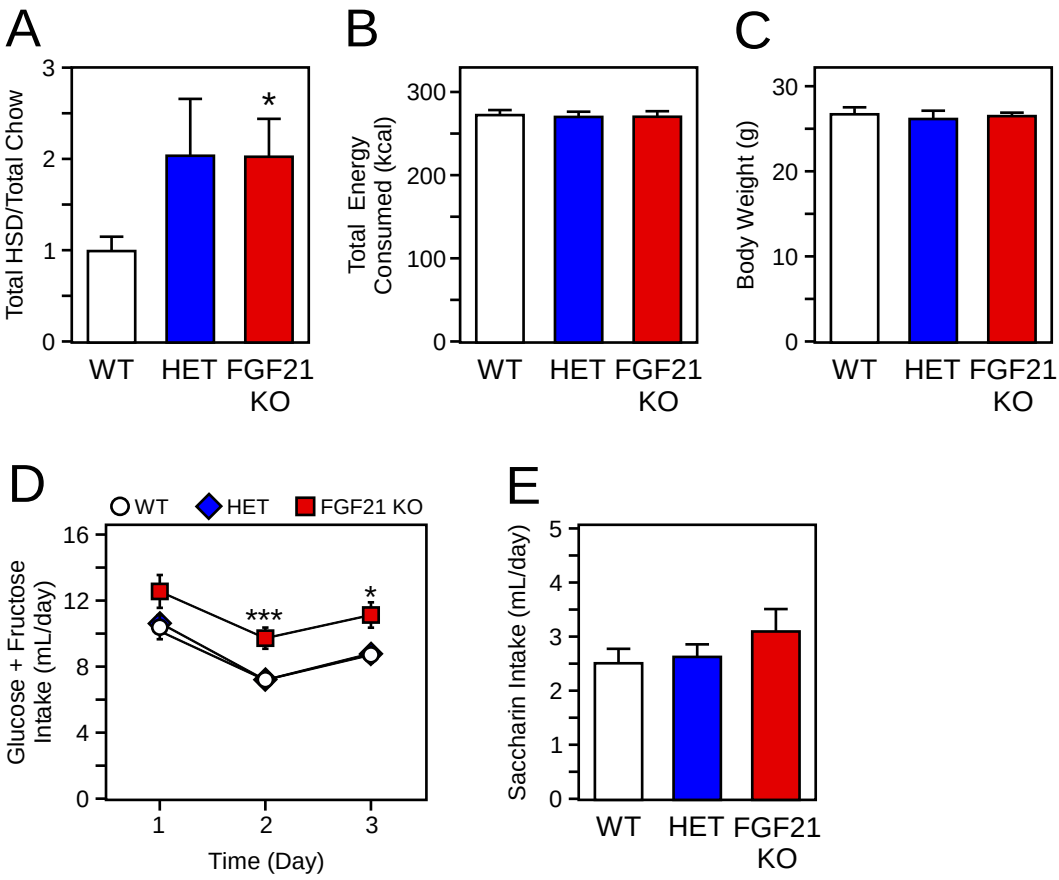
A
Total HSD/Total Chow
3
2
1
0
*
WT
HET
FGF21 KO
B
Total Energy Consumed (kcal)
300
200
100
0
WT
HET
FGF21 KO
C
Body Weight (g)
30
20
10
0
WT
HET
FGF21 KO
D
WT
HET
FGF21 KO
Glucose + Fructose Intake (mL/day)
16
12
8
4
0
***
*
1
2
3
Time (Day)
E
Saccharin Intake (mL/day)
5
4
3
2
1
0
WT
HET
FGF21 KO

