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Fibroblast Growth Factor 21 Corrects Obesity in Mice

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Fibroblast growth factor 21 (FGF21) is a metabolic regulator that provides efficient and durable glycemic and lipid control in various animal models. However, its potential to treat obesity, a major health concern affecting over 30% of the population, has not been fully explored. Here we report that systemic administration of FGF21 for 2 wk in diet-induced obese and *ob/ob* mice lowered their mean body weight by 20% predominantly via a reduction in adiposity. Although no decrease in total caloric intake or effect on physical activity was observed, FGF21-treated animals exhibited increased energy expenditure, fat utilization, and lipid excretion, reduced hepatosteatosis, and ameliorated glycemia. Transcriptional and blood cytokine profiling studies revealed effects consistent with the ability of FGF21 to ameliorate insulin and leptin resistance, enhance fat oxidation and suppress *de novo* lipogenesis in liver as well as to activate futile cycling in adipose. Overall, these data suggest that FGF21 exhibits the therapeutic characteristics necessary for an effective treatment of obesity and fatty liver disease and provides novel insights into the metabolic determinants of these activities. (*Endocrinology* 149: 6018–6027, 2008)

BASED ON THE data obtained in a variety of animal models, fibroblast growth factor 21 (FGF21) (1) has recently emerged as an effective metabolic regulator of glucose and lipid homeostasis in the context of insulin resistance, glucose intolerance, and dyslipidemia (2–5). When tested in rodents and nonhuman primates, FGF21 provides sustained glucose and lipid control, amelioration of insulin resistance, improvements in β-cell function and mass, and beneficial changes in other lipoprotein and cardiovascular risk factor profiles. Of equal importance, these striking metabolic outcomes do not appear to coincide with adverse effects, such as hypoglycemia, mitogenicity, or increased adiposity (6). Limited prior evidence also suggests that FGF21 may have the potential to favorably impact obesity. Indeed, FGF21 transgenic mice are resistant to the weight and fat accumulation-promoting effects of a high-fat diet and age-related weight gain (2). Slight but significant weight reduction was also recently shown in a pharmacological study with FGF21 in diabetic nonhuman primates (7). Although the effects and underlying mechanisms behind glucose and lipid-lowering actions of FGF21 *in vivo* are now becoming better understood, there is a paucity of knowledge regarding its ability to regulate energy balance and body weight in the context of obesity or the underlying molecular mechanisms that could affect these potential attributes of FGF21.

Here we have addressed the hypothesis that exogenous FGF21 can mediate favorable effects on body adiposity in the context of established obesity and that it might do so via beneficial effects on energy metabolism. For this purpose, we studied diet-induced obese (DIO) mice, a specific animal model of obesity with nearly normal glucose and circulating lipids, and obese and overtly diabetic *ob/ob* mice. Systemic administration of FGF21 with a range of doses/delivery routes led to a profound dose-dependant weight loss, attenuated adiposity/water content, and improved hepatosteatosis in both of these animal models. We then explored the mechanisms of FGF21 action on energy balance via functional assays and at the molecular level by studying FGF21-induced changes in gene expression in various tissues and analyzing hormonal profiles in blood. This information provides novel mechanistic insights into FGF21 antiobesity and glucose/lipid-lowering effects. Taken together, our data suggest that FGF21 is a potent pharmacological agent that has the potential to effectively treat not only diabetes and dyslipidemia but obesity and fatty liver disease as well.

Materials and Methods

Animals

All experimental animal protocols in this study were approved by Eli Lilly and Co. Animal Use and Care Committee. DIO male C57/BL6 mice (Harlan, Indianapolis, IN) were maintained on a calorie-rich diet consisting of 40% fat, 39% carbohydrate, and 21% protein caloric content (TD95217; Harlan Teklad, Madison, WI) from weaning and had free access to food and water for at least 7 wk before randomization by weight and treatment. Male *ob/ob* mice (Harlan) were maintained on Purina 5001 chow with free access to food and water for a minimum of 2 wk before randomization by weight and treatment. Animals were individually housed in a temperature-controlled (24 C) facility with 12-h light, 12-h dark cycle (lights on at 2200 h). Body weights ranged from 34–40 g. Mice were treated with vehicle or various doses of FGF21, as indicated, via continuous sc infusion with miniosmotic pumps (Alzet, Cupertino, CA) or once-daily sc injections, as indicated. Food and body weights were recorded daily before dark photoperiod. Cumulative body weight change was calculated as the daily body weight minus body weight before treatment for each animal and presented as an average for

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Abbreviations: AGRP, Agouti-related peptide; aP2, adipogenic fatty acid-binding protein 2; BAT, brown adipose tissue; ChREBP, carbohydrate-responsive element-binding protein; CoA, coenzyme A; DGAT, diglyceride acyltransferase-1; DIO, diet-induced obese; FGF21, fibroblast growth factor 21; HNF4α, hepatocyte nuclear factor 4α; NPY, neuropeptide Y; PEPCK, phosphoenolpyruvate carboxykinase; PGC1α, PPARγ coactivator 1α; PPARγ, peroxisome proliferator-activated receptor γ; RQ, respiratory quotient; SCD1, stearoyl-CoA desaturase 1; UCP1, uncoupling protein 1; WAT, white adipose tissue.

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the group. Food intake was measured daily for each animal and combined with the total amount of food consumed by that animal from all previous days to yield cumulative food intake, which was then averaged for the group.

Protein

FGF21 was generated as described (2).

Analysis of metabolites and circulating factors

Glucose and plasma triglyceride levels were determined using Precision G Blood Glucose Testing System (Abbott Laboratories, Abbott Park, IL) and Hitachi 912 Clinical Chemistry analyzer (Roche Diagnostics, Indianapolis, IN), respectively. T_3, T_4, and TSH levels were measured by Ani Lytics Inc. (Gaithersburg, MD). Rodent multi-analyte profile (MAP) panel was performed by Rules Based Medicine (Austin, TX), and cytokines profiled were apolipoprotein A1, β2-microglobulin, calbindin, CD40, CD40 ligand, clusterin, C-reactive protein, cystatin-C, epidermal growth factor, endothelin1, eotaxin, factor VII, fibroblast growth factor (FGF)2, FGF9, fibrinogen, granulocyte-macrophage colony-stimulating factor, granulocyte chemotactic protein 2, GH, growth-regulated gene product α, glutathione S-transferase-α and -μ, haptoglobin, interferon-γ, IgA, IL-10, IL-11, IL-12p70, IL-17, IL-18, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, insulin, inducible protein 10, leptin, leukemia inhibitory factor, lymphotactin, monocyte chemoattractant protein 1 (MCP1), MCP3, MCP5, macrophage colony-stimulating factor, macrophage-derived chemokine, macrophage inflammatory protein 1α (MIP1α), MIP1β, MIP1γ, MIP2, MIP3β, matrix metalloprotease 9, myoglobin, neutrophil gelatinase-associated lipocalin, oncostatin M, osteopontin, RANTES (Regulated upon Activation, Normal T Expressed, and Presumably Secreted), stem cell factor, serum glutamic oxalacetic transaminase, tissue inhibitor of metalloproteinases 1, tissue factor, TNFα, thrombopoietin, vascular cell adhesion molecule 1, vascular endothelial growth factor, and von Willebrand factor.

