Fibroblast Growth Factor 21 Controls Glycemia via Regulation of Hepatic Glucose Flux and Insulin Sensitivity

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Fibroblast growth factor 21 (FGF21) is a novel metabolic regulator shown to improve glycemic control. However, the molecular and functional mechanisms underlying FGF21-mediated improvements in glycemic control are not completely understood. We examined FGF21 effects on insulin sensitivity and glucose fluxes upon chronic (daily injection for 8 d) and acute (6 h infusion) administration in *ob*/+ and *ob*/*ob* mice. Results show that chronic FGF21 ameliorated fasting hyperglycemia in *ob*/*ob* mice via increased glucose disposal and improved hepatic insulin sensitivity. Acute FGF21 suppressed hepatic glucose production, increased liver glycogen, lowered glucagon, and improved glucose clearance in *ob*/+ mice. These effects were blunted in *ob*/*ob* mice. Neither chronic nor acute FGF21 altered skeletal muscle or adipose tissue glucose uptake in either genotype. In conclusion, FGF21 has potent glycemic effects caused by hepatic changes in glucose flux and improved insulin sensitivity. Thus, these studies define mechanisms underlying anti-hyperglycemic actions of FGF21 and support its therapeutic potential. **(*Endocrinology* 150: 4084–4093, 2009)**

Fibroblast growth factor 21 (FGF21) is a potent metabolic regulator shown to improve glucose and lipid metabolism as well as to reduce overall body weight and adipose mass (1–8). Importantly, beneficial effects associated with therapeutic administration and/or transgenic overexpression of FGF21 occurred without concomitant hypoglycemia or mitogenicity characteristic of several current antidiabetic drugs (9). Accordingly, FGF21 is now considered a potential therapeutic agent to treat a variety of metabolic diseases including hyperglycemia and dyslipidemia (9).

Based on evidence that chronic FGF21 administration lowers plasma insulin and improves glucose tolerance in diabetic rodents and nonhuman primates (2, 4–6, 8), it is likely that FGF21 ameliorates insulin resistance, and this outcome contributes to the striking anti-hyperglycemic effects of FGF21. This concept may be important considering that insulin resistance is a hallmark of numerous metabolic diseases and critically associated with defects in glucose flux. Indeed, several current therapies aim to treat hyperglycemia through modulation of insulin signaling (10). The link between FGF21 treatment and insulin sensitivity has been recently strengthened by findings that insulin sensitivity is improved in chronically treated high-fat-fed mice (11). This notion is further supported by recent evidence of insulin-FGF21 cross talk as well as a functional interplay between FGF21- and peroxisome proliferator-activated receptor (PPAR)-dependent mechanisms because PPAR agonists, which are potent insulin sensitizers, to a certain extent signal through changes in either FGF21 expression or action (8, 12–14).

In the present studies, FGF21 was administered sc to obese, diabetic *ob*/*ob* mice for 8 d and compared with

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Abbreviations: 2-DG, 2-[^{14}C]Deoxyglucose; endoR_a, endogenous R_a; FGF21, fibroblast growth factor 21; GIR, glucose infusion rate; G-6-Pase, glucose-6-phosphatase; NEFA, nonesterified fatty acids; PPAR, peroxisome proliferator-activated receptor; R_a, glucose appearance; R_d, glucose disappearance; R_g, glucose uptake.

vehicle-treated *ob*/*ob* and *ob*/+ mice to test whether, and to what extent, FGF21 improves insulin sensitivity and glucose flux in a mouse model of severe insulin resistance. This was examined in catheterized, conscious animals using hyperinsulinemic-euglycemic clamp techniques. In subsequent experiments, *ob*/+ and *ob*/*ob* mice were infused with FGF21 for 6 h, and blood glucose was clamped to assess the direct metabolic effects of FGF21 on glucose fluxes. In both experiments, isotopic tracer techniques were used to quantify whole-body and tissue-specific glucose fluxes. The present studies clearly demonstrate that FGF21 treatment improves insulin sensitivity in the liver.

Materials and Methods

Animals

Animal procedures were approved by the Vanderbilt University Animal Care and Use Committee. Male *ob*/*ob* and *ob*/+ mice on a C57BL/6 background (Harlan, Indianapolis, IN) at 7 wk of age were acclimated for 1 wk before surgery in an environmentally controlled facility with a 12-h light, 12-h dark cycle and free access to food and water.

Surgical techniques

A jugular vein catheter was surgically implanted 5 d before study using previously described techniques (15). Body weight was monitored daily, and only mice returning to within about 10% of presurgical body weight were studied.

Hyperinsulinemic-euglycemic clamp

Insulin sensitivity was assessed using previously described clamp techniques (15) in *ob*/*ob* mice treated daily via sc injection for 8 d with FGF21 (1 $mg \cdot kg^{-1}$) or vehicle. Vehicle-treated *ob*/+ mice were studied as an insulin-sensitive control. Body composition was assessed 24 h before study using NMR (Bruker-Optics). On the day of study, mice were placed in plastic restraint tube designed for rats at approximately 0800 h to begin a 5 h fast. At t = −90 min, a primed continuous infusion of HPLC-purified [3-^{3}H]glucose (5 μCi bolus + 0.05 $\mu Ci \cdot min^{-1}$) was started to measure glucose turnover. Basal blood samples from the cut tail for blood glucose, insulin, and glucose turnover were taken at t = −15 and −5 min. At t = 0 min, a continuous infusion of insulin ($10mU \cdot kg^{-1} \cdot min^{-1}$) was started to induce hyperinsulinemia, and the tracer infusion was increased to 0.1 $\mu Ci \cdot min^{-1}$ to minimize changes in specific activity. Blood glucose was measured at t = 5, 10, 15, and 20 min and every 10 min thereafter, and a variable glucose infusion rate (GIR) was adjusted as needed to maintain blood glucose at about 8.0 $mmol \cdot liter^{-1}$. At t = 78 min, a 12-μCi bolus of HPLC-purified 2-[^{14}C]deoxyglucose (2-DG) was given to assess tissue-specific glucose uptake. The steady-state clamp period was t = 80–120 min. Samples to determine plasma [3-^{3}H]glucose and 2-DG were taken every 10 min from t = 80–120 min. Samples to measure plasma insulin were taken at t = 100 and 120 min. After the final blood samples, mice were anesthetized using a bolus of sodium pentobarbital and tissues were removed and frozen in liquid nitrogen.

Acute effects of FGF21

Ob/*ob* and *ob*/+ mice were placed in a rat restraint tube at approximately 0600 h to begin a 5-h fast. At t = −90 min, a primed continuous infusion of HPLC-purified [3-^{3}H]glucose (5 μCi bolus + 0.05 $\mu Ci \cdot min^{-1}$) was started to assess glucose turnover. Basal samples for blood glucose, insulin, glucagon, and glucose turnover were taken at t = −15 and −5 min from the cut tail. At t = 0 min, a continuous infusion of FGF21 (1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was started and [3-^{3}H]glucose was increased to 0.1 $\mu Ci \cdot min^{-1}$ to minimize changes in specific activity. Samples to measure plasma glucose and determine glucose turnover were taken every 60 min for 5 h. A variable GIR was used to prevent a fall in glucose below about 8.0 $mmol \cdot liter^{-1}$. Samples for insulin and glucagon were taken at t = 150 and 300 min. At t = 318 min, a 12-μCi bolus of HPLC-purified 2-DG was given to assess tissue-specific glucose uptake, and blood samples were taken every 10 min from 320–360 min. At t = 360 min, mice were anesthetized using a bolus of pentobarbital, and a terminal blood sample was taken to measure plasma FGF21. Tissues were removed and frozen in liquid nitrogen.

