Fibroblast Growth Factor 21 Action in the Brain Increases Energy Expenditure and Insulin Sensitivity in Obese Rats

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OBJECTIVE—The hormone fibroblast growth factor 21 (FGF21) exerts diverse, beneficial effects on energy balance and insulin sensitivity when administered systemically to rodents with diet-induced obesity (DIO). The current studies investigate whether central FGF21 treatment recapitulates these effects.

RESEARCH DESIGN AND METHODS—After preliminary dose-finding studies, either saline vehicle or recombinant human FGF21 (0.4 μg/day) was infused continuously for 2 weeks into the lateral cerebral ventricle of male Wistar rats rendered obese by high-fat feeding. Study end points included measures of energy balance (body weight, body composition, food intake, energy expenditure, and circulating and hepatic lipids) and glucose metabolism (insulin tolerance test, euglycemic-hyperinsulinemic clamp, and hepatic expression of genes involved in glucose metabolism).

RESULTS—Compared with vehicle, continuous intracerebroventricular infusion of FGF21 increased both food intake and energy expenditure in rats with DIO, such that neither body weight nor body composition was altered. Despite unchanged body fat content, rats treated with intracerebroventricular FGF21 displayed a robust increase of insulin sensitivity due to increased insulin-induced suppression of both hepatic glucose production and gluconeogenic gene expression, with no change of glucose utilization.

CONCLUSIONS—FGF21 action in the brain increases hepatic insulin sensitivity and metabolic rate in rats with DIO. These findings identify the central nervous system as a potentially important target for the beneficial effects of FGF21 in the treatment of diabetes and obesity. *Diabetes* **59:1817–1824, 2010**

Fibroblast growth factor (FGF) 21 is a FGF family member produced by liver and other tissues that plays an important role in the control of energy balance and glucose metabolism (1). In addition, when administered at pharmacologic doses, FGF21 induces wide-ranging beneficial effects in animal models of obesity and diabetes (2). Specifically, in obese rodents, pharmacologic FGF21 treatment reduces body fat content and improves glucose tolerance, insulin sensitivity, and lipid parameters (both circulating and hepatic) (3–5). Consequently, FGF21 has emerged as a novel target for the treatment of obesity and associated metabolic dysfunction (2). FGF21-mediated weight loss appears to involve increased fat oxidation and metabolic rate with no change of food intake (3). Whether the insulin-sensitizing effects of FGF21 are dependent on reduced body fat or involve other, independent mechanisms has not been established. Interestingly, these insulin-sensitizing effects are attributable largely to enhanced insulin action in the liver (6), and yet recent evidence suggests that FGF21 regulates hepatic substrate metabolism via a mechanism that cannot be explained by a direct effect on hepatocytes (6).

The diverse and indirect nature of these pharmacologic effects raises the possibility that at least some actions of FGF21 might be mediated centrally. This hypothesis is consistent with growing evidence implicating the hypothalamus and other regions of the central nervous system (CNS) in adaptive adjustments of insulin sensitivity triggered by changing levels of key hormones and nutrients in the circulation (7). To determine whether metabolic effects observed during systemic FGF21 treatment might involve a central site of action, we infused FGF21 into the brain of diet-induced obesity (DIO) rats at a low dose that does not leak into the circulation in detectable amounts.

RESEARCH DESIGN AND METHODS

Male Wistar rats (250–275 g; Harlan, Indianapolis, IN) were individually housed and provided with ad libitum access to water and a high-fat diet (HFD) (60 kcal % from fat, D12492; Research Diets, New Brunswick, NJ) for 8 weeks before study. Lean LA/N rats (Vassar College, Poughkeepsie, NY) were maintained on standard rat chow (Purina, St. Louis, MO). All procedures were performed in accordance with National Institutes of Health Guidelines for the Care and Use of Animals and were approved by the university's Animal Care Committee.

Central FGF21 infusion. Under isoflurane anesthesia, each rat received a cannula (Alzet, infusion kit II; DURECT, Cupertino, CA) implanted stereotaxically into the right lateral ventricle (0.8 mm posterior to bregma, 1.5 mm lateral to the midline, and 3.5 mm below the surface of the skull), fixed onto the skull with dental cement and connected via polyethylene tubing to a subcutaneously implanted 14-day osmotic minipump (Alzet, model 2002). Minipumps were filled with either recombinant human (h) FGF21 (Prospec, Rehovot, Israel) dissolved in 0.1% BSA containing saline or vehicle only (0.1% BSA saline solution).

Body composition, locomotor activity, and indirect calorimetry. Determinations of total body lean and fat mass and liver triglyceride content were made using quantitative magnetic resonance spectroscopy (Echo Medical Systems, Houston, TX). Locomotor activity was assessed by the infrared beam breaks using an Opto-Varimetrix-3 sensor system (Columbus Instruments, Columbus, OH). Indirect calorimetry was performed with a computer-controlled calorimetry system (Oxymax; Columbus Instruments). V_{O_2} and V_{CO_2} were normalized to lean body mass. Energy expenditure (EE) was calculated based on $EE = 3.815 \times V_{O_2} + 1.232 \times V_{CO_2}$ (8).