Body composition analysis

Body composition of mice was determined using Quantitative Nuclear Magnetic Resonance analysis (ECHO MRI, 3-1 Composition Analyzer; Echo Medical Systems, Houston, TX) 1 d before initiation of treatment and on the last day of treatment. Fat-free mass was calculated by subtracting fat mass from total mass. Fat content of feces was determined using QNMR on the last day of treatment and calculated as fat mass normalized to total mass of feces (percent).

Energy homeostasis measurements

Indirect calorimeter chambers (OXYMAX; Columbus Instruments, Columbus, OH) were used to monitor various parameters of energy expenditure in treated animals over a 24-h period. Abdominally implanted telemetric temperature transmitters (model TA-F20; Data Science International, St. Paul, MN) were used to measure core body temperature every 5 min for 24 h after initiation of treatment. The beam breaker method was used to quantitate movement: ambulatory movement (new beam breaks per hour), fine movement (breaks within a beam per hour), and total movement (total beam breaks per hour).

RNA isolation, RT, and real-time quantitative PCR

RNA was isolated from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA) or by homogenization of frozen samples in Lysing Matrix D shaker tubes (MP Biomedicals, Santa Ana, CA) and was reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (PE Applied Biosystems, Foster City, CA). Reactions were performed in triplicate on an ABI Prism 7900HT (PE Applied Biosystems) and were normalized to either 36B4 mRNA or 18S rRNA. Results are expressed as fold induction relative to vehicle-treated samples. Primers and probes were from Biosearch Technologies Inc. (Novato, CA) or PE Applied Biosystems. Sequences for mouse 36B4 were forward primer 5′-GGCCCGAGAAGACCTCCTT-3′, reverse primer 5′-TCAATGGTGCCTCTGGAGATT-3′, and probe 5′-CCAGGCTTTGGGCATCACCACG-3′. Assays-on-Demand Gene Expression Products (PE Applied Biosystems) were as follows: ABCA1, Mm00442646_m1; ABCG5, Mm00446249_m1; ABCG8, Mm00445970_m1; ACC1, Mm01304285_m1; ACC2, Mm01204683_m1; AGRP, Mm00475829_g1; aP2, Mm00445880_m1; ApoA1, Mm00437569_m1; ApoA3, Mm00445670_m1; ATGL, Mm00503040_m1; βKlotho, Mm00473122_m1; CART, Mm00489086_m1; CCK, Mm00446170_m1; CD36, Mm00432403_m1; ChREBP, Mm00498811_m1; CIDEA, Mm00432554_m1; CPT1, Mm00550438_m1; CYP7A1, Mm00484152_m1; CYP8B1, Mm00501637_s1; D2, Mm00515664_m1; ERRα, Mm00433143_m1; FAS, Mm00433237_m1; FGF21, Mm00840165_g1; FoxA2, Mm00839704_mH; FXR, Mm00436419_m1; Glucagon, Mm00801712_m1; GPAT, Mm00833328_m1; HMGCS2, Mm00550050_m1; HMGR, Mm01282499_m1; HNF1α, Mm00493434_m1; HSL, Mm00495359_m1; INSIG1, Mm00463389_m1; INSIG2, Mm00460119_m1; Insulin, Mm01259683_g1; Insulin receptor, Mm00439693_m1; Leptin, Mm00434759_m1; Leptin receptor, Mm00440174_m1; LPAAT, Mm00479700_m1; LXR, Mm0443454_m1; NPY, Mm00445771_m1; PEPCK, Mm00440636_m1; PGC1α, Mm00447183_m1; POMC, Mm00445771_m1; PON1, Mm00599936_m1; PPARα, Mm00440939_m1; PPARγ, Mm00440945_m1; PPARδ, Mm00803186_g1; SCD1, Mm00772290_m1; SHP, Mm00442278_m1; SREBP1, Mm00550338_m1; UCP1, Mm01244860_m1; UCP2, Mm00495907_m1; and UCP3, Mm00494074_m1.

Statistical analysis

Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA, followed by Dunnett's multiple comparisons test. Significant differences of $P < 0.05$ are identified with an *asterisk* in the figures.

Results

FGF21 ameliorates obese phenotypes in DIO and ob/ob mice

To study potential antiobesity effects of exogenous recombinant human FGF21, we first administered the protein via continuous infusion with miniosmotic (Alzet) pumps to DIO mice for 2 wk. The daily doses of FGF21 of 0.1, 0.3, and 1 mg/kg attained steady-state circulating levels of 7.4 ± 2.9, 18.7 ± 5.2, and 42.7 ± 15.6 ng/ml, respectively. Compared with vehicle-treated animals, FGF21 led to a dose-responsive reduction in total body weight, reaching 20% at the highest used dose of FGF21 (Fig. 1A). Body/tissue composition measurements showed a profound FGF21-induced reduction of total body fat mass (Fig. 1B), liver weights (Fig. 1C), and liver fat content (Fig. 1D), whereas changes in total body fat-free mass were only minor, primarily due to reduction in water content (Fig. 1B). No significant modulation in total food consumption was observed in FGF21-treated DIO mice (Fig. 1E). However, when caloric intake was normalized to animal body weights, FGF21-treated animals consumed more food (Fig. 1F).

No significant changes in ambulatory, fine, or total movement were observed in treated DIO mice at any FGF21 dose, either during dark or light cycles (not shown). Although there was also no overt evidence of poor nutrient absorption in FGF21-treated mice, the fecal fat content in FGF21-dosed animals was on average modestly increased (30%) compared with vehicle-treated controls (Fig. 1G).

Consistent with previously published reports (2–7), FGF21 administration in DIO mice also led to blood glucose lowering. However, in contrast to body weight reduction which was dose dependent, the full glycemic effect was already observed at the lowest tested dose of FGF21 (Fig. 1H). No significant decrease in plasma triglycerides was detected in DIO mice (not shown).