Blood and plasma analyses

Blood glucose during each clamp was measured using an Accu-Chek meter (Roche, Indianapolis, IN). Plasma nonesterified fatty acids (NEFA) were measured using a kit (Wako Diagnostics, Osaka, Japan). Insulin and glucagon were determined by the Vanderbilt Mouse Metabolic Phenotyping Center Analytical Resources Core (16). FGF21 was measured using a previously described ELISA (6). Plasma [3-^{3}H]glucose and 2-DG radioactivity were measured using liquid scintillation counting (17).

Tissue analyses

Hepatic glycogen was measured enzymatically (18). The percentage of glucose disappearance in glycogen was determined by incorporation of [3-^{3}H]glucose in glycogen. Hepatic triglycerides were measured using a commercial assay (Pointe Scientific Inc., Canton, MI). Liver glucokinase and glucose-6-phosphatase (G-6-Pase) activity assays were performed as previously described (19). Tissue 2-DG was measured as previously described (17). Quantitative real-time PCR was performed using TaqMan Assay-on-Demand primers (Applied Biosystems, Foster City, CA) and normalized to 18S or 364B. Genes of interest are listed in Table 1.

Calculations and statistics

Whole-body glucose appearance (R_a), disappearance (R_d), and clearance were calculated as previously described (20). Tissue glucose uptake (R_g) was also calculated as previously described (17). Statistical comparisons were made using *t* tests or one-way ANOVA followed by the Fisher least significant difference test for *post hoc* comparisons. Data are presented as means ± SE. Statistical significance was defined as $P < 0.05$.

Results

FGF21 treatment for 8 d reduces fasting blood glucose and improves insulin sensitivity in *ob*/*ob* mice

The elevated fasting blood glucose, insulin, and NEFA levels characteristic of *ob*/*ob* mice were reduced by 8.0

TABLE 1. mRNA for genes of interest in *ob*/+ and *ob*/*ob* mice after hyperinsulinemic-euglycemic clamps or infusion with FGF21 or vehicle

	Insulin clamp			FGF21 clamp			
Genes of interest	***ob*/+**	***ob*/*ob***	***ob*/*ob***	***ob*/+**	***ob*/+**	***ob*/*ob***	***ob*/*ob***
FGF21	−	−	+	−	+	−	+
Liver							
AMPKα1	1.0 ± 0.1	0.9 ± 0.1	2.0 ± 0.5[a,b]	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
AMPKα2	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
βKlotho	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.2
CD36	1.0 ± 0.1	1.0 ± 0.1	1.5 ± 0.3	1.0 ± 0.1	1.1 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
CPT1α	1.0 ± 0.1	1.2 ± 0.2	1.8 ± 0.2[a,b]	1.0 ± 0.1	0.9 ± 0.1	1.5 ± 0.2	1.2 ± 0.2
CPT1β	1.0 ± 0.4	2.1 ± 0.2	2.2 ± 0.2[a]	1.0 ± 0.1	1.4 ± 0.1[b]	12.2 ± 0.5	11.6 ± 0.8
FOXA2	1.0 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	1.0 ± 0.1	1.7 ± 0.2[b]	1.0 ± 0.1	0.9 ± 0.1
G6Pase	1.0 ± 0.4	0.5 ± 0.1	2.1 ± 0.5[a,b]	1.0 ± 0.1	0.6 ± 0.1[b]	2.0 ± 0.9	0.9 ± 0.3[b]
Glucokinase	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	1.7 ± 0.2	1.7 ± 0.2
Glycogen synthase 1	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.7 ± 0.2	1.4 ± 0.2[b]
Glycogen synthase 2	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
Insulin receptor	1.0 ± 0.1	1.2 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.4 ± 0.1[b]	1.0 ± 0.1	0.9 ± 0.1
Leptin receptor	1.0 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	1.0 ± 0.2	2.3 ± 0.6[b]	1.0 ± 0.1	1.0 ± 0.2
MCD	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.8 ± 0.1[b]
PEPCK	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.7 ± 0.1
PPARα	1.0 ± 0.1	1.0 ± 0.1	1.3 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Heart							
Hexokinase II	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.1
Glut4	1.0 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.1	1.2 ± 0.2	0.8 ± 0.2	0.9 ± 0.1
βKlotho	ND	ND	ND	ND	ND	ND	ND
Gastrocnemius							
βKlotho	ND	ND	ND	ND	ND	ND	ND
Adipose							
βKlotho	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.2	0.9 ± 0.1

Mice were subjected to hyperinsulinemic (10 mU · kg^{-1} · min^{-1}), euglycemic (~8.0 mmol · liter^{-1}) clamps (insulin clamp; n = 8–10 in each group) or infused with FGF21 (1 ng · kg^{-1} · min^{-1}) or vehicle for 6 h (FGF21 clamp; n = 7–9 in each group). All mice were male, studied at 9–10 wk of age, and fasted for 5 h before study. Mice were euthanized using a bolus of sodium pentobarbital. Samples were run in duplicate using 18S or 36B4 as a reference gene and normalized to *ob*/+ mice infused with vehicle. Data are presented as mean ± SE, and statistical significance is established at $P < 0.05$. ND, No message detected.

[a] Differences compared with vehicle-infused *ob*/+ mice.

[b] Differences within a genotype.

mmol · liter^{-1}, 1.8 ng · ml^{-1}, and 0.3 mmol · liter^{-1}, respectively, with FGF21 treatment (Fig. 1, A–C). Absolute glucose and insulin levels in fasted *ob*/*ob* mice treated with FGF21 were, however, still higher compared with *ob*/+ mice (Fig. 1, A and C). Body weight in *ob*/*ob* mice was not affected by FGF21 treatment (FGF21- and vehicle-treated *ob*/*ob* mice were 45.6 ± 0.7 and 45.4 ± 0.7 g, respectively). Body weight was higher in *ob*/*ob* mice compared with *ob*/+ mice (26.5 ± 0.3 g) due to higher fat mass (22.7 ± 0.5 g in both FGF21- and vehicle-treated *ob*/*ob* mice *vs.* 2.7 ± 0.1 g in vehicle-treated *ob*/+ mice).

Hyperinsulinemic-euglycemic clamps were done to test the effect of 8 d FGF21 treatment on insulin sensitivity in *ob*/*ob* mice. As shown in Fig. 1C, plasma insulin during the clamp was similarly elevated in vehicle- and FGF21-treated *ob*/*ob* mice and remained significantly higher than levels in *ob*/+ mice. NEFA levels were suppressed by hyperinsulinemia in *ob*/+ mice but were unchanged in *ob*/*ob* mice (Fig. 1B). Blood glucose was clamped in all groups during the steady-state period (Fig. 1D). The GIR required to clamp blood glucose was 4-fold greater in FGF21-treated *ob*/*ob* mice, indicating improved insulin sensitivity (Fig. 1E). The increased GIR in FGF21-treated *ob*/*ob* mice was about 20% of vehicle-treated *ob*/+ mice indicating a modest improvement in insulin sensitivity (Fig. 1E).

