, FGF19 transgenic/lean; ▲, nontransgenic/obese (UCP-DTA); △, FGF19 transgenic/obese (UCP-DTA). Males, n = 4–17/group. The difference in body weights between the UCP-DTA nontransgenic mice and the other three groups reaches and maintains statistical significance at 12 wk and onward ($P < 0.05$, Tukey-Kramer test). C, FGF19 prevents the development of diabetes in the UCP-DTA mice. ■, Control, UCP-DTA; □, transgenic FGF19, UCP-DTA. Males, n = 4–6/group. D, Transgenic expression of FGF19 (transgenic) but not five daily injections of recombinant FGF19 (subchronic) increased BAT levels of the mRNA encoding citrate lyase and SE. *Left panel*, ■, control; □, transgenic FGF19. *Right panel*, FVB (HFD) mice injected daily for 5 d with: ■, vehicle; or □, recombinant FGF19. Males, n = 6/group. D, E, *, $P < 0.05$; #, $P < 0.01$.

TABLE 1. Metabolic rate values in mice treated for 6 d with FGF19

	VO_2 (liters 0_2/h/kg$^{0.75}$)				Energy expenditure (KJ/h)			
	HFD		Chow		HFD		Chow	
	Dark	Light	Dark	Light	Dark	Light	Dark	Light
Vehicle	1.53 ± 0.06	1.18 ± 0.07	2.10 ± 0.07	1.86 ± 0.07	2.30 ± 0.08	1.76 ± 0.13	2.89 ± 0.08	2.47 ± 0.08
FGF19	1.93 ± 0.05	1.49 ± 0.07	2.61 ± 0.06	2.04 ± 0.05	2.89 ± 0.04	2.22 ± 0.13	3.56 ± 0.13	2.72 ± 0.08
P	a	a	a	NS	a	a	a	NS

Data derived from the experiment described in Fig. 1A. a, $P < 0.001$, Student's *t* test.

measurably change in response to acute treatment with FGF19 but does decrease after five daily injections (Fig. 5C).

We measured the expression of other candidate genes by RT-PCR. The expression of ACC2 was acutely decreased by FGF19 in FVB(HFD) mice and leptin-deficient ob/ob mice treated for 5 d (Fig. 5C). We also noted that ob/ob mice have higher ACC2 expression, compared with lean mice, and that FGF19 returned ACC2 expression in ob/ob mice to a level that was not significantly different from the vehicle-treated lean mice (data not shown). CNS delivery of FGF19 also led to a decrease in liver expression of ACC2 (Fig. 5C). A decrease in liver ACC2 would be expected to result in an increase in liver lipid oxidation, and, as noted above, mice treated with FGF19 have a reduced RQ and lower serum triglyceride. To further explore increased lipid oxidation (Fig. 1B) as a mechanism underlying the effects of FGF19, chow-fed FVB mice were treated with FGF19, injected with a lipid emulsion, and the time-dependent changes in FFAs determined. The FFA excursion in the FGF19-treated mice is

TABLE 2. Changes in BAT and liver mRNA transcripts in FGF19 transgenic mice as compared to nontransgenic siblings

Expression relative to control	BAT (Low fat)	Liver (Low fat)	Liver (High fat)
<0.1	Neuronatin	Aquaporin 8; est mv49d06.r1; Alpha-1-antitrypsin;	Aquaporin 8, est mv49d06.r1
0.1 to 0.4	RGS-2, prostoglandin D synthetase, G0/G1 switch regulatory protein, leptin, kallikrein-binding protein, I-Aβ, Na^+ channel β1, tropomyosin, H-FABP, Apo-E, troponin 1, MUC18, β-enolase, LMP-7k	G0S2, CYP7a1, CYP4A14 est mw79h01.r1, CD10 neutral endopeptidase, glucokinase, aldehyde dehydrogenase Ahd-2, CD36, Gas2, est ms56b05.r1 procathepsin E, Glut2, Pw1, LIM REC2, acetyl CoA synthetase	Cyp4a14, CYP7a1, α-1-antitrypsin, est mw79h01.r1, CD10 neutral endopeptidase, glucokinase, aldehyde dehydrogenase Ahd-2, CD36, Gas2, est ms56b05.r1, Ileal Na-dependent bile acid transporter, LIM protein, aquaporin 4, est mq81e04.r1, SGK kinase, stearoyl CoA desaturase 1, transcription factor S-II, MHC Q1b, CYP7b1, SREBP1
2.5 to 5	11β-Hydroxysteroid dehydrogenase, farnesyl pyrophosphate synthetase, keratinocyte lipid binding protein, acetyl CoA synthetase, estradiol 17β-dehydrogenase, 6-phosphofructokinase, citrate lyase	3-Ketosteroid reductase (Hsd3b), IL-1rn antagonist, IGF binding protein-1; zif/268, squalene epoxidase, est mu56h09.r1, KC, est vc48e03.r1, GADD45, α-amylase, testosterone 16-α-hydroxylase, purine nucleotide binding protein, stearoyl CoA desaturase 2, prominin, Δ-5-3-β hydroxysteroid dehydrogenase/Δ-5-> Δ-4 isomerase, vas deferens androgen related protein (MVDP)	Id1, serum amyloid SAA3, IL-1m antagonist, leptin receptor, 24P3, IGF binding protein-1, zif/268, Est mz99h12.r1, est vj03d05.r1, leukocyte protease inhibitor, RGS-r, HMG CoA reductase, fructose bisphosphatase, aldolase, metalloelastase
>5	Squalene epoxidase, stearoyl CoA desaturase 2, CIG30, Glut 5	17-α Hydroxylase/C17–20 lyase, 24P3, leptin receptor, Est mw30e02.r1, vas deferens androgen-related protein (MVDP), alpha fetoprotein	17-α Hydroxylase/C17–20 lyase, squalene epoxidase, BTG3