FGF21 also induced dose-dependent weight loss in DIO mice when administered via once daily sc injections. In this

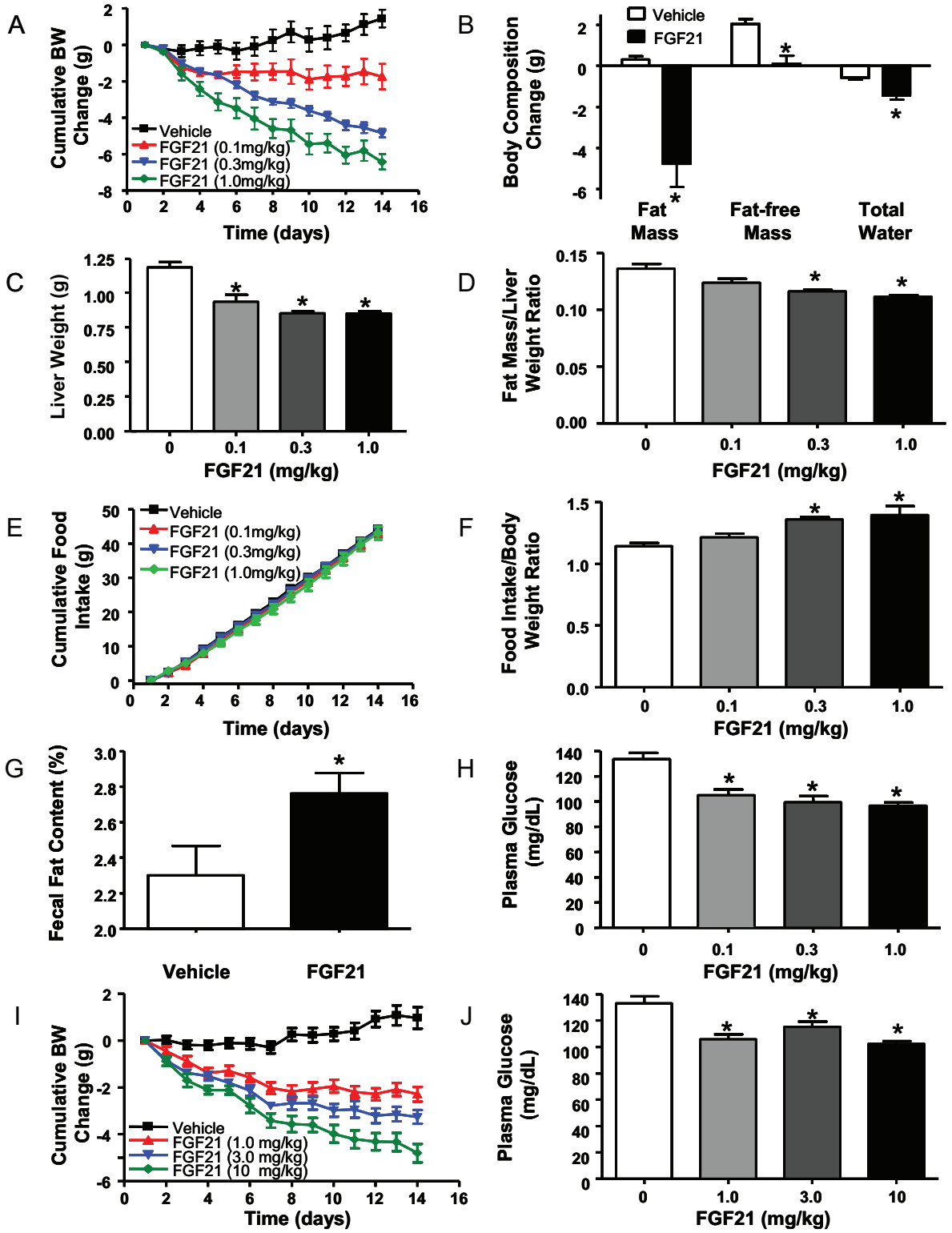

FIG. 1. FGF21 reverses diet-induced obesity and hyperglycemia. DIO mice received vehicle or various doses of FGF21 (as indicated) via continuous sc (A–H) or bolus daily administration (I–J). Body weight and food intake were measured daily; total body, liver and fecal composition, liver weight, and plasma glucose were measured on d 14. Data represent means ± SEM (seven to eight animals per group). All statistical comparisons were done by one-way ANOVA followed by Dunnett's multiple-comparison test. *, $P < 0.05$ compared with vehicle. A, Cumulative change in body weight (BW); B, change in body composition, with data from 1 mg/kg · d FGF21 dose group shown; C, change in liver weight; D, change in liver fat mass adjusted to liver weight; E, cumulative food intake; F, food intake on d 14 adjusted to body weight; G, change in fat content of feces, with data from 1 mg/kg · d FGF21 dose group shown; H, change in plasma glucose levels; I, cumulative change in body weight (BW); J, change in plasma glucose levels.

case, however, approximately a 10-fold greater dose of FGF21 was required to achieve an equivalent weight reduction compared with FGF21 administration via Alzet pumps (Fig. 1, A *vs.* I). Nevertheless, complete glucose lowering was again achieved with the lowest employed 1 mg/kg daily sc dose of FGF21 (Fig. 1J).

Finally, FGF21 also exerted antiobesity effects in leptin-deficient *ob/ob* mice. When a dose of 1 mg/kg · d was delivered via continuous infusion, animals lost approximately 3 g of their body weight (Fig. 2A). As in DIO mice, this effect was primarily due to a reduction of total fat mass and water (Fig. 2B). Decreases in liver weights mirrored reductions in liver fat content (Fig. 2, C and D), and no modulation of total caloric intake was detected (Fig. 2E).

FGF21 induces energy expenditure

Given that we observed no appreciable attenuation of total caloric intake or physical activity, and only modest increases in fecal fat content, we speculated that FGF21 might enhance