Fasting endogenous R_a (endoR_a) and R_d were increased by FGF21 treatment in *ob*/*ob* mice (Fig. 2, A and B) and fasting whole-body glucose clearance was fully restored to rates found in *ob*/+ mice (Fig. 2C). As expected, suppression of endoR_a by hyperinsulinemia was nearly complete in *ob*/+ mice (Fig. 2A). EndoR_a was not suppressed in *ob*/*ob* mice treated with vehicle (Fig. 2A). FGF21 treatment in *ob*/*ob* mice restored the magnitude in suppression of endoR_a to equal that of *ob*/+ mice, indicating improved hepatic insulin sensitivity (Fig. 2A). Clamp R_d (Fig. 2B) and glucose clearance (Fig. 2C) were similar in vehicle- and FGF21-treated *ob*/*ob* mice but remained well below rates of *ob*/+ mice. The index of skeletal muscle and adipose tissue glucose uptake (R_g) after the clamp was unchanged in *ob*/*ob* mice treated with FGF21 (Fig. 2, D and

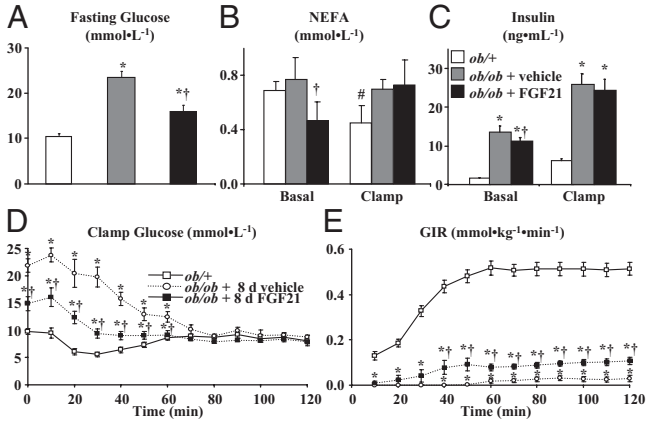

FIG. 1. Blood glucose (A), insulin (B), and NEFA (C) from hyperinsulinemic (10 mU · kg^{-1} · min^{-1})-euglycemic (~8.0 mmol · $liter^{-1}$) clamps in conscious, 5-h fasted *ob*/+ and *ob*/*ob* mice (n = 11–13 in each group). *Ob*/*ob* mice were sc injected with vehicle or FGF21 (1 mg · kg^{-1} · d^{-1}) for 8 d, and jugular vein catheters were implanted 5 d before study. Vehicle-treated *ob*/+ were studied as an insulin-sensitive control. Blood samples were taken at indicated time points from the cut tail to measure blood glucose (D). The GIR (E) was adjusted as needed to maintain euglycemia. Mice were euthanized using a bolus of sodium pentobarbital at t = 120 min, and tissues were dissected and quickly frozen. All mice were male and studied at 9–10 wk of age. Data are presented as mean ± SE, and the steady-state period was defined as t = 80–120 min during the clamp. Statistical significance was established at $P < 0.05$. *, Comparison to *ob*/+ mice; †, comparison between *ob*/*ob* mice treated with vehicle or FGF21; #, comparison within a group between basal and clamp conditions.

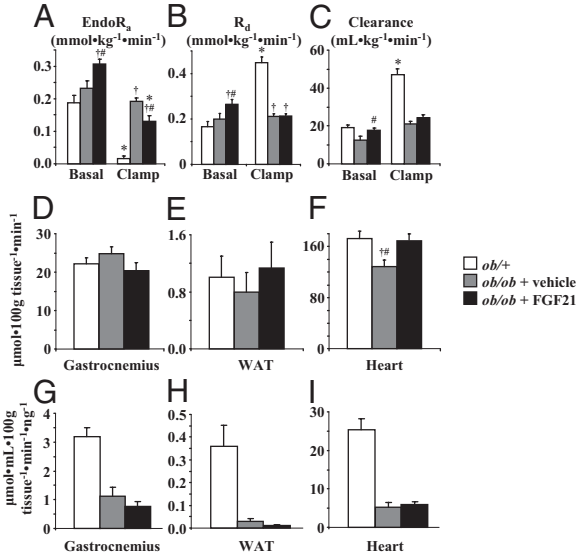

FIG. 2. $EndoR_a$ (A), R_d (B), and glucose clearance (C) during a 120-min hyperinsulinemic (10 mU · kg^{-1} · min^{-1})-euglycemic (~8.0 mmol · $liter^{-1}$) clamp in conscious, 5-h fasted *ob*/+ and *ob*/*ob* mice (n = 11–13 in each group). *Ob*/*ob* mice were sc injected with vehicle or FGF21 (1 mg · kg^{-1} · d^{-1}) for 8 d, and jugular vein catheters were implanted 5 d before study. Vehicle-treated *ob*/+ were studied as an insulin-sensitive control. [3-^{3}H]Glucose (0.1 μCi · min^{-1}) was infused starting at t = −90 min, and plasma samples were taken from the cut tail at time points indicated in each *panel*. Mice were euthanized at t = 120 min using sodium pentobarbital. D–F, R_g in the gastrocnemius (D), heart (E), and white adipose tissue (WAT; F) was assessed using 2-DG at t = 78 min; G–I, R_g values normalized to clamp insulin, respectively. Mice were male, studied at 9–10 wk of age, and fasted 5 h before study. *, Differences between basal and clamp; †, differences compared with *ob*/+ mice; #, differences compared with vehicle-treated *ob*/*ob* mice.

E). Absolute R_g rates in *ob*/*ob* mice were similar to *ob*/+ mice but were reduced when normalized for clamp insulin (Fig. 2, G and H). Cardiac R_g was increased in *ob*/*ob* mice treated with FGF21 and restored to absolute levels seen in *ob*/+ mice (Fig. 2F). Cardiac R_g was, however, not different when normalized to clamp insulin (Fig. 2I).