Euglycemic-hyperinsulinemic clamps. Rats underwent all surgical procedures (intracerebroventricular cannulation and implantation of arterial and venous catheters) at least 6 days before study, as previously described (9). After a 16-h fast, rats were placed into an animal enclosure that allowed simultaneous sampling of arterial blood while infusing into the jugular vein. The clamp protocol (9) included a 2-h basal period during which only [3-^{3}H]glucose tracer was infused. Two hours after the basal period, a primed continuous infusion of regular human insulin (60 mU/kg bolus followed by 1.8 mU · kg^{-1} · min^{-1} Humulin R (Eli Lilly, Indianapolis, IN) was administered at $t = 0$ min, and a 50% dextrose solution was infused to maintain euglycemia. Serial blood samples were obtained throughout the basal and clamp periods for determination of plasma glucose and insulin levels and for calculation of glucose turnover rates as described (9).

Insulin and glucose tolerance tests. After 10 days of intracerebroventricular infusion, an insulin tolerance test (ITT) was performed after a 4-h fast in the midlight cycle by an injection of insulin (0.75 units/kg i.p. for DIO rats and 0.5 units/kg i.p. for lean rats; Humulin R), with glucose levels determined at indicated time points on blood samples taken from tail vein blood using a hand-held glucometer (Accu-Chek; Roche, Basel, Switzerland). Glucose tolerance tests were also conducted after an overnight fast. After an intraperitoneal injection of 30% D-glucose (2.5 g/kg) (Abbott Laboratories, North Chicago, IL), glucose levels were measured in blood obtained from the tail vein at the indicated times points.

Serum and liver analysis. Plasma insulin and hFGF21 levels were determined by ELISA (Crystal Chem, Downers Grove, IL, and BioVendor, Modrice, Czech Republic, respectively). Plasma free fatty acids (FFAs) and triglycerides were measured using colorimetric assay (Wako Chemicals, Richmond, VA). Analysis of liver glycogen content was performed using an calorimetric assay according to the manufacturer's instructions (BioVision, Mountain View, CA).

RT-PCR. RNA extraction, reverse transcription and real-time PCR for amplification of *Pepck*, *G6Pase*, *ACC2*, *Foxa2*, *PGC1α*, *PPARα*, *SCD1*, and *SREBP-1c* was performed as previously described (9).

Statistical analysis. All results are expressed as means ± SEM. A one-way ANOVA with a least significant difference post hoc test was used to compare mean values between multiple groups and a two-sample, unpaired Student t test was used for two-group comparisons. $P < 0.05$ was considered significant.

RESULTS

Effect of intracerebroventricular hFGF21 infusion on energy balance and body composition in DIO rats.
Our first goal was to identify a dose of hFGF21 that does not enter the plasma in detectable amounts when administered centrally. To confirm our ability to detect hFGF21 immunoreactivity in the circulation, plasma from mice receiving a continuous subcutaneous hFGF21 (0.2 mg/kg/day) infusion was analyzed. As expected, hFGF21 immunoreactivity was readily detected (14.6 ± 0.2 μg/ml) using an hFGF21-specific ELISA on plasma taken from these mice. We next administered either vehicle or one of three doses of hFGF21 (4.0, 0.40, and 0.04 μg/day; each well below doses that induce metabolic effects during systemic administration) (3,4) as a continuous infusion into an indwelling cannula inserted into the lateral ventricle of adult male Wistar rats. For 8 weeks before brain cannulation, rats were rendered obese by consuming HFD, and after 14 days of intracerebroventricular infusion, leakage of hFGF21 from brain into plasma was assessed. Although hFGF21 was clearly detected in the plasma of rats receiving continuous intracerebroventricular infusion of the highest dose (4.0 μg/day) of hFGF21 (0.91 ± 0.86 μg/ml), neither of the lower doses (0.04 or 0.40 μg/day) detectably increased plasma levels (not shown). Therefore, hFGF21 does not detectably enter plasma during continuous intracerebroventricular infusion of up to 0.4 μg/day.

Continuous intracerebroventricular infusion of hFGF21 at doses of either 0.04 or 0.40 μg/day was without effect on body weight or body composition throughout a 14-day period of observation (mean body weight = 581.9 ± 10.4 g) compared with weight-matched, vehicle-treated rats consuming an identical HFD (Fig. 1*A* and *B*). Given

this outcome, we were somewhat surprised to observe that animals receiving intracerebroventricular hFGF21 at 0.40 μg/day consumed 20–30% more food than vehicle-treated controls from days 4–10 of infusion (Fig. 1*C* and *D*). To explain these divergent findings, we hypothesized that intracerebroventricular hFGF21 infusion also increases energy expenditure, as occurs with systemic administration (3), such that neutral energy balance is maintained. To test this hypothesis, we used indirect calorimetry to measure rates of V_{O_2} and V_{CO_2} in rats after 10 days of continuous intracerebroventricular infusion of either vehicle or hFGF21 (0.40 μg/day) while consuming the same HFD. Both V_{O_2} and energy expenditure tended to be higher in the hFGF21-treated group compared with controls throughout the observation period (Fig. 1*E* and *F*), and this effect achieved statistical significance during the light cycle (when animals are relatively inactive), but not during the dark cycle ($P < 0.05$). This pattern of increased energy expenditure in the light cycle suggests but does not establish that resting metabolic rate was increased by central hFGF21 infusion. By comparison, respiratory quotient (a measure of fat vs. carbohydrate oxidation) and ambulatory activity were not different between groups (data not shown).