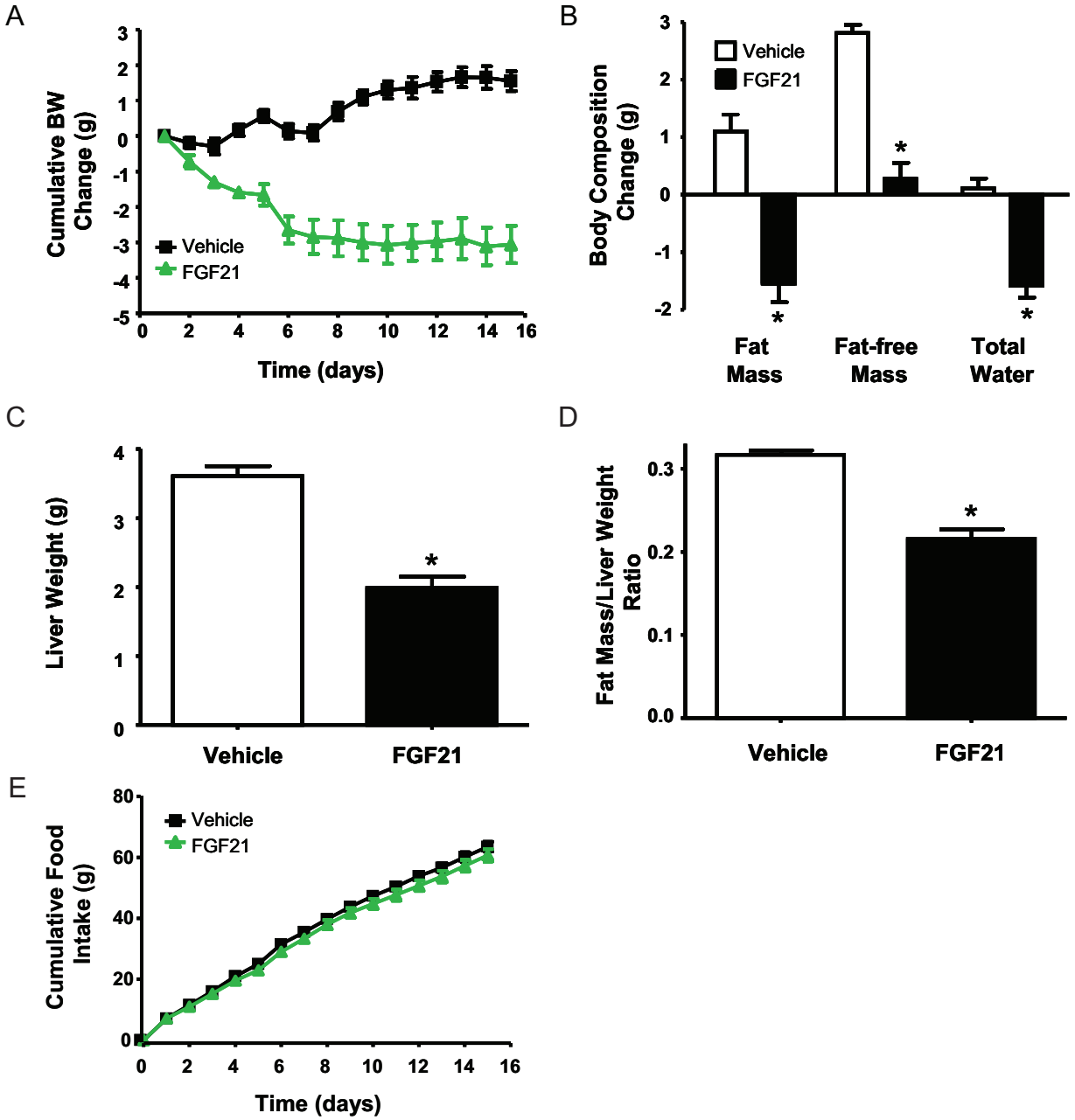

FIG. 2. FGF21 metabolic effects in *ob/ob* mice. The *ob/ob* mice received vehicle or 1 mg/kg · d FGF21 via continuous sc administration. Body weight and food intake were measured daily; total body and liver composition and liver weight were measured on d 15. Data represent means ± SEM (10 animals per group). All statistical comparisons were done by one-way ANOVA followed by Dunnett's multiple-comparison test. *, $P <$ 0.05 compared with vehicle. A, Cumulative change in body weight (BW); B, change in body composition; C, change in liver weight; D, change in liver fat mass adjusted to liver weight; E, cumulative food intake.