The effect of FGF21 treatment to improve hepatic insulin sensitivity in *ob*/*ob* mice, based on increased insulin-mediated suppression of $endoR_a$, corresponded with increased hepatic Akt activation (p-Akt/Akt) (Fig. 3A). Consistent with normalized R_g data, Akt activation was not different in heart, gastrocnemius, or adipose tissue of FGF21-treated *ob*/*ob* mice (Fig. 3, B–D). FGF21 treatment also increased hepatic glycogen (Fig. 3E) and the percentage of R_d accounted for in glycogen (Fig. 3F). FGF21 treatment had no significant effect on liver triglyceride in *ob*/*ob* mice, although there was a trend for reduced levels ($P = 0.07$) (Fig. 3G). Hepatic glycogen and triglyceride concentrations remained lower and higher, respectively, compared with *ob*/+ mice (Fig. 3, F and G). Hepatic glucokinase activity was increased in FGF21-treated *ob*/*ob* mice by about 15% (4.5 ± 0.1 *vs.* 5.5 ± 0.3 μmol · min^{-1} · g^{-1}, $P = 0.02$, in vehicle- and FGF21-treated animals, respectively), whereas G-6-Pase activity was unaffected (7.6 ± 0.9 *vs.* 7.0 ± 0.4 μmol · min^{-1} · g^{-1}, $P = 0.55$, in vehicle- and FGF21-treated mice, respectively). Expression of key genes involved in hepatic metabolic regulation was investigated based on evidence of a liver phenotype and were indeed altered in FGF21-treated *ob*/*ob* mice. As shown in Table 1, mRNA levels for G-6-Pase, AMPKα1, CPT1α, and CPT1β were increased in FGF21-treated *ob*/*ob* mice. There were no changes in the cardiac expression of hexokinase II or glucose transporter 4 (Glut4) (Table 1) associated with FGF21 treatment consistent with R_g normalized to insulin. Expression of βKlotho, the cofactor needed for FGF21 activity, in liver and adipose tissue was unaffected by FGF21 treatment and was undetectable in heart and gastrocnemius (Table 1).

Acute effects of FGF21 on glucose fluxes in *ob*/+ and *ob*/*ob* mice

To test the acute effects of FGF21 on glucose fluxes, FGF21 was infused in *ob*/+ and *ob*/*ob* mice for 6 h while GIR was adjusted to maintain steady-state conditions. Steady-state blood glucose and GIR were obtained within 3 h (Fig. 4, A and B). Although endogenous FGF21 levels were below limits of detection in vehicle-infused mice, continuous infusion led to pharmacological FGF21 levels of 53 ± 3 and 46 ± 2 ng · ml^{-1}, respectively, in *ob*/+

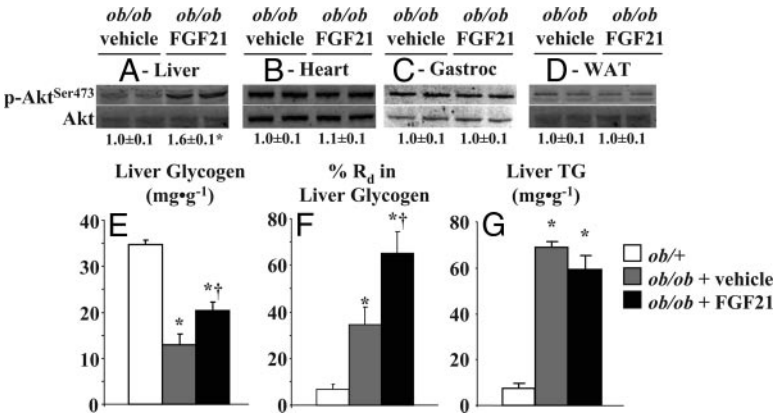

FIG. 3. Hepatic Akt phosphorylation at serine 473 normalized to total Akt protein content in liver (A), heart (B), gastrocnemius (C), and adipose (D) after 120 min hyperinsulinemic (10 mU · kg^{-1} · min^{-1})-euglycemic (~8.0 mmol · liter^{-1}) clamps in conscious, 5-h fasted *ob*/+ and *ob*/*ob* mice (n = 11–13 in each group). Mice were sc injected with either vehicle or FGF21 (1 mg · kg^{-1} · d^{-1}) for 8 d before study. Liver glycogen (E), percentage of R_d in liver glycogen (F), and triglyceride (TG; G) were measured after the clamp using enzymatic techniques and incorporation of [3-^{3}H]glucose into glycogen. Mice were euthanized at t = 120 min using sodium pentobarbital. Mice were male, studied at 9–10 wk of age, and fasted 5 h before study. *, Differences compared with *ob*/+ mice; #, differences compared with vehicle-treated *ob*/*ob* mice. WAT, White adipose tissue.

and *ob*/*ob* mice. FGF21 had a potent glycemic effect in *ob*/+ mice requiring a GIR of approximately 0.14 mmol · kg^{-1} · min^{-1} to maintain steady-state blood glucose (Fig. 4B). The blood glucose response in *ob*/*ob* mice to FGF21 was comparatively modest, requiring a GIR that was only about 20% of that in *ob*/+ mice (Fig. 4B). NEFA levels were not changed by FGF21 in either genotype (Fig. 4C). Glucagon was decreased after 5 h in *ob*/+ mice but not in *ob*/*ob* mice infused with FGF21 (Fig. 4D). Although insulin fell during the clamp in both *ob*/+ and *ob*/*ob* mice, this was due to the length of the fast and was not affected by FGF21 (Fig. 4E). Liver glycogen was increased in *ob*/+ mice infused with FGF21, but this effect was absent in *ob*/*ob* mice (Fig. 4F). Liver triglycerides were reduced by a similar decrement in *ob*/+ and *ob*/*ob* mice infused with FGF21 (Fig. 4G).

EndoR_a in *ob*/+ mice was reduced by FGF21 infusion but was unchanged in *ob*/*ob* mice (Fig. 5A). R_d and glucose clearance were increased in *ob*/+ mice infused with FGF21 (Fig. 5, B and C). These rates were unchanged in *ob*/*ob* mice at steady state (Fig. 5, B and C). Cardiac R_g in *ob*/+ mice infused with FGF21 was increased about 7-fold compared with about 2-fold in *ob*/*ob* mice (Fig. 5D). FGF21 infusion had no effect on skeletal muscle or adipose tissue R_g in *ob*/+ and *ob*/*ob* mice (Fig. 5, E and F). FGF21-mediated suppression of endoR_a corresponded to increased hepatic Akt phosphorylation in *ob*/+ mice (Fig. 5G). FGF21 also increased hepatic Akt activation in *ob*/*ob* mice, but this increase remained well below that of *ob*/+ mice (Fig. 5G). Expression of the insulin receptor, leptin receptor, and Foxa2 in the liver of *ob*/+ mice was increased by FGF21 infusion (Table 1). In *ob*/*ob* mice, hepatic MCD and glycogen synthase mRNA levels were decreased and increased, respectively, by FGF21 infusion, but other genes were unaffected (Table 1). Infusion of FGF21 had no effect on cardiac expression of hexokinase II or Glut4. Expression of βKlotho was unchanged by FGF21 treatment in liver and adipose and was undetectable in heart and gastrocnemius (Table 1).

Discussion

Metabolic diseases such as type 2 diabetes, obesity, and metabolic syndrome are characterized by impairments in insulin sensitivity and glucose flux. Amelioration of these symptoms is considered to be beneficial in treating metabolic abnormalities. Recent studies have shown that FGF21 reduces hyperglycemia and insulin resistance (9, 11). However, the mechanisms by which FGF21 propagates its function to control glucose and its role, if any, in regulating insulin sensitivity and glucose flux are not well defined. Thus, we examined the chronic and acute mechanisms underlying FGF21 antihyperglycemic activities using *in vivo* clamp techniques in *ob*/*ob* and *ob*/+ mice. We hypothesized that the mode of action for FGF21 includes regulation of glucose flux through improvements in hepatic insulin sensitivity.