Effect of central hFGF21 infusion on insulin sensitivity in DIO rats.
As a first step to determine whether hFGF21 action in the brain increases insulin sensitivity, we performed an ITT after 5 days of continuous intracerebroventricular infusion of either hFGF21 or saline in rats fed a HFD. Insulin-induced glucose lowering was increased by intracerebroventricular hFGF21 at the 0.40 μg/day dose relative to vehicle (Fig. 2*A*), suggesting improved insulin sensitivity, although differences of baseline (4 h food-deprived) blood glucose and plasma insulin levels were not detected (Fig. 2*C* and *D*). We also found no differences in liver triglyceride content (Fig. 2*E*), and although liver glycogen content tended to be higher in intracerebroventricular hFGF21-infused rats (Fig. 2*F*), this difference did not reach statistical significance ($P = 0.06$).

To further investigate the effect of centrally administered hFGF21 on insulin sensitivity, we performed euglycemic-hyperinsulinemic clamp studies in separate groups of weight-matched, HFD-fed rats on day 7 of continuous intracerebroventricular infusion of either saline or hFGF21 (0.40 μg/day). As in our previous studies, intracerebroventricular infusion of hFGF21 had no effect on body weight (despite increased cumulative food intake), nor did it alter fasting plasma insulin, triglyceride, or FFA levels (Table 1). Although fasting glucose levels tended to be lower in hFGF21-treated rats, this effect did not achieve statistical significance ($P = 0.08$).

During the clamp procedure, arterial glucose and plasma insulin concentrations were matched between groups (by design) (Fig. 2*H*, Table 1), but the glucose infusion rate (GIR) needed to maintain euglycemia was increased by 70% in animals receiving intracerebroventricular hFGF21 compared with vehicle (Fig. 2*G*, Table 1), establishing that hFGF21 action in the brain increases insulin sensitivity in obese, HFD-fed rats. This was accompanied by an effect of intracerebroventricular hFGF21 to enhance the effect of insulin to lower the rate of glucose appearance (measured as the difference in R_a between the basal and clamped states) (Fig. 3*A*) relative to rats receiving intracerebroventricular vehicle, such that insulin-mediated suppression of hepatic glucose production was increased by twofold (Fig. 3*B*). By comparison, the rate of
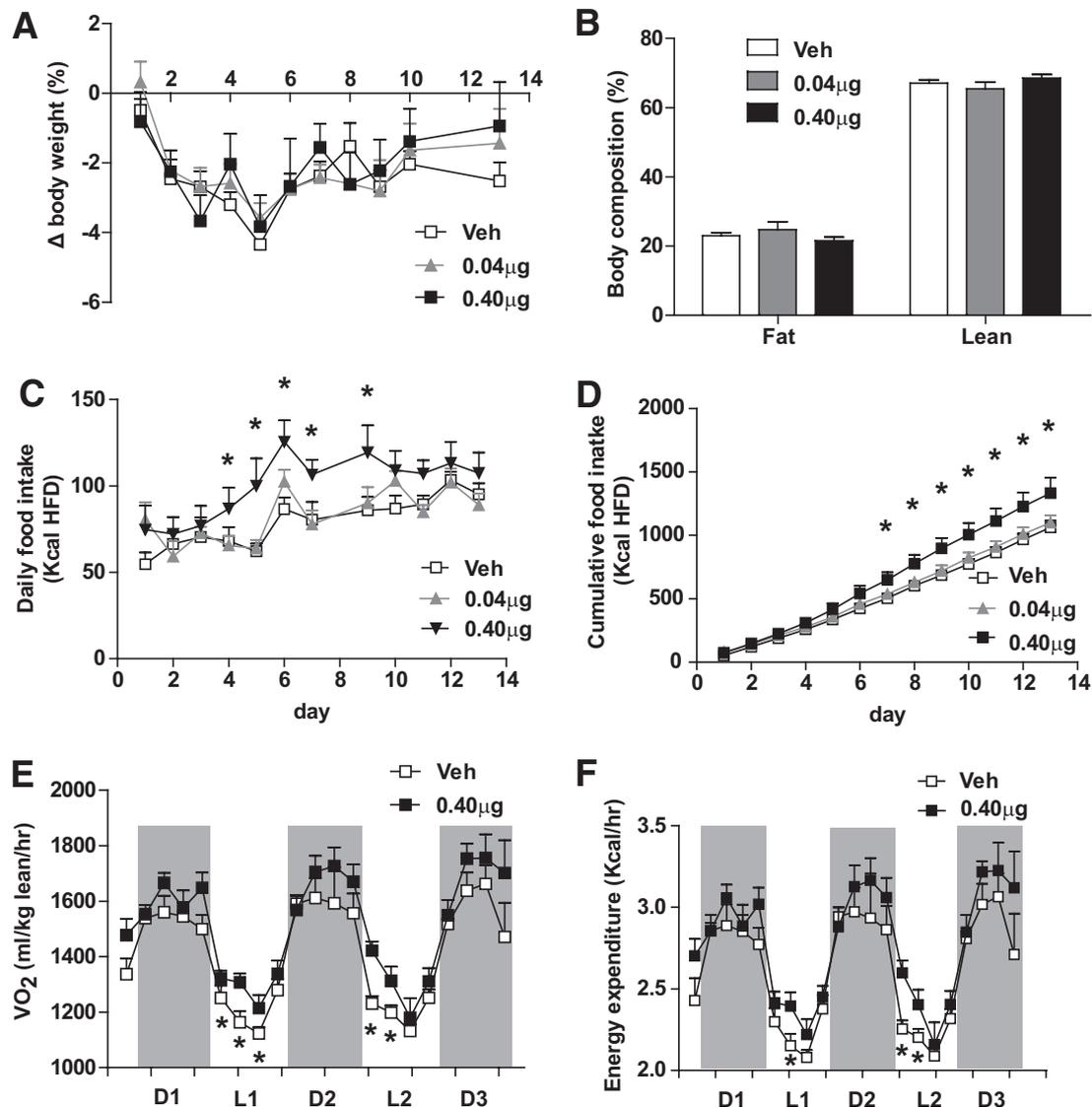
FIG. 1. Effect of central hFGF21 infusion on energy homeostasis in DIO rats. Percent body weight change (*A*), body composition (*B*), daily food intake (*C*), cumulative food intake (*D*), Vo_2 (*E*), and energy expenditure (*F*) in adult male rats with DIO (*n* = 6 per group) receiving continuous intracerebroventricular infusion of either hFGF21 (0.04 and 0.40 μg/day) or vehicle. For clarity, Vo_2 and energy expenditure data in *E* and *F* are presented as 3-h averages over three consecutive 12-h dark (D1–3) and two 12-h light (L1–2) cycles. *$P < 0.05$ vs. vehicle (Veh).