Figure S1

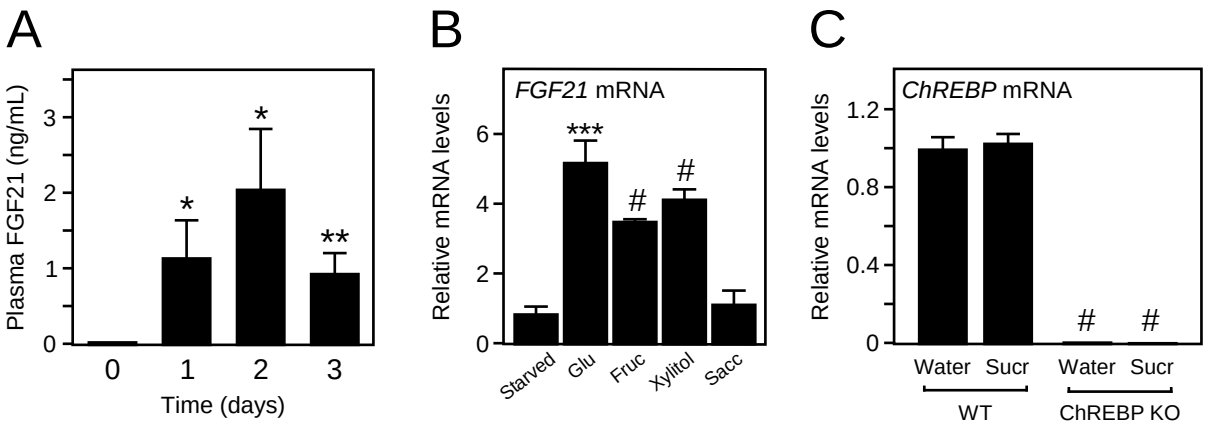
A
Plasma FGF21 (ng/mL)
0
1
2
3
*
*
**
0
1
2
3
Time (days)
B
FGF21 mRNA
Relative mRNA levels
0
2
4
6
***
#
#
Starved
Glu
Fruc
Xylitol
Sacc
C
ChREBP mRNA
Relative mRNA levels
0
0.4
0.8
1.2
#
#
Water
Sucr
Water
Sucr
WT
ChREBP KO

Figure S2

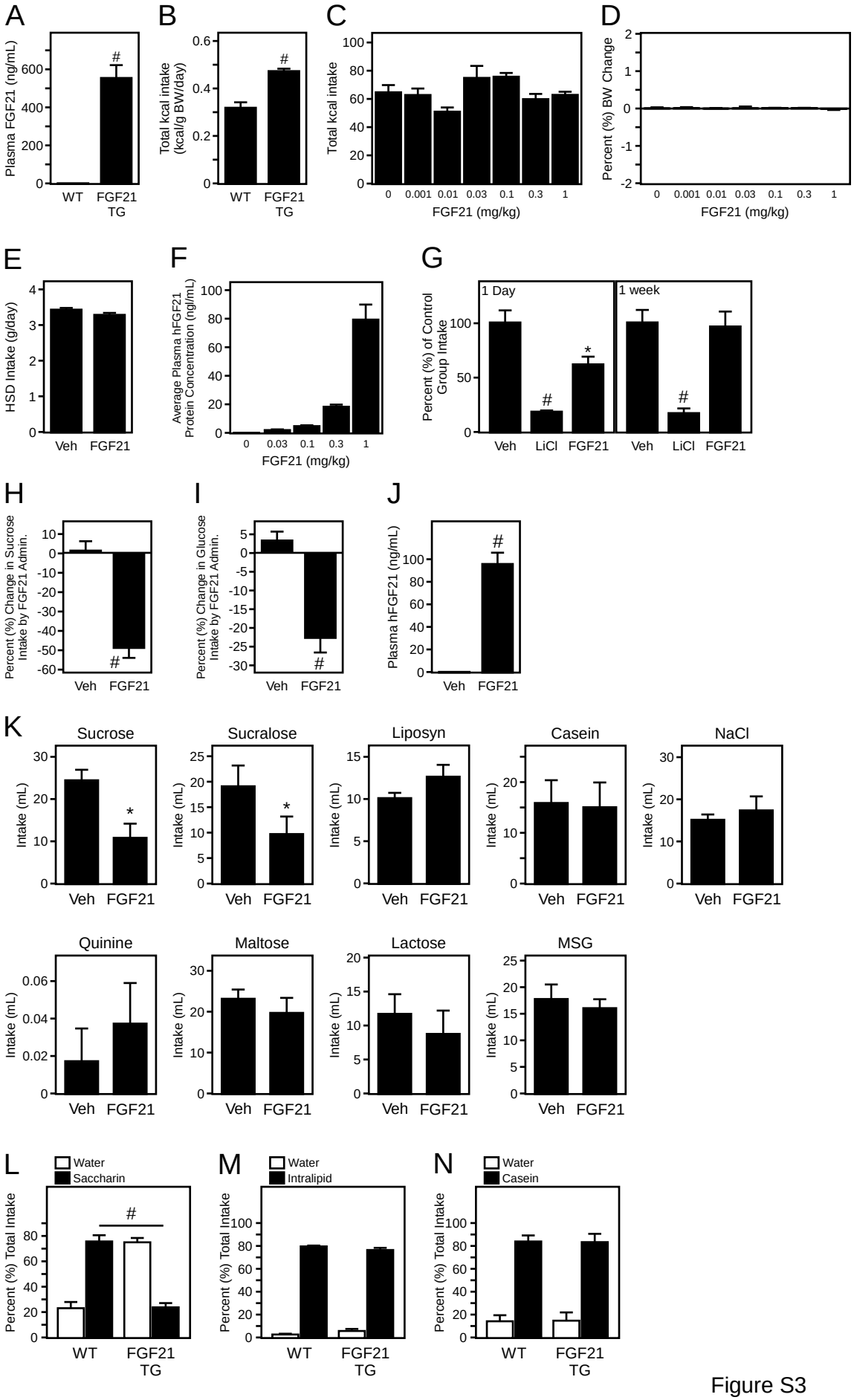
A
Plasma FGF21 (ng/mL)
WT
FGF21 TG
B
Total kcal intake (kcal/g BW/day)
C
Total kcal intake
FGF21 (mg/kg)
D
Percent (%) BW Change
E
HSD Intake (g/day)
Veh
FGF21
F
Average Plasma hFGF21 Protein Concentration (ng/mL)
G
Percent (%) of Control Group Intake
1 Day
1 week
Veh
LiCl
FGF21
H
Percent (%) Change in Sucrose Intake by FGF21 Admin.
I
Percent (%) Change in Glucose Intake by FGF21 Admin.
J
Plasma hFGF21 (ng/mL)
K
Sucrose
Sucralose
Liposyn
Casein
NaCl
Quinine
Maltose
Lactose
MSG
Intake (mL)
L
Water
Saccharin
M
Water
Intralipid
N
Water
Casein
Percent (%) Total Intake