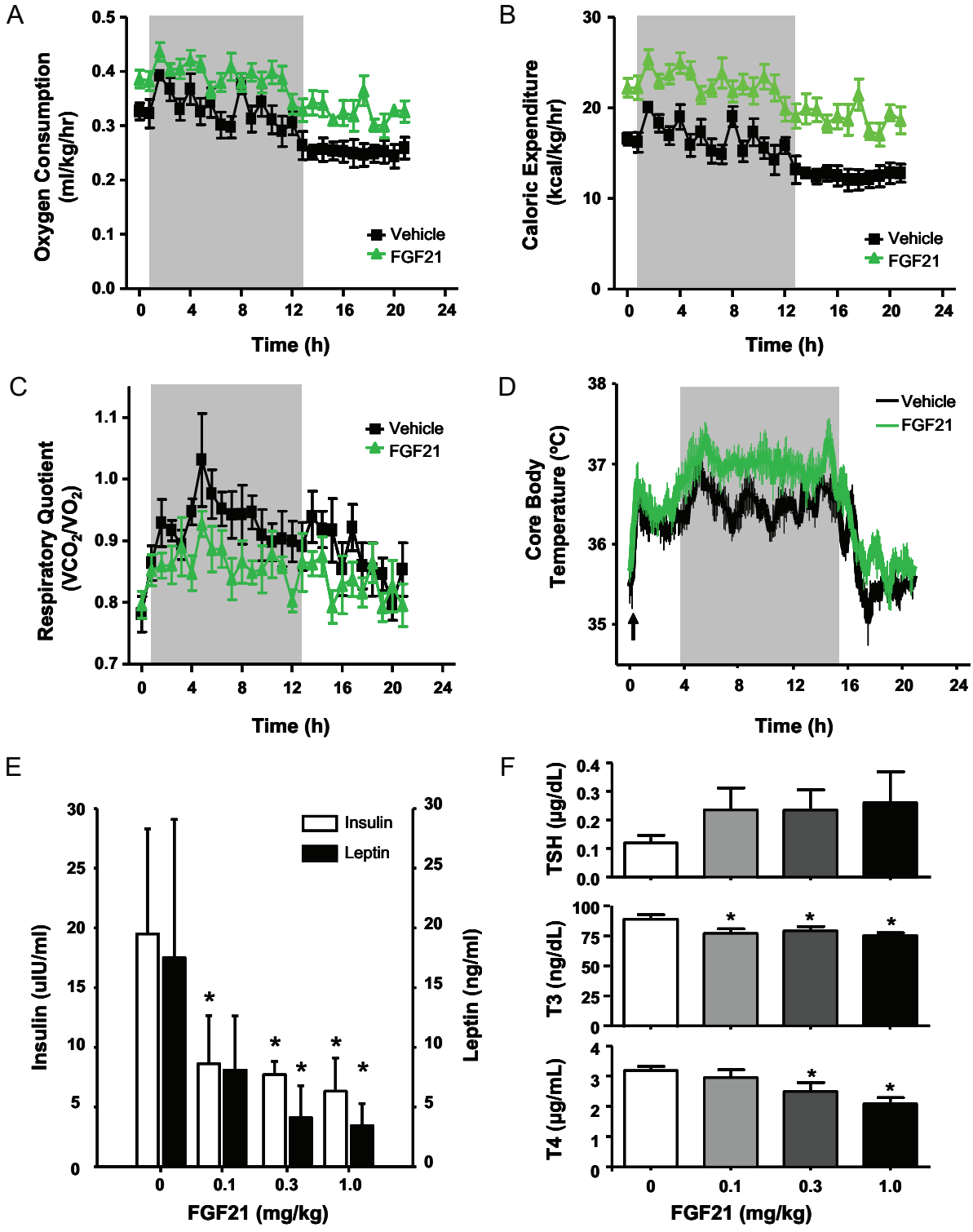

FIG. 3. FGF21 modulates energy metabolism and hormonal profiles in DIO mice. DIO mice received vehicle, various doses of FGF21 (as indicated) via continuous sc administration (A–C, E, and F), or one bolus sc injection (as indicated by *arrow*) of vehicle or FGF21 (10 mg/kg) for core body temperature experiment (D). On d 7, various parameters of energy expenditure were measured (A–C, only 1 mg/kg · d dose is shown). On d 14, cytokine and thyroid hormone profiles in blood were measured (E and F). Data represent means ± SEM (eight animals per group). All statistical comparisons were by one-way ANOVA followed by Dunnett's multiple-comparison test. *, $P < 0.05$ compared with vehicle. A, Oxygen consumption; B, caloric expenditure; C, RQ; D, core body temperature; E, insulin and leptin levels in blood; F, thyroid hormone levels in blood.

resting energy expenditure. Indeed, as measured by indirect calorimetry, animals that were continuously infused with FGF21 (1 mg/kg · d) for 5 d consumed more oxygen (Fig. 3A), had a significantly higher energy expenditure rate (Fig. 3B) during both light and dark cycles, and a had lower respiratory quotient (RQ) in the dark cycle (Fig. 3C) as well as a small but significant elevation of core body temperature beginning 4 h after initiation of treatment and lasting for approximately 10 h (Fig. 3D), compared with the vehicle-treated mice.

FGF21 alters hormonal profiles in blood

FGF21-induced changes in the levels of polypeptide factors in mouse blood were examined using a mouse multianalyte profiles kit (Rules-Based-Medicine). Only insulin and leptin were found to be lowered in DIO mice (Fig. 3E), whereas no significant changes in levels of 65 other circulating polypeptides examined, including GH, were observed (not shown).

FGF21 triggers gene expression changes

Because FGF21 is believed to exert its net effects through regulation of gene transcription (2), we examined gene expression changes in tissues from control and FGF21-dosed DIO mice after 2 wk administration. Animals were not fasted at the time of tissue collection, and expression was measured using quantitative PCR. We focused this line of research on liver, fat, and pancreas, which represent primary target tissues for FGF21 action because they express βKlotho (8) and various FGF receptors (9), the required components of FGF21 receptor machinery (10–12), and respond to FGF21 stimulation in a direct way (2, 3, 5, 10, 13, 14). Expression profiles of genes involved in the regulation of lipid and glucose metabolism as well as selected genes that have been implicated as part of the FGF21 signaling pathway were profoundly altered in all these tissues. We also profiled gene changes in hypothalamus because FGF21-dosed animals appeared to be mildly hyperphagic (Fig. 1F).

In liver (Table 1), the transcripts for peroxisome proliferator-activated receptor γ (PPARγ) and PPARα were reduced, whereas hepatocyte nuclear factor 4α (HNF4α) expression was increased at each dose of FGF21. In contrast, the mRNA levels of other profiled nuclear hormone receptors, HNF1α, PPARδ, liver X receptor (LXR), farnesoid X receptor (FXR), and estrogen-related receptor α (ERRα) were not changed.

FGF21 administration led to a substantial reduction in mRNAs encoding acetyl-coenzyme A (CoA) carboxylases 1 (ACC1) and ACC2 as well as the transcript levels for several lipogenic genes such as stearoyl-CoA desaturase 1 (SCD1), glycerol-3-phosphate acyltransferase (GPAT), and fatty acid synthase (FAS). In contrast, forkhead transcription factor A2 (FoxA2) mRNA was dose-dependently elevated. No change in diglyceride acyltransferase-1 (DGAT1), carnitine palmitoyltransferase 1 (CPT1), lysophosphatidic acid acyltransferase (LPAAT), and PPARγ coactivator 1α (PGC1α) mRNAs were observed.

FGF21 also induced a modulation of various genes involved in cholesterol/bile acid metabolism. A trend toward lowering of a rate-limiting enzyme within the cholesterol synthesis pathway, 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), was observed. This also coincided with an elevation in insulin-induced gene 2 (INSIG2) that controls HMGR expression; however, no change in INSIG1 was detected. The mRNA levels of cholesterol 12α-hydroxylase (CYP8B1) were reduced in a dose-dependent manner. Changes in mRNAs encoding other critical molecules within these pathways, cholesterol 7α-hydroxylase (CYP7A1) and small heterodimer partner (SHP), cholesterol transporters, ATP-binding cassette transporter, subfamily A, member 1 (ABCA1), ATP-binding cassette, subfamily G, member 5 (ABCG5) and 8 (ABCG8), as well as sterol regulatory element-binding protein 1 (SREBP1) and 3-hydroxy-3-methylglutaryl CoA synthase (HMGCS2) were not observed.

Scavenger receptor CD36 and paraoxonase 1 (PON1) mRNAs, both involved in the regulation of lipoprotein