glucose disposal (R_d) was not altered by intracerebroventricular hFGF21 during either basal or clamp periods (Fig. 3*C* and *D*). Moreover, plasma hFGF21 immunoreactivity was not detected in either group, confirming that intracerebroventricular-infused hFGF21 did not leak into the periphery (Table 1). Samples taken after the completion of the clamp revealed no difference in liver triglyceride content between groups (18.3 ± 1.2 vs. 17.2 ± 1.4% for hFGF21 vs. vehicle; NS), nor were there differences in liver glycogen content (Table 1). Thus, hFGF21 action in the CNS improves hepatic insulin sensitivity of rats with DIO without altering either total body or hepatic fat content.

To gain further insight into the mechanism underlying the insulin-sensitizing effects of intracerebroventricular hFGF21, we measured expression of two key gluconeogenic genes, glucose-6-phosphatase (*G6Pase*) and phosphoenolpyruvate carboxykinase (*Pepck*), in liver samples obtained both in the basal state (after a 4-h fast) and after the completion of clamp (insulin-stimulated). Among clamped animals, hepatic expression of both

G6Pase and *Pepck* mRNA was significantly reduced (by 52 and 47%, respectively) in animals receiving intracerebroventricular hFGF21 (Fig. 4*A*), whereas in the basal state, *Pepck* mRNA levels were unchanged and expression of *G6Pase* mRNA was slightly increased by intracerebroventricular hFGF21 (Fig. 4*B*). Thus, intracerebroventricular hFGF21 augments insulin-mediated inhibition of hepatic *G6Pase* and *Pepck* expression, an effect that was absent (if not reversed) in the absence of insulin stimulation.