Figure S3

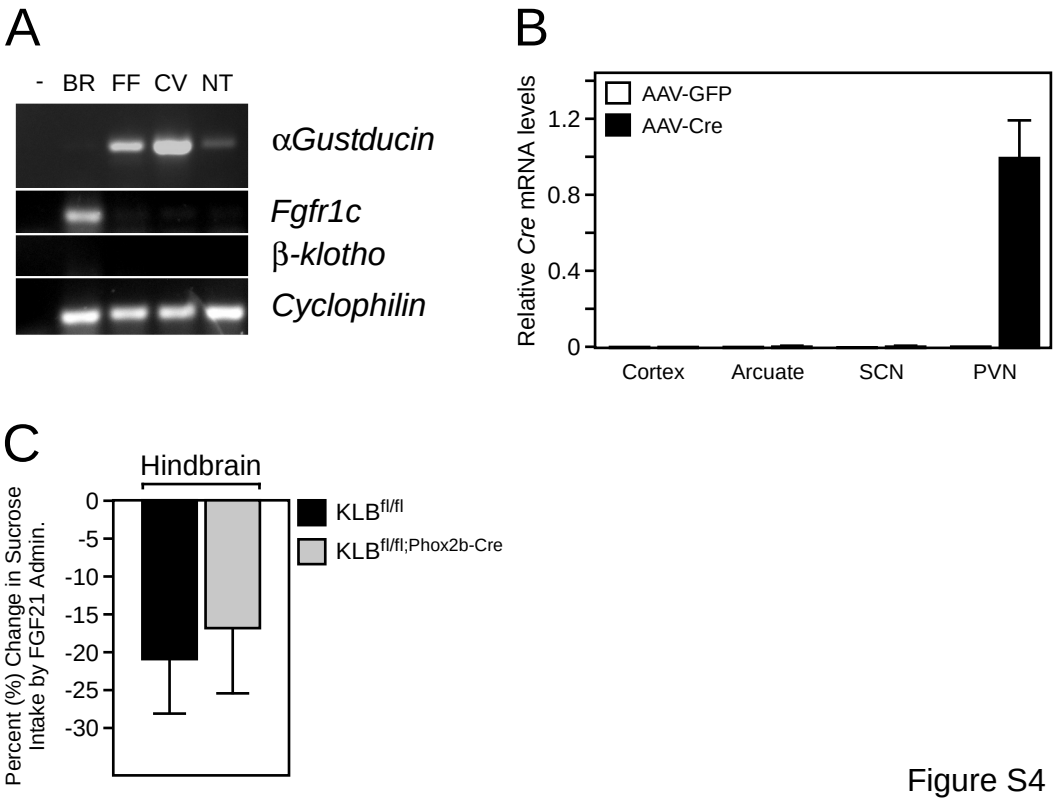
A
- BR FF CV NT
αGustducin
Fgfr1c
β-klotho
Cyclophilin
B
AAV-GFP
AAV-Cre
Relative Cre mRNA levels
1.2
0.8
0.4
0
Cortex
Arcuate
SCN
PVN
C
Hindbrain
Percent (%) Change in Sucrose Intake by FGF21 Admin.
0
-5
-10
-15
-20
-25
-30
KLBfl/fl
KLBfl/fl;Phox2b-Cre