TABLE 1. FGF21-stimulated transcription changes in liver of DIO mice

Gene of interest	Vehicle	FGF21 (mg/kg)		
		0.1	0.3	1.0
FGF21	1	0.558	0.155[a]	0.123[a]
βKlotho	1	1.074	1.129	1.028
Insulin receptor	1	1.365[a]	1.421[a]	1.308[a]
Leptin receptor	1	1.339	2.838[a]	3.294[a]
Fatty acid oxidation				
ACC1	1	0.749	0.568[a]	0.407[a]
ACC2	1	0.652[a]	0.436[a]	0.292[a]
Lipogenesis				
GPAT	1	0.835[a]	0.789[a]	0.693[a]
DGAT1	1	0.915	0.833	0.811
SCD1	1	0.482[a]	0.169[a]	0.092[a]
FAS	1	0.950	0.941	0.825
FoxA2	1	1.612[a]	1.613[a]	2.418[a]
LPAAT	1	0.853	0.945	0.831
PGC1α	1	0.850	1.063	0.918
CPT1	1	0.852	0.876	0.774[a]
Lipoprotein metabolism				
CD36	1	0.560	0.434[a]	0.286[a]
PON1	1	1.247	1.365[a]	1.408[a]
ApoA3	1	1.033	1.051	1.084
ApoA1	1	0.989	1.051	0.962
Cholesterol/bile acid metabolism				
HMGR	1	0.899	0.886	0.696[a]
INSIG2	1	1.175	1.228[a]	1.281[a]
INSIG1	1	0.945	0.810	0.739
CYP8B1	1	0.634	0.473[a]	0.407[a]
CYP7A1	1	1.220	1.214	1.033
ABCA1	1	0.961	0.925	0.907
ABCG5	1	1.047	1.034	1.079
ABCG8	1	0.994	1.150	1.271
SREBP1	1	1.051	0.966	0.909
HMGCS2	1	0.813	0.749	0.726
SHP	1	0.762	0.682	0.812
Nuclear hormone receptors				
HNF4α	1	1.246[a]	1.448[a]	1.491[a]
PPARα	1	0.858	0.805	0.710[a]
PPARγ	1	0.783	0.477[a]	0.320[a]
PPARδ	1	1.106	1.411	1.105
ERRα	1	1.001	0.904	0.851
LXR	1	1.005	1.052	0.993
FXR	1	1.092	1.124	1.015
HNF1α	1	1.107	0.927	1.000

[a] $P \leq 0.05$.

TABLE 2. FGF21-stimulated transcription changes in WAT of DIO mice

Gene of interest	Vehicle	FGF21 (mg/kg)		
		0.1	0.3	1.0
FGF21	1	0.438[a]	0.396[a]	0.404[a]
βKlotho	1	0.885	1.321	1.160
Insulin receptor	1	1.185	1.279[a]	1.338[a]
Leptin receptor	1	1.513[a]	1.561[a]	1.645[a]
Leptin	1	0.732[a]	0.479[a]	0.351[a]
Fatty acid oxidation				
ACC1	1	1.442[a]	1.655[a]	1.567[a]
ACC2	1	1.555[a]	1.776[a]	1.701[a]
Lipogenesis				
DGAT1	1	1.178	1.474[a]	1.311[a]
SCD1	1	1.659[a]	1.681[a]	1.506[a]
PGC1α	1	1.352[a]	1.765[a]	1.557[a]
CPT1	1	1.063	0.994	0.817[a]
Lipid metabolism				
CIDEA	1	1.108	1.090	1.114
ChREBP	1	1.176	1.499[a]	1.779[a]
CD36	1	1.222	1.009	0.949
HSL	1	1.323[a]	1.246[a]	1.319[a]
aP2	1	1.344[a]	1.282[a]	1.363[a]
ATGL	1	1.500[a]	1.513[a]	1.536[a]
UCP1	1	1.163	3.427[a]	4.996[a]
UCP2	1	0.753	0.833	0.758
UCP3	1	0.835	0.764[a]	0.487[a]
PEPCK	1	3.134[a]	3.537[a]	3.326[a]
Nuclear hormone receptors				
PPARα	1	1.438[a]	1.864[a]	1.532[a]
PPARγ	1	1.143	1.424	1.044
PPARδ	1	1.077	1.094	1.030

[a] $P \leq 0.05$.

metabolism, were profoundly modulated; however, the levels of apolipoproteins A3 (ApoA3) and A1 (ApoA1) were unchanged.

Both insulin and leptin receptor mRNA was elevated at each dose and reached 40 and 325% increases *vs.* control, respectively, at the highest concentration of FGF21. No change in βKlotho mRNA was detected, whereas FGF21 transcript levels were dramatically decreased.

In white adipose tissue (WAT) (Table 2), FGF21 administration also led to alterations in the mRNA profiles of several genes involved in lipid metabolism. Among these, a dramatic up-regulation of uncoupling protein 1 (UCP1) was observed. Consistent with this observation, the transcript for PGC1α was also increased at each dose of FGF21. No change in mRNA levels of UCP2 and cell death-inducing DFFA-like effector A (CIDEA) were observed, whereas UCP3 trended downward. The transcripts for adipogenic fatty acid-binding protein 2 (aP2), carbohydrate-responsive element-binding protein (ChREBP), SCD1, DGAT1, ACC1, ACC2, PPARα, and PEPCK were all induced upon FGF21 treatment. No change in PPARγ, PPARδ, CPT1, and CD36 mRNAs was detected. The mRNA levels of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) were elevated.

Similar to observations made with liver tissue samples, both insulin and leptin receptor transcripts were induced with FGF21 administration in WAT but to a smaller extent compared with liver. Leptin mRNA was also dose-dependently reduced consistent with lower leptin levels measured in blood of FGF21-dosed animals. Finally, the FGF21 transcript was also dramatically decreased with the full effect reached at the lowest tested dose of FGF21. No change in βKlotho mRNA was detected.

In brown adipose (BAT) (Table 3), FGF21 administration led to an elevation of UCP1 and ACC2 transcripts, whereas ACC1, CPT1, and DGAT1 mRNAs were unchanged. The level of deiodinase type 2 (D2), the critical enzyme in activation of thyroid hormone function, was elevated by almost 4-fold. Nevertheless, no significant increase in TSH, but rather a reduction in the circulating levels of T_3 and T_4 was observed (Fig. 3F).

In total pancreas (Table 3), FGF21 administration led to a significant reduction in FGF21 mRNA, whereas βKlotho transcript was increased at every dose of FGF21. No FGF21-induced changes in glucagon, insulin, and insulin receptor mRNAs were detected.

Because FGF21-dosed animals are hyperphagic (Fig. 1F), we determined whether FGF21 administration might affect hypothalamic neuropeptides involved in the regulation of appetite and satiety. Notably, agouti-related peptide (AGRP) and neuropeptide Y (NPY) mRNAs were significantly elevated in FGF21-administered animals, whereas cocaine-and amphetamine-regulated transcript (CART), proopiomelanocortin (POMC), and cholecystokinin (CCK) did not change. High levels of βKlotho mRNA (Table 3) as well as transcripts for various FGF receptors (not shown) were also expressed in hypothalamus, but their mRNA levels were not modulated by FGF21 administration.