Our observation of increased caloric intake despite no change in total body or hepatic fat content in rats receiving intracerebroventricular hFGF21 led us to ask whether hepatic expression of genes involved in lipid metabolism is altered by this treatment. In the insulin-stimulated state (postclamp), hepatic mRNA expression of the lipolytic gene forkhead box A2 (*Foxa2*) was increased (by 77%), whereas mRNA expression levels of the lipogenic genes sterol regulatory element-binding protein-1c (*SREBP-1C*) and stearoyl-CoA desaturase-1 (*SCD1*) were reduced (by 50 and 55%, respectively) by intracerebroventricular
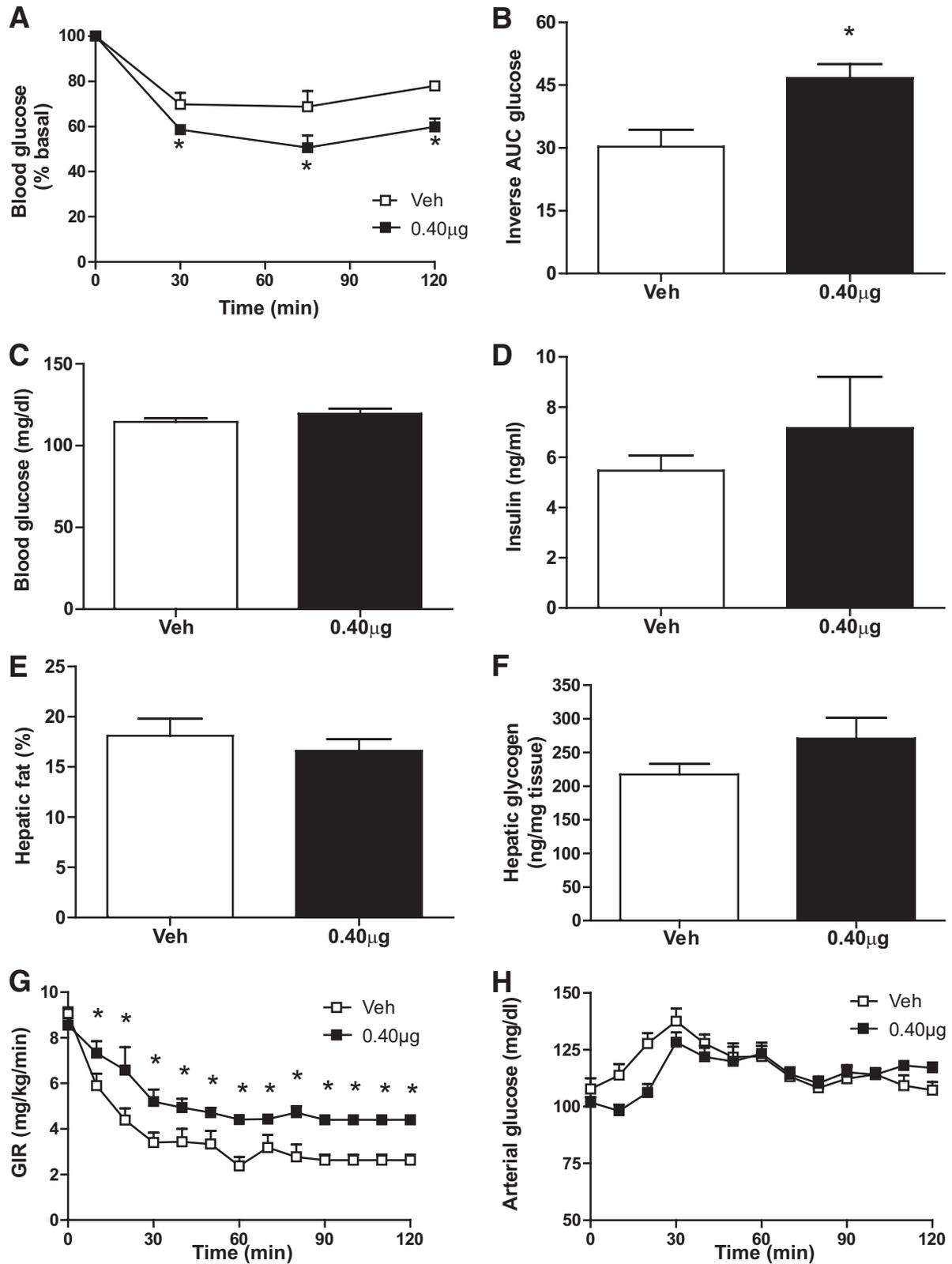
FIG. 2. Central infusion of hFGF21 improves insulin sensitivity in DIO rats. Percent basal blood glucose levels (*A*), inverse area under the curve (*B*) during ITT, 4-h fasted blood glucose (*C*), plasma insulin levels (*D*), hepatic fat (*E*), and glycogen content (*F*) obtained from male Wistar rats with DIO (*n* = 6 per group) that received continuous intracerebroventricular infusion of either hFGF21 (0.40 μg/day) or vehicle for 2 weeks. Glucose infusion rate (GIR) (*G*) and arterial glucose levels (*H*) were measured during the euglycemic-hyperinsulinemic clamp on day 7 of intracerebroventricular infusion in separate groups of rats. *$P < 0.05$ vs. vehicle (Veh).

hFGF21 compared with vehicle (Fig. 4*A*). In contrast, in the basal state (4-h fast), these effects were reversed such that intracerebroventricular hFGF21 induced a slight but

significant increase of hepatic *SREBP-1C* mRNA levels and a doubling of acetyl-CoA carboxylase-2 (*ACC2*) mRNA content (Fig. 4*B*), consistent with decreased lipid oxida-

TABLE 1
Basal and clamp characteristics of DIO rats receiving continuous intracerebroventricular infusion of either hFGF21 or vehicle

	Vehicle	hFGF21
n	6	7
Basal		
Body weight (g)	527.1 ± 18.9	561.1 ± 26.7
Δ Body weight (g)	-23.0 ± 3.2	-25.6 ± 4.4
HFD intake (g/5 days)	62.6 ± 4.2	$84.6 \pm 4.8^*$
Plasma hFGF21	N.D.	N.D.
Arterial plasma glucose (mg/dl)	112.2 ± 4.0	104.0 ± 5.0
Plasma insulin (ng/ml)	2.40 ± 0.68	2.12 ± 0.54
Plasma nonesterified fatty acids (mmol/l)	0.39 ± 0.020	0.41 ± 0.014
Plasma triglycerides (mg/dl)	41.0 ± 4.3	48.3 ± 6.0
Clamp		
Arterial plasma glucose (mg/dl)	110.7 ± 5.4	116.0 ± 2.4
Plasma insulin (ng/ml)	6.6 ± 0.35	7.1 ± 0.90
GIR (mg/kg/min)	2.6 ± 0.24	$4.4 \pm 0.10^*$
Hepatic glycogen (ng/mg tissue)	144.1 ± 12.4	152.7 ± 12.5

Data are means $\pm$ SEM. N.D., not determined. $*P < 0.05$ vs. vehicle.

tion. Together, these observations suggest that centrally infused hFGF21 may protect against hepatic lipid accumulation in the insulin-stimulated state, but not in settings where insulin levels are not elevated.