Figure S4

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. FGF21 KO Mice Have Increased Sugar, But Not Energy, Intake. (A) Total HSD intake/total chow intake and (B) total kcal intake in wild-type (WT), FGF21 heterozygous (HET), and FGF21 total KO (FGF21 KO) littermates (n = 7-8/group). (C) Body weights of WT, HET, and FGF21 KO mice (n = 6-9/group) used in two-bottle choice versus water. (D) Glucose plus fructose intake in male WT, HET, and FGF21 KO mice in two-bottle choice versus water (n = 6-14/group). (E) Saccharin (0.2%) intake in two-bottle choice versus water in WT, HET, and FGF21 KO mice (n = 6-9/group). Values are mean +/- SEM. (*, $P< 0.05$; ***, $P<0.005$).

Figure S2, related to Figure 2. Carbohydrate Acts Directly on Hepatocytes to Increase FGF21. (A) Plasma FGF21 levels in 12-week old, male wild-type C57Bl/6 mice provided *ad libitum* access to 10% sucrose and chow for the indicated time (n = 4/group). (B) *FGF21* mRNA levels in HepG2 cells treated with 5 mM glucose, 5 mM fructose, 0.2% saccharin, or 5 mM xylitol for 4 hours (performed in triplicate twice). (C) Hepatic *Chrebp* mRNA levels in WT and ChREBP KO mice *ad libitum* fed chow and 10% sucrose *ad libitum* for 24 hours (n = 5-8/group). Values are mean +/- SEM. (*, $P< 0.05$; **, $P< 0.01$; #, $P< 0.001$ compared to WT).

Figure S3, related to Figure 3. Increasing Plasma FGF21 Levels Reduces a Preference for Sweet Taste. (A) Plasma FGF21 levels in WT and FGF21 transgenic mice (n = 5-7/group). (B) Total kcal intake in 12-16 week old WT and FGF21 transgenic mice (n = 7-12/group). (C) Total kcal intake and (D) percent body weight (BW) change in WT C57BL/6 mice receiving the indicated amount of recombinant human FGF21 via s.c. injection (n = 4/group). (E) Single choice high sucrose diet (HSD) intake per day in WT C57Bl/6 mice administered vehicle or FGF21 (1 mg/kg) following an acclimation period of a two-choice diet between chow and HSD. (F) Average plasma hFGF21 protein levels WT C57BL/6 mice receiving the indicated amount of recombinant human FGF21 via s.c. injection (n = 4/group). (G) Percent sucralose intake relative to control group 1 day and 1 week after conditioning and treatment with vehicle

(control), FGF21 (1 mg/kg), or LiCl (0.3M, 10 ml/kg) (n = 8-9/group). (H) Percent change in sucrose intake in a two-bottle choice before and after administration of vehicle or FGF21 (1 mg/kg) (n = 7-8/group). (I) Percent change in glucose intake in a two-bottle choice before and after administration of vehicle or FGF21 (1 mg/kg) (n = 7/group). (J) Plasma human FGF21 levels in WT C57Bl/6 male mice implanted with osmotic minipumps delivering vehicle or recombinant human FGF21 (3 mg/kg/day). (K) Intake of maltose (100mM), lactose (100mM), sucrose (100mM), liposyn (20%), casein (8%), monosodium glutamate (100mM), quinine (1.5mM), sodium chloride (0.1%), or sucralose (10mM) in WT C57Bl/6 mice implanted with osmotic minipumps providing vehicle or recombinant human FGF21 (3 mg/kg/day) (n = 3-4/group). Intake of (L) 0.2% saccharin, (M) 20% intralipid, and (N) 10% casein in 12-16 week old male WT and FGF21 transgenic mice (n = 5-7/group). Values are mean +/- SEM. (*, $P< 0.05$; #, $P< 0.001$ compared to WT)