Discussion

Although FGF21 has been implicated in the regulation of glucose and lipid homeostasis, ketogenesis, and adaptation to starvation (2–7), little is known about the ability of FGF21 to regulate body weight and adiposity *in vivo* in the context of obesity. Here we have clearly demonstrated that exoge-

TABLE 3. FGF21-stimulated transcription changes in pancreas, BAT, and hypothalamus of DIO mice

Gene of interest	Vehicle	FGF21 (mg/kg)		
		0.1	0.3	1.0
Pancreas				
FGF21	1	0.537[a]	0.401[a]	0.361[a]
βKlotho	1	2.083[a]	3.563[a]	3.238[a]
Insulin receptor	1	0.820	0.965	0.862
Insulin	1	0.920	0.750	0.780
Glucagon	1	1.171	1.462	1.498
BAT				
ACC1	1	NA	NA	1.034
ACC2	1	NA	NA	2.516[a]
UCP1	1	NA	NA	3.033[a]
CPT1	1	NA	NA	0.809
DGAT1	1	NA	NA	0.799
D2	1	NA	NA	3.553[a]
Hypothalamus				
AGRP	1	NA	NA	2.701[a]
CART	1	NA	NA	1.001
NPY	1	NA	NA	1.538[a]
POMC	1	NA	NA	1.342
CCK	1	NA	NA	0.832
βKlotho	1	NA	NA	1.251

NA, Sample not tested.
[a] $P \leq 0.05$.

nous FGF21 can exert substantial metabolic effects resulting in reversal of obesity phenotypes in mice.

When administered to obese mice via continuous infusion, FGF21 induced dramatic and dose-dependent weight lowering that was primarily due to a reduction in total adiposity coupled with a minor loss of total body water (Fig. 1, A and B). The latter observation provides preclinical evidence to suggest that future attempts to develop FGF21 as a therapy for type 2 diabetes will not be hampered by edema as a side effect, a common adverse consequence seen with some antidiabetic therapies (15).

FGF21 when administered via daily bolus sc injections also induced a substantial weight-loss effect. However, the protein is significantly more efficacious when delivered via miniosmotic (Alzet) pumps (compare Fig. 1, A and I). Administration via infusion allows for the continuous presence of circulating bioactive FGF21 throughout the course of the study, whereas daily bolus sc delivery leads to a temporary spike in FGF21 blood levels because FGF21 half-life in mice is only about 1 h (7). Thus, the uninterrupted activation of FGF21 signaling is required to achieve maximal weight-lowering effect.

No evidence of decreased total caloric intake (Fig. 1E), changes in physical activity, or overt impairment of nutrient absorption was noted, suggesting that FGF21 is likely to exert its antiobesity effects primarily through the modulation of basal metabolic rate. Indeed, the systemic delivery of FGF21 in DIO mice increased oxygen consumption and overall energy expenditure (Fig. 3, A and B). Importantly, the magnitude of FGF21 effects on energy expenditure and oxygen consumption were comparable during both dark and light cycles, reflecting continuous actions of FGF21, which argues against the dependence of FGF21 actions on diurnal variations in the animals' metabolic status.

Consistent with its effects to increase net energy expenditure, FGF21 administration also led to a small but significant elevation of core body temperature (Fig. 3D). It is important to note that this observation contrasts with an earlier report (5) where body temperature lowering was observed, which is likely due to different experimental approaches employed by Inagaki *et al.* (5). In that report, lowering of core body temperature was observed only in lean, starved, and therefore energy-deprived, FGF21 transgenic mice; no such effects were reported in fed animals. Our studies were conducted using obese animals with *ad libitum* access to food, and body temperatures were measured in the fed state.

Decreased total adiposity, improved hepatosteatosis, and modest increase in fecal fat content in FGF21-dosed obese animals indicated that FGF21's actions were mediated by underlying regulation of lipid metabolism, as also suggested by prior studies employing different experimental models (2, 4, 5, 7, 16). This concept is further supported by our finding of lowered RQ in treated obese mice (Fig. 3C), which reflects a relative increase in lipid oxidation rate and the preferential use of fat as a fuel source.

Even though FGF21 did not alter total food consumption, FGF21 administration led to a steady reduction in body weights during the course of the study (Fig. 1A). Thus, FGF21-dosed mice revealed the development of mild hyperphagia as noted when their caloric intake was normalized to animals' body weights (Fig. 1F). This is reminiscent of earlier observations made in FGF21 transgenic mice (2).

In harmony with the earlier reported ability of FGF21 to regulate blood glucose and its disposal in tolerance tests (2–7), we observed normalization of hyperglycemia in DIO mice even at the lowest tested dose of FGF21 (Fig. 1H). A dose of 0.1 mg/kg·d administered via miniosmotic (Alzet) pumps achieved steady-state blood concentrations of about 7.4 ng/ml; thus, levels of about 5–10 ng/ml appear to be sufficient for nearly complete glucose normalization in DIO mice. In contrast, FGF21-induced weight loss was further potentiated with doses greater than 0.1 mg/kg·d, indicating that maximal antiobesity effects require greater FGF21 exposure (Fig. 1A).

To understand the mechanistic determinants of FGF21 effects to induce energy expenditure and promote weight loss in obese mice, we performed extensive studies using blood and tissues derived from FGF21-dosed and control animals. In these studies, we demonstrated alterations in levels of circulating hormones and key changes in gene expression that shed important new light on the mechanisms underlying FGF21 actions.

Of 67 total hormones and cytokines profiled in blood, the levels of only two polypeptides were significantly modulated by FGF21 administration (Fig. 3E). As expected based on previously reported results (2, 4, 5, 7), insulin levels were suppressed, and this effect reached its maximum at the lowest dose of FGF21. Leptin levels in these obese mice were dramatically lowered in proportion to progressive weight loss and reduced adiposity. Given the lack of effects on a variety of additional circulating factors and the likelihood that these effects on insulin and leptin could be implicated as secondary to interrelated changes in insulin/leptin sensitivity and adiposity, primary effects on hormones and cytokines do not appear to represent major mechanisms for FGF21 antiobesity actions. FGF21-dependent increases in the transcript levels of insulin and leptin receptors observed in liver (Table 1) as well as a decrease in leptin mRNA in WAT (Table 2) could also represent secondary consequences of changes in adiposity and insulin sensitivity.

FGF21 signaling requires the presence of FGF receptors and a critical cofactor, βKlotho, which is selectively expressed in tissues implicated as direct targets for FGF21 action (10–12). A careful analysis of gene expression profiles in these target organs revealed key changes that are likely to represent the primary mechanisms underlying the antiobesity effects of FGF21.

In liver, the most prominently affected gene was SCD1, a key lipogenic enzyme involved in the conversion of saturated to monounsaturated fatty acids (17); hepatic SCD1 mRNA expression was dramatically suppressed by FGF21 in a dose-dependent manner. Importantly, SCD1-null animals are lean, metabolically fit, and have lower liver fat content (18), similar to the phenotype of FGF21-treated DIO animals. Furthermore, FGF21's ability to suppress SCD1 mRNA expression was compromised in leptin-deficient *ob/ob* mice compared with DIO animals with normal leptin signaling (not shown). These data further suggest that a reduction in SCD1 contributes to the antiobesity efficacy of FGF21.