Effect of central hFGF21 infusion on energy homeostasis and glucose metabolism in lean rats. To determine whether effects of central hFGF21 infusion on energy homeostasis and insulin sensitivity are limited to obese rats, either saline or hFGF21 (0.40 μg/day) was infused for 7 days into the lateral ventricle of lean, chow-fed rats. Although no net changes in body weight were observed over the 7-day infusion period (Fig. 5A),

FIG. 3. Central infusion of hFGF21 improves insulin sensitivity by increasing insulin-mediated suppression of endogenous glucose production. Rate of endogenous glucose appearance (Endo R_a) during the basal (□) and clamp (■) periods (A), percent suppression of hepatic glucose production (B), rate of glucose disposal during the basal and clamped periods (C), and percent increase in glucose utilization (D) during euglycemic-hyperinsulinemic clamps performed during continuous intracerebroventricular infusion of hFGF21 (0.40 μg/day) or vehicle as in Fig. 2. $*P < 0.05$ vs. vehicle (Veh).
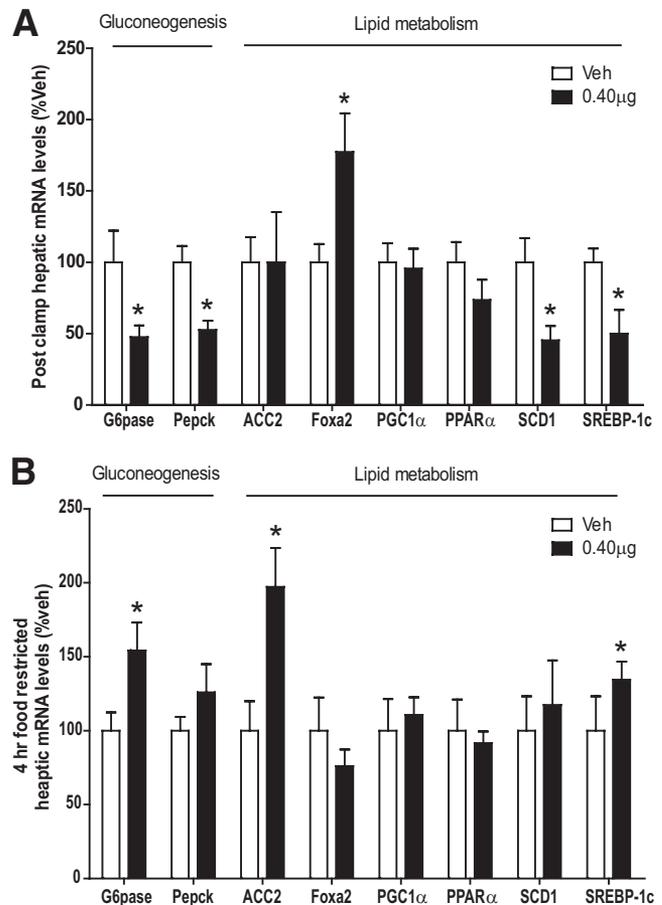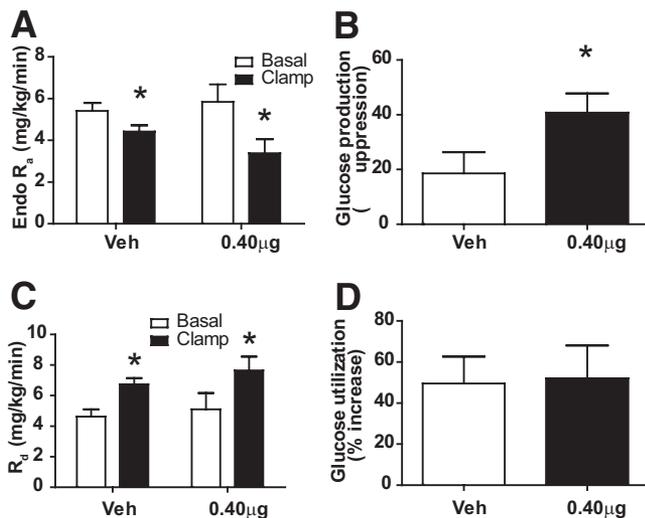
FIG. 4. Effect of central hFGF21 infusion on hepatic genes involved in gluconeogenesis and lipid metabolism. Hepatic levels of mRNA encoding gluconeogenic and lipid metabolic genes measured in either the presence (e.g., after the clamp) (A) or absence of hyperinsulinemia (e.g., after a 4-h fast) (B) in rats ($n = 6-7$ per group) receiving continuous intracerebroventricular infusion of hFGF21 (0.40 μg/day) or vehicle. $*P < 0.05$ vs. vehicle (Veh).

daily food intake was increased on days 5 through 7 in animals receiving intracerebroventricular hFGF21 relative to vehicle (Fig. 5B), analogous to what was observed in DIO rats (Fig. 1). After 5 days of continuous intracerebroventricular infusion, insulin-induced glucose lowering was unchanged in the hFGF21-infused rats relative to vehicle during an ITT (data not shown). Because differences in insulin tolerance can be difficult to see in already insulin-sensitive lean rats, we also subjected them to an intraperitoneal glucose tolerance test (IPGTT) and found that glucose tolerance was increased in intracerebroventricular hFGF21-treated rats relative to vehicle (Fig. 5C). No differences in plasma insulin (4 h after the IPGTT) or glucose levels (4-h food-deprived or fasted) were observed between intracerebroventricular hFGF21 vs. vehicle-infused rats (data not shown). Hepatic glycogen content tended to be higher in hFGF21-infused rats relative to vehicle (93.9 ± 4.1 vs. 72.4 ± 10.8 ng/mg tissue for hFGF21 vs. vehicle), but this effect did not achieve statistical significance ($P = 0.06$).