These results demonstrate that chronic FGF21 treatment (daily sc injection of 1 mg · kg^{-1} for 8 d) reduced fasting hyperglycemia, hyperinsulinemia, and NEFA levels in *ob*/*ob* mice. Such improvements in glycemic control and lipid metabolism are consistent with previous studies in obese and diabetic animals chronically treated with FGF21 (1, 2, 5, 6, 8, 11). We show that reduced fasting blood glucose associated with FGF21 treatment was due to improved basal glucose disposal (Fig. 2B). Considering the marked decrease in blood glucose, it was surprising that FGF21 treatment increased endoR_a (Fig. 2A). Additional analyses of livers from *ob*/*ob* mice treated with FGF21 reveal increased glycogen content (accounting for ~65% of whole-body R_d; Fig. 3F), increased glucokinase activity, and unaffected G-6-Pase activity indicating a potential futile cycling situation favoring glucose uptake. A net influx of glucose into the liver could explain how blood glucose is lowered despite higher endoR_a.

A critical finding was that chronic FGF21 administration improved insulin sensitivity in *ob*/*ob* mice based on increased GIR needed to maintain euglycemia during hy-

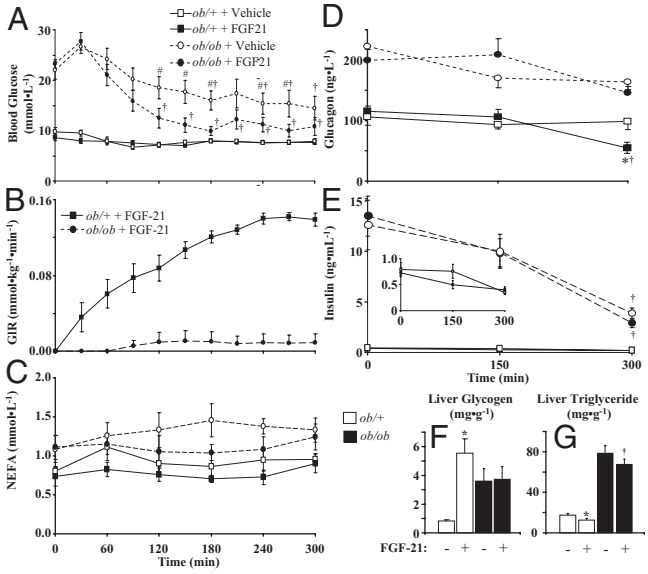

FIG. 4. Whole blood glucose (A), GIR (B), glucagon (C), and insulin (D) in *ob*/+ and *ob*/*ob* mice infused with FGF21 (1 ng · kg^{-1} · min^{-1}) or vehicle for a total of 6 h (n = 7–9 in each group). *Inset* in E shows rescaled insulin levels for *ob*/+ mice. Samples were taken at time points indicated in each *panel*, and t = 0 is the mean of t = −15 and −5 min samples. Figures show data for the first 5 h before the bolus infusion of 2-DG at t = 318 min to measure tissue-specific glucose uptake. Glycogen (F) and triglyceride (G) were measured after euthanasia via a bolus of pentobarbital. Jugular vein catheters used for infusion purposes were surgically implanted in all mice 5 d before study, and blood samples were taken from the cut tail. All mice were male, studied at 9–10 wk of age, and fasted for 5 h before study. Data are presented as mean ± SE, and statistical significance is established at $P < 0.05$. *, Differences between vehicle- and FGF21-infused *ob*/+ mice; #, differences between vehicle- and FGF21-infused *ob*/*ob* mice; †, differences compared with basal values.

perinsulinemic-euglycemic clamps. This technique is the gold standard for assessing insulin sensitivity because it is possible to normalize differences in blood glucose, which often complicate interpretation of glucose and insulin tolerance tests. The present improvements in insulin sensitivity are largely ascribed to hepatic FGF21 effects because suppression of endoR_a was increased (Fig. 2A), but R_g was unchanged in skeletal muscle, heart, and adipose tissue when normalized for clamp insulin levels. Hepatic effects, at least in part, may be due to insulin-sensitizing effects of FGF21 treatment at a molecular level. Akt phosphorylation, a key step in hepatic insulin and FGF21 signaling (8, 21), was increased during the clamp in FGF21-treated *ob*/*ob* mice (Fig. 4G). In this context, this likely reflects improved insulin sensitivity considering the approximately 1-h half-life of FGF21 (6). mRNA levels for key mediators of hepatic fuel flux such as AMP-activated protein kinase-α (AMPKα), carnitine palmitoyl-transferase-1α (CPT1α), CD36, and G-6-Pase were also elevated by chronic FGF21 treatment in *ob*/*ob* mice (Table 1). These changes may contribute to a FGF21-dependent mechanism to regulate hepatic insulin sensitivity (22–25).

FGF21 is previously shown to inhibit adipose tissue lipolysis and postulated as a potential mechanism to explain improvements in insulin sensitivity (1). This result, however, contrasts findings that FGF21 stimulates adipose tissue lipolysis (4). The contribution of reduced basal NEFA levels (Fig. 1B) in FGF21-treated *ob*/*ob* mice to improvements in insulin sensitivity is difficult to discern in the current study because lipid flux was not assessed. These studies do demonstrate that FGF21 treatment did not improve insulin-mediated suppression of lipolysis in *ob*/*ob* mice (Fig. 1C).

It is important to note that there were no differences in steady-state insulin levels in *ob*/*ob* mice regardless of treatment (Fig. 1B). This is critical to consider when interpreting insulin clamp data (26). Moreover, there were no differences in body weight or composition due to FGF21 treatment in *ob*/*ob* mice. Although previous studies in high-fat-fed and *ob*/*ob* mice have shown dose-dependent effects of FGF21 on body weight and adipose mass (3, 11), we did not observe a FGF21 weight-lowering effect. The latter finding is consistent with the fact that higher doses (>1 mg · kg^{-1} · d^{-1}), longer duration of administration, and/or alternative administration routes are needed to reveal the ability of FGF21 to lower body weight/adipose tissue mass (3, 6). Thus, the FGF21 insulin-sensitizing effects observed here are not secondary to changes in adiposity or differences in insulin levels but are rather a direct result of FGF21 action.