The expression of the indicated genes was lower (<0.1 and 0.1–0.4) or higher (2–5 and >5) in the BAT or liver of FGF19 transgenic mice as compared to nontransgenic littermate controls.

reduced, compared with the mice treated with vehicle (Fig. 5A). FGF19-treated mice [both FVB (HFD) and ob/ob] also have decreased liver triglycerides (Fig. 5B).

Discussion

Treatment of mice with FGF19 increased metabolic rate, decreased weight and lipids, and improved glucose homeostasis. It is likely that the latter effects are a secondary response to the increase in metabolic rate. With acute (1 d) or subchronic (7 d) exposure to FGF19, there was no measurable change in food intake, so this appears not to be the cause of the weight loss and lower serum insulin and glucose. It might have been expected that the significant increase in metabolic rate and energy expenditure produced by FGF19 would have led to a compensatory increase in food intake. This was found to be the case in FGF19 transgenic mice (16) but not in the 7-d experiments reported here. In the subchronic 7-d experiments, the drive to increase food intake may be balanced by a decrease in leptin or insulin.

Both peripheral and CNS administration of FGF19 increased the metabolic rate of HFD mice. It is possible that central delivery can lead to systemic exposure. However, because the dose that produces a significant effect when given via the CNS (0.5 μg) had a negligible effect when given peripherally, it appears unlikely that spillover could account for this result. Whereas the hypothalamus is an obvious candidate for mediating the central effects of FGF19, proteins injected into the lateral ventricle can also reach other brain regions, and it is possible that FGF19 impacts these structures. Whether FGF19 alters the activity of the neural network that regulates appetite and thermogenesis is being investigated.

Although it is unlikely that the amount of protein used in the central delivery of FGF19 produced significant systemic levels of the protein, the converse cannot be ruled out. Similar questions regarding central *vs.* peripheral effects of leptin have also been a challenge to unravel. The rapid gene expression response in the liver to peripheral delivery of FGF19 is consistent with a direct effect on the liver. However, it is also possible that systemic exposure to FGF19 leads to a CNS response that is then rapidly transmitted to the liver via neural or endocrine pathways. Resolving these issues will require a more detailed knowledge of the receptor(s) involved in FGF19 signaling. Although FGFR4 is the only known receptor that can independently bind FGF19 in a cell-free system (21), mice that have the FGFR4 gene deleted are still sensitive to FGF19 (John, L. M., and T. A. Stewart, manuscript in preparation).

The FGF19-mediated increase in metabolic rate takes several days to become obvious (Figs. 1A and 4A). This is consistent with a change in gene expression being an important mechanism and is in contrast to the acute metabolic effects obtained with leptin (*e.g.* see Ref. 42). The gene expression changes that might contribute to the effects of FGF19 were investigated by a both a broad screen and a more targeted focus on particular genes. We believe that a key result is the finding that FGF19 produced a rapid (within 6 h) decrease in the liver expression of ACC2. We note that mice treated with