DISCUSSION

The wide-ranging effects of systemic hFGF21 administration on body composition, energy balance, substrate use, and insulin sensitivity suggest a central site of action. This hypothesis is strengthened by evidence that the hepatic
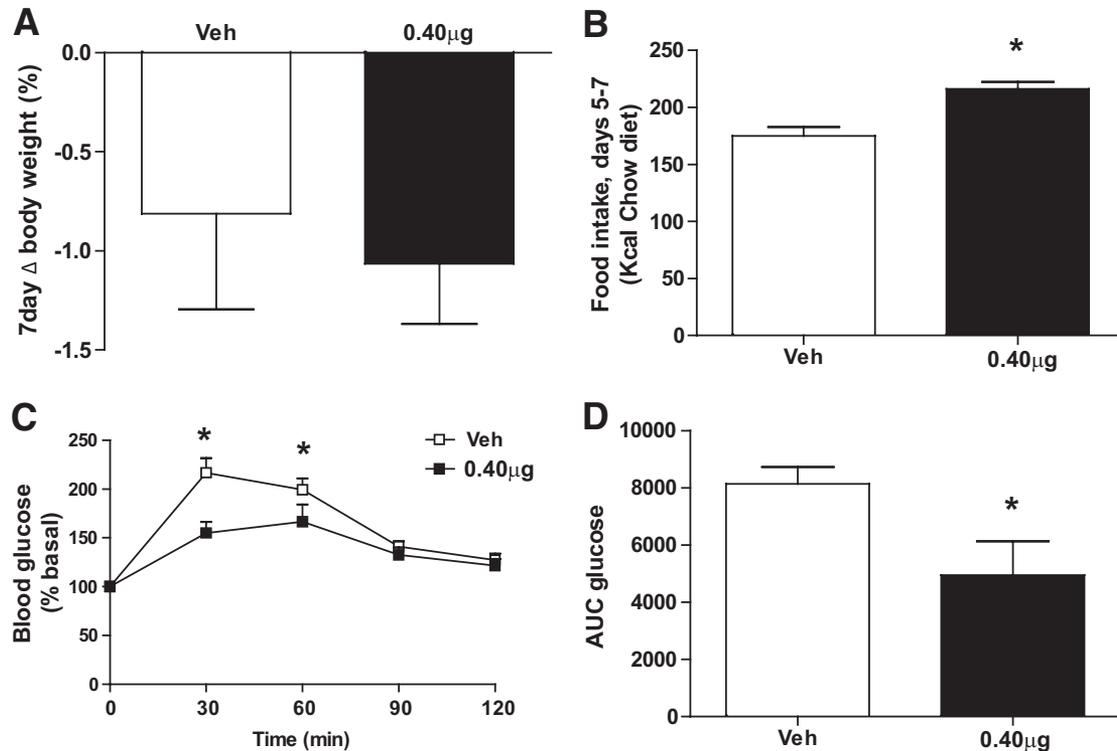
FIG. 5. Effect of central hFGF21 infusion on energy homeostasis and glucose metabolism in lean rats. Percent body weight change (*A*), cumulative food intake (days 5–7) (*B*), and results obtained during an IPGTT, including percent change from basal blood glucose levels (*C*) and area under the curve (AUC) (*D*) in chow-fed adult male rats (*n* = 4 per group) receiving continuous intracerebroventricular infusion of either hFGF21 (0.40 μg/day) or vehicle for 7 days *P < 0.05 vs. vehicle (Veh).

effects of systemically administered hFGF21 appear to be mediated indirectly (6) and that FGF21 enters the brain from the circulation (10). Here we report that chronic central infusion of hFGF21 in rats with DIO increases insulin sensitivity selectively in the liver and that this effect is not due to reduced body fat mass.

Our finding that intracerebroventricular hFGF21 infusion enhances insulin-mediated suppression of hepatic glucose production, while having no effect on glucose utilization, is reminiscent of the action of several other hormone- and nutrient-related signals that regulate glucose metabolism through a central mechanism. These include insulin, leptin, FFAs such as oleate, and glucose itself (11–17), each of which enhances hepatic insulin sensitivity via an action involving the hypothalamus. Moreover, the effect of each of these interventions to increase hepatic insulin sensitivity is accompanied (and potentially mediated) by enhanced insulin-mediated suppression of hepatic *G6Pase* and *Pepck* expression (11–14), as was observed for intracerebroventricular hFGF21. Because fibroblast growth factor receptor (FGFR)-1, one of a family of FGFRs that mediate FGF21 signal transduction, is concentrated in hypothalamic areas such as the arcuate and ventromedial nuclei of the hypothalamus that mediate the metabolic effects of insulin, leptin, oleate, and glucose (16–19), we hypothesize that neurocircuits involved in the response to FGF21 overlap with those mediating the action of key hormone- and nutrient-related stimuli, and studies to test this hypothesis are a high priority.