The current finding that chronic FGF21 treatment improves insulin sensitivity is generally consistent with data in FGF21-treated high-fat-fed mice (11). In agreement with our results, this study found that FGF21 lowered fasting blood glucose and insulin and improved hepatic insulin sensitivity. In contrast, Xu *et al.* (11) found that FGF21 treatment improved insulin-stimulated glucose uptake in heart, adipose tissue, and skeletal muscle in a dose-dependent manner. It is worthwhile to note that interpreting these data are difficult because details concerning fast duration and specific muscles used to assess glucose uptake are not provided (11). These and other methodological concerns about clamp techniques used in Xu *et al.* (11) have been previously discussed (15). Additionally, clamp glucose and insulin levels were significantly different between groups in Xu *et al.* (11). For example, clamp glucose in high-fat-fed mice treated with 0.1 and 10 mg · kg^{-1} · d^{-1} FGF21 were about 20 and 25% lower, respectively, than vehicle-treated control mice. Clamp insulin levels were also about 23 and 51% lower, respectively, in FGF21-treated high-fat-fed mice compared with vehicle-treated controls. These differences in clamp conditions complicate comparing between groups. It is noteworthy that normalizing for clamp insulin would augment only effects ascribed to FGF21, resulting in profound ef-

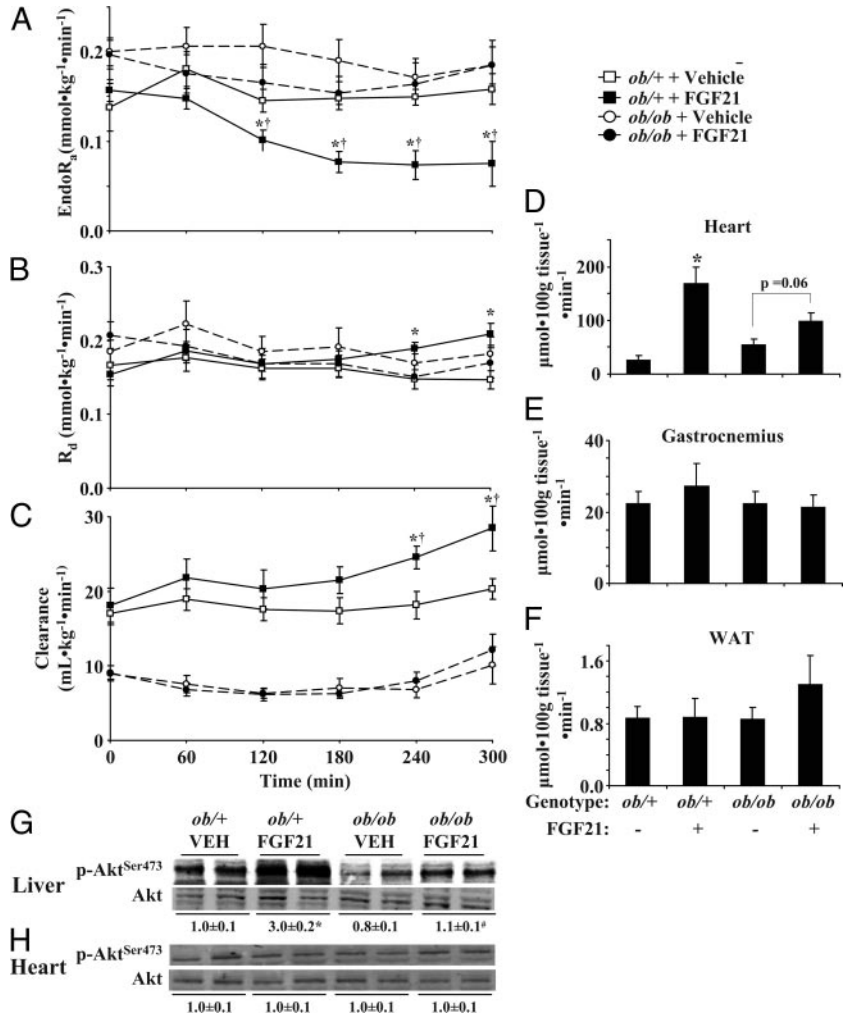

FIG. 5. EndoR$_a$ (A), R$_d$ (B), and glucose clearance (C) in *ob*/+ and *ob*/*ob* mice infused with FGF21 (1 ng · kg^{-1} · min^{-1}) or vehicle for 6 h (n = 7–9 in each group). Time points are data from t = 0–300 min before the bolus infusion of 2-DG at t = 318 min. [3-^{3}H]Glucose (0.1 μCi · min^{-1}) was infused starting at t = −90 min, and plasma samples were taken from the cut tail at time points indicated in each *panel* where t = 0 min is the mean of t = −15 and −5 min samples. R$_g$ in the gastrocnemius (D), heart (E), and white adipose tissue (WAT; F) was assessed using 2-DG. Hepatic Akt phosphorylation at serine 473 (p-Akt for 473) normalized to total Akt protein content in liver and heart is shown in G and H, respectively. Mice were euthanized at t = 360 min using sodium pentobarbital. Mice were male, studied at 9–10 wk of age, and fasted 5 h before study. *, Differences between vehicle (VEH)- and FGF21-infused *ob*/+ mice; †, differences compared with basal values.

fects on muscle glucose uptake. This is curious considering that βKlotho, the cofactor required for FGF21 activity, is not known to be expressed in skeletal muscle. Nevertheless, our studies and those of Xu *et al.* (11) do generally agree that FGF21 treatment improves glycemic control and has potent hepatic effects. An additional consistent finding was that the lower dose of FGF21 did not stimulate adipose tissue glucose uptake (11). βKlotho is expressed in this tissue, and FGF21 is reported to increase glucose flux into adipose tissue (5, 11). Our results, however, do not support this effect *in vivo*.

To understand the actions of FGF21 in the fasted state and further define sites of action, we next tested whether an acute 6-h constant infusion of FGF21 directly impacts glucose flux. In this novel protocol, FGF21 had rapid and potent metabolic effects in healthy *ob*/+ mice. FGF21 lowered endoR$_a$ and increased whole-body glucose clearance in *ob*/+ mice (Fig. 5, A and C). There were no changes in plasma NEFA (Fig. 4C) or adipose tissue R$_g$ (Fig. 5F) associated with infusion of FGF21, suggesting that metabolic effects are not secondary to direct actions on adipose tissue. Remarkably, the GIR required to maintain steady-state euglycemia was comparable to exogenous glucose requirements during a 2.5 mU · kg^{-1} · min^{-1} insulin clamp in C57BL/6 mice (15). Consistent with the chronic FGF21 treatment results, the acute studies demonstrate a potent effect on the liver. FGF21 reduced liver triglyceride in both *ob*/*ob* and *ob*/+ mice by about 25% (Fig. 4G), consistent with a role for FGF21 in amelioration of hepatosteatosis (2, 3, 11). FGF21 also led to a remarkable increase in liver glycogen (Fig. 4F), accounting for 72 ± 2% of whole-body R$_d$ in *ob*/+ mice. FGF21 also increased hepatic Akt activation (Fig. 4G) and expression of the insulin receptor, leptin receptor, and FoxA2 in *ob*/+ mice consistent with improvements in insulin signaling (Table 1).

The current data indicate that the actions of acutely infused FGF21 on the liver are predominantly direct rather than secondary to effects in other tissues. This conclusion is based on evidence that FGF21 did not alter insulin levels (Fig. 4E), NEFA (Fig. 4C), or R$_g$ in skeletal muscle or adipose tissue (Fig. 5, E and F). It is important to note that the acute infusion of FGF21 in *ob*/+ mice was associated with effects in tissues other than the liver based on increased cardiac R$_g$ (Fig. 5D) and decreased plasma glucagon (Fig. 4D). This effect in the heart is consistent with absolute R$_g$ rates in chronically treated *ob*/*ob* mice (Fig. 2F) and previous studies (11). This finding is somewhat surprising given that βKlotho is not known to be expressed in the heart. To test whether FGF21 directly impacts the heart, we assessed Akt activation and βKlotho transcript in acutely infused and chronically treated mice.