In addition to a reduction in SCD1 mRNA, we observed transcript lowering for ACC1/2 as well as of GPAT and FAS

and a prominent decrease in PPARγ expression (Table 1). This was suggestive of a substantial net reduction in hepatic *de novo* lipogenesis coupled with a potential increase in long-chain fatty acid oxidation (via suppression of ACC1/2 expression, which would be predicted to decrease malonyl CoA levels). Along with PPARγ lowering, we also noted modest reductions in PPARα mRNA levels. Together, these effects further implicate these nuclear receptors as important parts of FGF21 signaling pathways as also suggested by other reported results (4, 5, 14, 16, 19).

Two additional key hepatic transcriptional networks were also affected by FGF21, HNF4α and Foxa2, which were induced by up to 1.5- and 2.5-fold, respectively. HNF4α has an important role in the maintenance of glucose metabolism in liver and is also able to regulate ApoA1 gene expression (20). The latter may explain the ability of FGF21 to induce high-density lipoprotein cholesterol that was previously reported in primates (7). Foxa2 is a master regulator of glucose and lipid homeostasis, which when overexpressed as a constitutively active variant in mice can augment insulin sensitivity, normalize plasma glucose, and improve hepatosteatosis (21). Thus, Foxa2 induction in liver is also a likely contributor to the observed net effects of FGF21 actions.

Additional hepatic actions of FGF21 treatment were consistent with the proposed role that βKlotho may have in cholesterol/bile acid metabolism (22). These effects included a downward trend in HMGR mRNA levels as well as a parallel increase in INSIG2, the HMGR expression inhibitor. In addition, mRNA for CYP8B1, which catalyzes cholic acid synthesis downstream of HMGR, was lowered in a dose-dependant manner (Table 1). Such an effect might contribute to elevated fat content in the feces of FGF21-treated mice via possible changes in individual pools of bile acids. Further studies will be required to address this question.

Taken together, gene expression data derived from the analysis of livers from treated mice are indicative of specific FGF21 actions within a broad network of metabolic regulatory pathways. Effects mediated by these pathways would be predicted to reduce *de novo* lipogenesis, lower triglyceride secretion, augment hepatic fat utilization, enhance hepatic insulin sensitivity, and reverse hepatosteatosis.

In WAT, FGF21 induced the up-regulation of PGC1α, a master regulator of mitochondrial biogenesis/oxidative metabolism and its downstream target UCP1, a critical mediator of nonshivering thermogenesis (23). In contrast to the down-regulation of lipogenic pathway genes in liver, several of the same genes were induced by FGF21 in WAT. These effects included the induction of transcripts encoding SCD1, DGAT1, ACC1, ACC2, ChREBP, aP2, and phosphoenolpyruvate carboxykinase (PEPCK). Given that some of these genes are also PPARγ targets, an increase in PPARγ activity can be implicated, consistent with the earlier finding (14). Because HSL gene expression in WAT was also up-regulated, a parallel increase in both lipogenesis and lipid mobilization can be inferred. Taken together with the increase in UCP1 and PGC1α, it appears likely that FGF21 treatment may induce a state of increased futile cycling and energy expenditure in this, metabolically activated, adipose tissue.

Several effects similar to transcriptional readouts in WAT were observed in BAT, including the induction of UCP1, ACC1, and ACC2 transcripts. Interestingly, mRNA levels for deiodinase type 2 (D2), the enzyme controlling local thyroid hormone activation, were also substantially elevated. Taking into account a recent concept on bile acids serving as energy expenditure regulators via D2 activation (24), the potential for FGF21/βKlotho to modulate bile acid synthesis (22) (Table 1) as well as FGF21 effects on energy expenditure seen in this study (Fig. 3B), we suggest that FGF21 may coordinately regulate BAT activity via both direct and indirect actions that could involve bile acids, thyroid hormone action, and UCP1 activity. Although the mechanistic details of these interactions are currently unclear, it is important to mention that the effect of FGF21 on thyroid hormone action is likely to take place only locally, in BAT, as rather a modest reduction in circulating T_3 and T_4 levels was observed (Fig. 3F).

Modest hyperphagia (Fig. 1F) induced in animals by FGF21 administration was indicative of FGF21-mediated effects on central regulation of feeding. Therefore, we studied gene expression changes in hypothalamus of FGF21-dosed DIO mice. The observed elevation in mRNAs for appetite-promoting hypothalamic neuropeptides, AGRP and NPY, suggests that the observed hyperphagia may occur via a compensatory nutrient sensing-mediated process (25) in response to FGF21-induced increases in energy expenditure. Alternatively, the elevation in orexigenic AGRP and NPY in hypothalamus raises an intriguing possibility of direct FGF21 actions in brain. Indeed, FGF receptors (not shown) and βKlotho (Table 3), the required components of FGF21 receptor, are expressed in the hypothalamus, and FGF21 has recently been shown to penetrate the blood-brain barrier (26).

Finally, we also detected a prominent effect of exogenous FGF21 treatment on endogenous FGF21 mRNA expression in liver, adipose, and pancreas (6, 19). Notably, FGF21 transcript levels were substantially lowered in each of these tissues after FGF21 administration. These results indicate that FGF21 *in vivo*, either directly or through an indirect mechanism, is able to feed back and down-regulate its own expression.

In conclusion, this report is the first to reveal a profound antiobesity effect of exogenous FGF21 when administered systemically in murine models of obesity. The antiobesity efficacy of FGF21 primarily derives from reduced total adiposity and a modest decrease in total body water, and it occurs despite mild hyperphagia, implicating a primary impact on nutrient metabolism. Although beneficial effects on glucose homeostasis and hepatic steatosis were also observed, the dose-response relationships we established in the course of these studies also suggest that FGF21 effects on glucose-, lipid-, and body-weight-regulating functions can be achieved independently. Our data clearly indicate that FGF21 actions to decrease body weight and adiposity in obese mice were likely to be mediated by an increase in energy expenditure and preferential fat utilization as evidenced by elevated oxygen consumption, increased core body temperature, and a reduction in RQ. A careful analysis of FGF21-mediated changes in gene expression in tissues implicated as potential direct targets of FGF21 suggests that potentiation of fatty acid oxidation and suppression of *de novo* lipogenesis may occur in liver. In WAT, predicted effects of FGF21 include the induction of futile cycling because genes affecting lipogenesis, lipid mobilization, and uncoupling

were all coordinately induced. The effects demonstrated in these studies lend further support to the concept that FGF21 might have potential therapeutic benefits in obese humans.

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