Our understanding of how FGFRs mediate FGF21 signal transduction is incomplete. Studies from cell culture models have demonstrated that FGFR1, FGFR2, and FGFR3 can mediate cellular responses to FGF21, including phosphorylation of the receptor, receptor substrate, and mito-

gen-activated protein kinase, leading to induction of *Egr-1* gene expression, and have identified β-klotho as an indispensable FGF21 cofactor (20–22, 24). The recent finding that FGF21 can induce signal transduction even in β-klotho knockout mice (23), however, suggests that at least in some tissues, β-klotho may not be required for FGF21-mediated signaling. Future studies are warranted both to determine whether FGFR1 and β-klotho are essential mediators of the effects of FGF21 in the CNS and to identify the neuronal subsets involved. In addition, because several other FGF family members can signal through the same FGFRs, it will be of interest to determine whether they exert central effects that alter energy balance or glucose metabolism.

One key difference between our findings and effects reported during peripheral hFGF21 infusion is that systemic, but not intracerebroventricular, FGF21 administration reduces body fat content. Interestingly, this effect is mediated by increased energy expenditure with no change of food intake (3). Although both central and systemic hFGF21 treatment increases metabolic rate, we observed a sustained increase of food intake during intracerebroventricular hFGF21 infusion that prevented any overall change in energy balance or body composition. Although the mechanisms underlying this hyperphagia await further study, we hypothesize that this response was elicited as a result of increased metabolic rate. This hypothesis is supported by the relatively late onset of hyperphagia, which became evident only after 3 days of intracerebroventricular infusion, along with the fact that during peripheral infusion, energy expenditure increases within the first 24 h of FGF21 administration (our unpublished data and ref. 4). However, we cannot at this time exclude the possibility that the hyperphagia we observed is a direct

consequence of hFGF21 action in the brain rather than a secondary response to increased energy expenditure, and further studies are warranted to investigate this question. Combined with our finding that unlike systemic treatment (4), intracerebroventricular hFGF21 infusion did not increase physical activity, these observations suggest that a peripheral site of action is required for the capacity of FGF21 to promote negative energy balance and weight loss but not to increase either metabolic rate or liver insulin sensitivity.

Although central hFGF21 infusion had no effects on either hepatic fat content or respiratory quotient (a measure of total body fat oxidation), analysis of its effect on hepatic expression of genes involved in lipid metabolism suggest that it may nonetheless promote lipid oxidation while decreasing lipid storage in the insulin-stimulated state (an effect that is lost in the nonstimulated state). Further, our finding that the effect of intracerebroventricular hFGF21 on liver gene expression resembles that reported during peripheral infusion of much larger FGF21 doses (3,4) suggests that peripheral FGF21 affects the liver at least in part via an action in the CNS. Because FGF21 reduces liver fat content after peripheral but not central administration, it is possible that in the latter setting, improvements in hepatic lipid metabolism are offset by increased caloric intake. Alternatively, peripheral actions of FGF21 may promote the clearance of liver fat via mechanisms additional to those involving the CNS. In either case, amelioration of hepatic steatosis is not required for the insulin-sensitizing action of hFGF21 in the brain. Lastly, we note that changes in hepatic expression of genes involved in both gluconeogenesis and lipid metabolism induced by intracerebroventricular hFGF21 were sensitive to ambient insulin levels. Similarly, the effect of centrally acting hFGF21 on insulin sensitivity was only reliably detectable during the clamp conditions in which plasma insulin levels are raised to values in the high physiologic range. This outcome again is reminiscent of what is seen with other hormones and nutrients that act in the hypothalamus to increase peripheral insulin sensitivity (3,4) and raise interesting possibilities for future study.

Although this study was undertaken primarily to determine whether central hFGF21 infusion mimics the beneficial effects observed during systemic administration in rats with DIO, our findings suggest that the metabolic effects of central FGF21 action are not limited to obese animals consuming a HFD. Our preliminary results in lean, chow-fed rats show that intracerebroventricular hFGF21 increased food intake while having no net effect on body weight, as was observed in rats rendered obese by high-fat feeding. Although metabolic rate was not measured in lean animals, this combination of findings is suggestive of increased energy expenditure, as was observed in DIO rats. Similarly, glucose tolerance was increased by intracerebroventricular hFGF21 administration to lean, chow-fed animals, although insulin sensitivity has yet to be formally assessed in this setting, and additional studies are warranted to more definitively investigate this response.

A substantial physiologic role for FGF21 in nutrient metabolism is suggested by evidence that FGF21-deficient mice are prone to late-onset weight gain and exhibit metabolic defects including decreases of ketogenesis, glucose tolerance, and metabolic rate (1). In light of our current work, the observation that circulating FGF21 levels increase during high-fat feeding in type 2 diabetes and during both fasting and refeeding (25–27) raises the question of whether physiologic responses induced by changing FGF21 levels in these conditions involve a central site of action. Similarly, our findings support the possibility that a central mechanism mediates the effect of peripherally administered hFGF21 to improve hepatic insulin sensitivity (25–27), a testable hypothesis relevant to ongoing and future efforts to investigate the utility of FGF21 in the treatment of human obesity and diabetes.

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