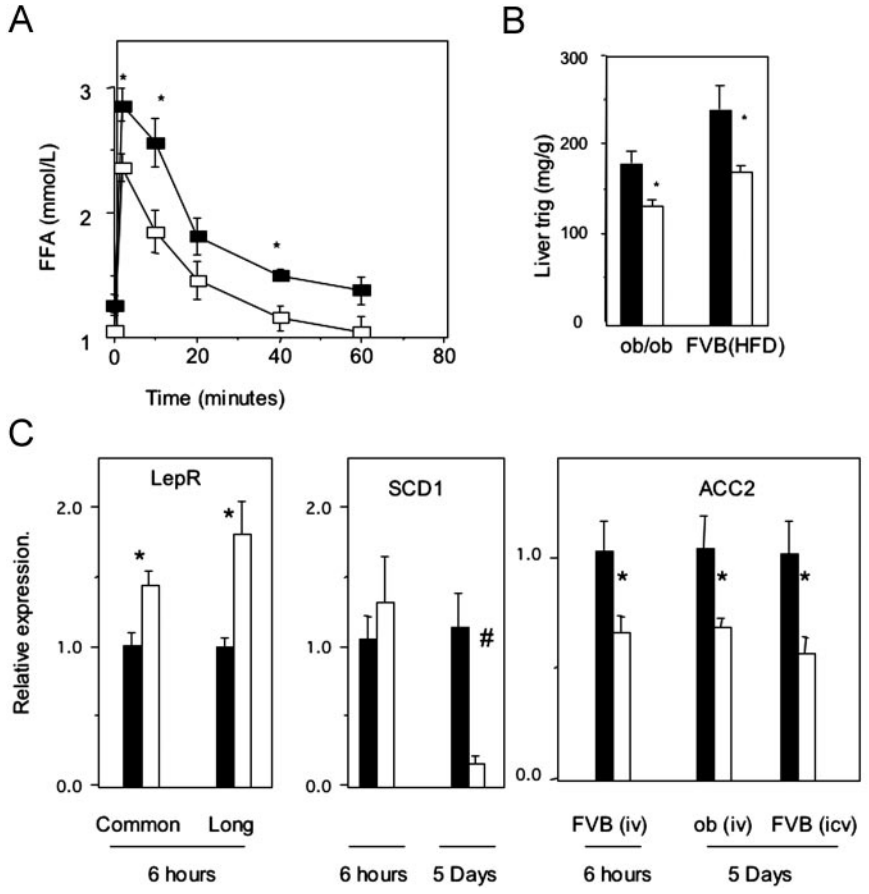

FIG. 5. FGF19-mediated increase in lipid oxidation is associated with decreased expression of ACC2. A, Recombinant FGF19 (1 mg/kg·d for 6 d) decreased FFA excursion after an iv injection of lipids; males, n = 6. ■; control; □, FGF19. B, Recombinant FGF19 decreased liver triglycerides in both obese (ob/ob) and FVB (HFD) mice. Symbols as in A. Males, n = 7/group. C, FGF19 induced expression of the leptin receptor and reduces expression of SCD1 and ACC2. *Left panel*, FGF19 induction of the common and long forms of the leptin receptor in FVB (HFD) mice within 6 h of an initial injection. *Center panel*, FGF19 suppressed SCD1 expression after five daily injections. *Right panel*, FGF19 suppressed ACC2 in FVB (HFD) mice (6 h after the first injection), ob/ob mice, and FVB mice in which FGF19 is delivered via an icv injection. Symbols as in A. A and B, n = 8/group. C, n = 4–6/group. A–C, *, $P < 0.05$; #, $P < 0.01$.

FGF19 have a metabolic profile similar to mice in which the gene for ACC2 has been deleted (43). These observations support a model in which FGF19 reduces expression of ACC2, and, as a direct consequence, carnitine palmitoyl transferase 1 activity and fatty acid oxidation are increased. This leads to depletion of internal fat stores, initially the local liver triglycerides, and then as a secondary consequence adipose tissue. An FGF19-mediated increase in liver fatty acid oxidation is consistent with the reduced RQ, FFA excursion after injection of a lipid emulsion, and liver triglycerides. A possible signaling route between the FGF19 receptor (in either the CNS or liver) and the ACC2 promoter will require further investigation. We also note that liver SCD1 mRNA levels are reduced in mice treated with FGF19. Whereas this does not appear to be an acute effect of FGF19 (Fig. 5), the observation that mice with diminished SCD1 are hypermetabolic (41) suggests that this phenomenon may also contribute to the physiological changes induced, directly or indirectly, by FGF19.

We also found that FGF19 increased the expression of the long form of the leptin receptor in the liver. However, mice with no liver leptin receptors are similar to normal mice, whereas the mice with no hypothalamic receptor are obese (44). Thus, whereas leptin acutely regulates the expression of several metabolically important genes in the liver (45), it is unlikely that all of the effects of FGF19 could be mediated through increased liver expression of the leptin receptor. Furthermore, at least some of the reported leptin-induced effects (*e.g.* Cyp7a) are in the opposite direction from the effects of FGF19 reported here.