Cardiac Akt activation was unchanged, and βKlotho expression was undetectable in the heart (Table 1) despite increased R_g associated with FGF21. These findings suggest that the changes in cardiac glucose flux linked to FGF21 are indirect and likely due to the fact that the heart is constantly active and sensitive to changes in substrate availability. Subtle changes in NEFA availability, for example, may cause the heart to rely on more glucose as energetic substrate. This notion of indirect FGF21 effects on cardiac glucose flux is supported by recent evidence (11). Despite the apparent indirect nature of the effect, this is potentially an important observation considering that individuals with diabetes are at a higher risk for cardiovascular disease (27). Additional studies are needed to explore metabolic changes responsible for this effect and to test the role, if any, of FGF21 in the treatment of cardiovascular disease.

The action of FGF21 to lower glucagon in *ob*/+ mice is consistent with previous findings and expression of the required receptor complex in the pancreas (5). In the present studies, it is unclear whether this is a direct effect on the α-cell, an indirect effect, or both. It is, however, unlikely that the glucagon-lowering effect is mediated by the β-cell because FGF21 did not change insulin levels during the acute infusion studies (Fig. 4E). FGF21 has also been shown *in vitro* not to influence insulin secretion in healthy islets (8). Nevertheless, we cannot rule out that additional factors contribute to glucagon-lowering effects. Although it is possible that the fall in glucagon contributed to hepatic changes, the fact that the fall in endoR_a preceded changes in the hormone further indicate liver-specific effects of FGF21.

An additional important finding from the acute infusion studies was that, with the exception of lowered hepatic triglycerides, all effects linked to FGF21 in *ob*/+ mice were blunted or absent in *ob*/*ob* mice. Importantly, steady-state plasma FGF21 levels were increased to similar pharmacological levels in both genotypes. FGF did have a modest glucose-lowering effect in *ob*/*ob* mice (Fig. 4A), but there were no significant acute effects on steady-state endoR_a or whole-body glucose clearance (Fig. 5, A and C). Blood glucose also fell during the experiment in vehicle-infused *ob*/*ob* mice (Fig. 4A), but this was likely due to the progressive length of the fast, which was 11 h at the final time point. FGF21 did increase hepatic Akt activation in *ob*/*ob* mice, although this was blunted compared with *ob*/+ mice (Fig. 5G). Acute FGF21 infusion was also associated with increased cardiac R_g in *ob*/*ob* mice (Fig. 5D), although this was not statistically significant ($P = 0.06$).

Taken together, these experiments using chronic treatments and acute infusions provide important and novel information about tissue-specific sites of action and functional outcomes of FGF21 treatment. In both settings, FGF21 is shown to regulate hepatic glucose flux primarily to stimulate Akt activation. These effects are consistent with studies demonstrating a powerful hepatic phenotype associated with changes in FGF21 (3–6, 8, 11) and support a direct mechanism of FGF21 action on the liver. These results are unique because they are based on *in vivo* studies performed using state-of-the-art clamp techniques under well-controlled conditions.

The effect of FGF21 to induce effects in the liver, and possibly the α-cell, also aligns with expression of FGF receptors and the required cofactor, βKlotho, in liver and pancreas (28–30). Moreover, absence of βKlotho in skeletal muscle and heart is consistent with findings that neither chronic treatment nor acute infusion of FGF21 directly altered R_g in gastrocnemius or heart. Expression of βKlotho in adipose tissue was, however, not linked to FGF21-mediated metabolic effects. As previously mentioned, this conflicts with *in vitro* data showing that FGF21 potently increases glucose uptake in adipocytes and *in vivo* results in white adipose tissue using 10-fold higher treatment doses (5, 11). It is possible that subtle changes were undetectable because adipose tissue glucose uptake is at least an order of magnitude lower than other tissues (Figs. 2 and 5) (11). Notwithstanding these methodological concerns, the current results indicate that FGF21 does not alter adipose tissue glucose uptake despite efforts to increase sensitivity (*e.g.* analyzing more tissue to achieve higher counts). In regard to expression of βKlotho, an additional finding was that FGF21 did not alter expression of βKlotho in liver or adipose tissue and was undetectable in heart and skeletal muscle, consistent with prior evidence (28–30).

The striking contrast between marked metabolic improvements in *ob*/*ob* mice treated with FGF21 for 8 d compared with blunted pharmacological response in the 6-h FGF21 infusion study suggests that FGF21 may induce chronic and acute effects via different mechanisms. In a chronic setting, FGF21 appears to regulate glycemia via improvements in insulin sensitivity. This conclusion is based on FGF21-mediated effects to lower insulin (Fig. 1C), increase basal glucose clearance (Fig. 2C), and increase GIR needed during an insulin clamp (Fig. 1E). This is supported at a molecular level by increased hepatic Akt phosphorylation (Fig. 2G) and may have contributions from elevations of the insulin receptor, an effect that has been previously reported after chronic FGF21 treatment (3). Consistent with these previous findings, acute infusion of FGF21 induced marked increases in the hepatic expression of transcripts for insulin receptor, Foxa2, and leptin receptor (Table 1) as well as hepatic Akt phosphorylation (Fig. 5G). Overall, these results support the con-

cept that FGF21 functions primarily through transcriptional activation (9).

Differences between genotypes in acute infusion studies also show that *ob*/*ob* mice are resistant to acute FGF21 actions. Recent evidence shows that plasma FGF21 protein is elevated in obese and/or diabetic individuals (31, 32), and FGF21 tissue transcript is increased in mouse models of endocrine disease (7, 28, 33). These findings have led to suggestions of FGF21 resistance in the metabolically compromised state. Our data investigating the effects of FGF21 in *ob*/*ob* mice compared with healthy *ob*/+ mice support this concept and provide the first experimental evidence of an attenuated functional response to FGF21 in a disease state.

In summary, this report directly links the glucose-lowering function of FGF21 with improvements in insulin resistance and changes in glucose flux. These studies show that the liver is the primary target organ associated with FGF21 action. Furthermore, although our evidence suggests a partial resistance to acute effects of FGF21 in metabolically abnormal *ob*/*ob* mice *vs.* healthy *ob*/+ mice, repeated administration of FGF21 resulted in vast metabolic improvements in *ob*/*ob* mice indicating that FGF21 resistance can be overcome by FGF21 treatment. Together, these results are critical to better understand the FGF21 mode of action and add significantly to the concept of FGF21 as a viable and promising therapeutic agent to treat metabolic disease.

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References

1. **Arner P, Pettersson A, Mitchell PJ, Dunbar JD, Kharitonenkov A, Rydén M** 2008 FGF21 attenuates lipolysis in human adipocytes: a possible link to improved insulin sensitivity. FEBS Lett 582:1725–1730
2. **Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E** 2007 Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 5:426–437
3. **Coskun T, Bina HA, Schneider MA, Dunbar JD, Hu CC, Chen Y, Moller DE, Kharitonenkov A** 2008 FGF21 corrects obesity in mice. Endocrinology 12:6018–6027
4. **Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, Li Y, Goetz R, Mohammadi M, Esser V, Elmquist JK, Gerard RD, Burgess SC, Hammer RE, Mangelsdorf DJ, Kliewer SA** 2007 Endocrine regulation of the fasting response by PPARα-mediated induction of fibroblast growth factor 21. Cell Metab 5:415–425
5. **Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, Sandusky GE, Hammond LJ, Moyers JS, Owens RA, Gromada J, Brozinick JT, Hawkins ED, Wroblewski VJ, Li DS, Mehrbod F, Jaskunas SR, Shanafelt AB** 2005 FGF-21 as a novel metabolic regulator. J Clin Invest 115:1627–1635
6. **Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, Hansen BC, Shanafelt AB, Etgen GJ** 2007 The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 148:774–781
7. **Lundåsen T, Hunt MC, Nilsson LM, Sanyal S, Angelin B, Alexson SE, Rudling M** 2007 PPARα is a key regulator of hepatic FGF21. Biochem Biophys Res Commun 360:437–440
8. **Wente W, Efanov AM, Brenner M, Kharitonenkov A, Köster A, Sandusky GE, Sewing S, Treinies I, Zitzer H, Gromada J** 2006 Fibroblast growth factor-21 improves pancreatic β-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. Diabetes 55:2470–2478
9. **Kharitonenkov A, Shanafelt AB** 2008 Fibroblast growth factor-21 as a therapeutic agent for metabolic diseases. BioDrugs 22:37–44
10. **Zhang BB, Moller DE** 2000 New approaches in the treatment of type 2 diabetes. Curr Opin Chem Biol 4:461–467
11. **Xu J, Lloyd DJ, Hale C, Stanislaus S, Chen M, Sivits G, Vonderfecht S, Hecht R, Li YS, Lindberg RA, Chen JL, Jung DY, Zhang Z, Ko HJ, Kim JK, Véniant MM** 2008 FGF21 reverses hepatic steatosis, increases energy expenditure and improves insulin sensitivity in diet-induced obese mice. Diabetes 58:250–259
12. **Izumiya Y, Bina HA, Ouchi N, Akasaki Y, Kharitonenkov A, Walsh K** 2008 FGF21 is an Akt-regulated myokine. FEBS Lett 582:3805–3810
13. **Moyers JS, Shiyanova TL, Mehrbod F, Dunbar JD, Noblitt TW, Otto KA, Reifel-Miller A, Kharitonenkov A** 2007 Molecular determinants of FGF-21 activity-synergy and cross-talk with PPARγ signaling. J Cell Physiol 210:1–6
14. **Muise ES, Azzolina B, Kuo DW, El-Sherbeini M, Tan Y, Yuan X, Mu J, Thompson JR, Berger JP, Wong KK** 2008 Adipose fibroblast growth factor 21 is up-regulated by PPARγ and altered metabolic states. Mol Pharmacol 74:403–412
15. **Ayala JE, Bracy DP, McGuinness OP, Wasserman DH** 2006 Considerations in the design of hyperinsulinemic-euglycemic clamps in the conscious mouse. Diabetes 55:390–397
16. **Morgan CR, Lazarow A** 1962 Immunoassay of insulin using a two-antibody system. Proc Soc Exp Biol Med Society 110:29–32
17. **Ayala JE, Bracy DP, Julien BM, Rottman JN, Fueger PT, Wasserman DH** 2007 Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice. Diabetes 56:1025–1033
18. **Chan TM, Exton JH** 1976 A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal Biochem 71:96–105
19. **Barzilai N, Rossetti L** 1993 Role of glucokinase and glucose-6-phosphatase in the acute and chronic regulation of hepatic glucose fluxes by insulin. J Biol Chem 268:25019–25025
20. **Altszuler N, De Bodo RC, Steele R, Wall JS** 1956 Carbohydrate metabolism of hypophysectomized dogs as studied with radioactive glucose. Am J Physiol 187:25–31
21. **Kharitonenkov A, Shanafelt AB** 2009 FGF21: a novel prospect for

the treatment of metabolic diseases. Curr Opin Investig Drugs 10: 359–364
22. **Ferre T, Pujol A, Riu E, Bosch F, Valera A** 1996 Correction of diabetic alterations by glucokinase. Proc Natl Acad Sci USA 93: 7225–7230
23. **Hajri T, Han XX, Bonen A, Abumrad NA** 2002 Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. J Clin Invest 109:1381–1389
24. **Park EA, Mynatt RL, Cook GA, Kashfi K** 1995 Insulin regulates enzyme activity, malonyl-CoA sensitivity and mRNA abundance of hepatic carnitine palmitoyltransferase-I. Biochem J 310(Pt 3):853–858
25. **Viollet B, Foretz M, Guigas B, Horman S, Dentin R, Bertrand L, Hue L, Andreelli F** 2006 Activation of AMP-activated protein kinase in the liver: a new strategy for the management of metabolic hepatic disorders. J Physiol 574:41–53
26. **Berglund ED, Li CY, Poffenberger G, Ayala JE, Fueger PT, Willis SE, Jewell MM, Powers AC, Wasserman DH** 2008 Glucose metabolism in vivo in four commonly used inbred mouse strains. Diabetes 57: 1790–1799
27. **Eckel RH, Kahn R, Robertson RM, Rizza RA** 2006 Preventing cardiovascular disease and diabetes: a call to action from the American Diabetes Association and the American Heart Association. Diabetes Care 29:1697–1699
28. **Kharitonenkov A, Dunbar JD, Bina HA, Bright S, Moyers JS, Zhang C, Ding L, Micanovic R, Mehrbod SF, Knierman MD, Hale JE, Coskun T, Shanafelt AB** 2008 FGF-21/FGF-21 receptor interaction and activation is determined by βKlotho. J Cell Physiol 215:1–7
29. **Ogawa Y, Kurosu H, Yamamoto M, Nandi A, Rosenblatt KP, Goetz R, Eliseenkova AV, Mohammadi M, Kuro-o M** 2007 βKlotho is required for metabolic activity of fibroblast growth factor 21. Proc Natl Acad Sci USA 104:7432–7437
30. **Suzuki M, Uehara Y, Motomura-Matsuzaka K, Oki J, Koyama Y, Kimura M, Asada M, Komi-Kuramochi A, Oka S, Imamura T** 2008 βKlotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor (FGFR) 1c and FGFR3c. Mol Endocrinol 22:1006–1014
31. **Chen WW, Li L, Yang GY, Li K, Qi XY, Zhu W, Tang Y, Liu H, Boden G** 2008 Circulating FGF-21 levels in normal subjects and in newly diagnose patients with type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 116:65–68
32. **Zhang X, Yeung DC, Karpisek M, Stejskal D, Zhou ZG, Liu F, Wong RL, Chow WS, Tso AW, Lam KS, Xu A** 2008 Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes 57:1246–1253
33. **Satapati S, He T, Inagaki T, Potthoff M, Merritt ME, Esser V, Mangelsdorf DJ, Kliewer SA, Browning JD, Burgess SC** 2008 Partial resistance to PPARα agonists in Zucker diabetic fatty (ZDF) rats is associated with defective hepatic mitochondrial metabolism. Diabetes 57:2012–2021

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