The gene expression array experiment also revealed that FGF19 altered the expression of a set of genes implicated in cholesterol metabolism and another set involved in the modification of steroid hormones. We report here [and in the FGF19 transgenic mice (16)] that FGF19 lowers cholesterol. In contrast, FGF19 suppresses the expression of the gene encoding the principal catabolic enzyme (cyp7a) and increases the expression of SE, a key synthetic enzyme. Holt *et al.* (24) recently reported that the ability of the transcription factor FXR to suppress cyp7a may be dependent on a primary induction of FGF19. It is not clear how FGF19 can lower circulating cholesterol while simultaneously decreasing the expression of genes encoding catabolic enzymes and increasing the expression of genes encoding synthetic enzymes. One obvious possibility is that a very early substrate for cholesterol synthesis (*e.g.* ATP or acetyl coenzyme A) becomes rate limiting.

The effect on ACC2 provides an obvious and plausible mechanism for the metabolic changes produced by FGF19. However, FGF19 also leads to changes in the expression of genes encoding steroid hormone-modifying enzymes (Table 2). Thus, changes in steroid hormone activity may also contribute to the response to FGF19. That alterations in steroid hormone levels can profoundly impact metabolic control is most clearly seen in patients suffering from Cushing's syndrome. In these patients excessive glucocorticoids can lead to

obesity, insulin resistance, and diabetes. Similarly in rodents, at least some forms of obesity and diabetes can be prevented by adrenalectomy (46, 47). Although the glucocorticoids are the most obvious candidate steroid hormones for altering metabolism, there are others. For example, we note that the expression of 3-ketosteroid reductase is decreased in the livers of FGF19 transgenic mice. The enzyme encoded by this gene functions as a 3-ketosteroid reductase converting an active androgen, dihydrotestosterone, into an inactive androgen, 5α-androstane-3 β,17 β-diol. In mice at least, obesity and diabetes are sex linked (48), with males being more sensitive to the effects of the diabetes mutation than females. We note, however, that the FGF19 males are fertile, suggesting that there are, at most, only small systemic changes produced by the decrease in expression of this gene. One other interesting possibility arises from the increased expression of Δ-5–3-β-hydroxysteroid dehydrogenase/Δ-5-> Δ-4 isomerase and 17-α hydroxylase/C17–20 lyase, the combined effect of which could lead to an increase in the synthesis of dehydroepiandrosterone. Dehydroepiandrosterone has been reported to have both antihyperglycemic and antiobesity effects in mice, although the specific results obtained are dependent on the genetic background of the mice being treated (49, 50). Whether, and how, the changes in the expression of steroid hormone-modifying enzymes may participate in mediating the metabolic effects of FGF19 will require further study.

The BAT is an important organ for regulating rodent metabolic rate (25) and is critical for many leptin effects (51, 52). Although the BAT from the transgenic FGF19 is clearly activated (*e.g.*, see the increase in CIG30, Table 2), this tissue appears to be dispensable for the effects of FGF19. Adiponectin (also known as APMI and ACRP30) is a recently described hormone produced primarily by adipose tissue (53, 54) that increases insulin sensitivity and decreases glucose (34). It is unlikely that the effects of FGF19 are mediated by an increase in adiponectin. Not only did we not detect any increase in serum levels of this hormone, but also in humans there appears to be no relationship between adiponectin and either RQ or metabolic rate (55).

We do not yet know whether the mouse homologue of FGF19 is a normal component of metabolic control. That this may be the case is supported by the following observations. First, the amount of FGF19 injected into the mice in the studies reported here is comparable with the amount of other hormones used in rodent studies (*e.g.* see Ref. 56 for the doses of ghrelin and GH used to alter the metabolic profile of mice). In addition, a normal role for the FGFs in the maintenance of physiological homeostasis is directly supported by human and mouse studies. Injection of recombinant FGF23 into mice reduces serum phosphate (20), and mutations in the coding region cause rickets. Furthermore, mice with a deficiency in FGFR4 have altered cholesterol metabolism (57). We note that the increased expression of cyp7a in the FGFR4 knockout mice is reflected by the decreased expression of cyp7a subsequent to FGF19 treatment. Whereas there is some support, the mouse homologue of human FGF19 will need to be identified before a normal role for this protein in rodent energy metabolism can be investigated.

The increase in metabolic rate induced by FGF19 is most obvious in mice during the night when they feed and in HFD mice. These data are most readily incorporated into a model in which the FGF19-mediated increase in metabolic rate is dependent on another, as-yet-undefined metabolic or hormonal condition. Part of this condition may involve a ready source of fuels. That is, under conditions in which intracellular levels of metabolizable substrates are reduced [low-fat diet (Fig. 1), diminished food intake (daytime, Fig. 1A), and during fasting (16)], there is minimal effect of FGF19. However, the diminished response to FGF19 under conditions of fuel insufficiency is not absolute because transgenic mice on a low-fat diet have a significant increase in metabolic rate, and the CNS injections of FGF19 were performed in mice fed a chow diet.

The effect of FGF19 on metabolic rate and the subsequent decreased weight gain and improvement in glucose homeostasis seen in three different mouse models is striking. It will be of significant interest to determine whether these effects are also seen in other species.

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