Figure S4, related to Figure 4. FGF21 Signals Directly to the PVN. (A) Semiquantitative RT-PCR for *α-gustducin*, *Fgfr1c*, *β-klotho* and *cyclophilin* using RNA prepared from fungiform (FF), circumvallate (CV), whole brain (BR), and non-taste epithelium taken from tongue (NT). (B) *Cre* mRNA expression as determined by QPCR in brain punches of the indicated regions in $KLB^{fl/fl}$ injected with AAV-GFP or AAV-Cre into the paraventricular nucleus (PVN). (C) Percent change in sucrose intake in female $KLB^{f/f;Phox2b}$ and littermate controls by i.p. administration of FGF21 (1 mg/kg) (n = 3-7/group). Values are mean +/- SEM.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Two-bottle choice studies

Mice were acclimated to cages and drinking tubes filled with water for 1 week prior to being given the first test fluid. Each test fluid was presented for 3 consecutive days, with the left-right positions of the test fluids alternated daily. Between each test series the mice were given two-bottle access to water for 3 or more days. Solutions were available 23 h/day and the fluid intake was recorded and tubes were refilled or replaced during the remaining hour similar to previously described (Sclafani et al., 2007). Food intake was measured as described (Butler et al., 2001).

For two-bottle choice studies in wild-type, FGF21 heterozygous, and FGF21 total KO mice, test fluids were offered in the following order: 0.2 % saccharin (Sigma), 2% maltodextrin (Solcarb), 20% intralipid (Baxter, Deerfield, IL), 10% fructose (Sigma), and then 10% sucrose (Sigma). A separate cohort of wild-type, FGF21 heterozygous, and FGF21 total KO mice were offered 10% glucose then glucose plus fructose. For two-bottle choice in WT and FGF21 TG mice, test fluids were offered in the following order: 0.2% saccharin, 20% intralipid, and then 10% casein. All solutions that were mixed were prepared with deionized water and served at room temperature. The spillage from drinking tubes was estimated daily by recording the change in weight of two drinking tubes that were placed on an empty cage where one tube contained that day's test solution, and the other tube contained the vehicle as described (Sclafani et al., 2007).

Assessment of plasma FGF21 levels

To assess FGF21 levels in mice, mice were individually caged, provided *ad libitum* access to chow (2920X; Teklad) and bottles of water, 10% glucose, 10% fructose, 10% sucrose, or 0.2% saccharin (all from Sigma Aldrich) for 24 hours (10 am to 10 am). All mice were sacrificed by decapitation and trunk blood was collected in EDTA-containing microvette tubes (Sarstedt), kept on ice, spun at 3000 rpm at 4°C for 20-45 minutes, and then plasma was collected and either immediately used for an ELISA or snap

frozen in liquid nitrogen. Mouse FGF21 levels were measured using a commercially available ELISA (Biovendor). Livers were immediately collected after decapitation and snap frozen in liquid nitrogen.

To determine mean circulating FGF21 values after subcutaneous dosing, peak plasma concentrations (C_{max}) were measured empirically. For all doses, C_{max} was achieved at 1 hour. Using the half-life of FGF21 ($t_{1/2}$=36 minutes) (Kharitonenkov et al., 2007), mean levels of FGF21 over the 24-hour period between doses were calculated.

To determine plasma FGF21 levels humans in response to acute and prolonged hyperglycemia, ten healthy subjects (8 male, 2 female, age 56±3 y, BMI 30.3±1.2 kg/m^2) underwent a 24 hour hyperglycemic clamp during which blood glucose levels were clamped 5.4 mM (90 mg/dL) above basal levels via an exogenous variable-rate glucose infusion (Solomon et al., 2012). Venous blood glucose was measured at the bed side every 5 minutes throughout (ABL 725; Radiometer, Copenhagen, Denmark). Blood samples were collected into EDTA-containing blood tubes at baseline, and after 2 and 24 hours of hyperglycemia. Plasma was separated by centrifugation and stored at -80 prior to analysis. Plasma samples were analyzed for FGF21 by ELISA according to the manufacturer's instructions (Biovendor).

Intracerebroventricular injections of FGF21

Guide cannula (Plastics One, Roanoke, VA) were obtained targeting the lateral ventricle (M/L: -1mm; A/P: -0.3mm; D/V: -2mm (cannula) and -2.5mm (cannula + injector)). After guide cannula placement, mice received post-operative analgesia (5mg/kg Rimadyl) and were given a week to recover. Mice were then given *ad libitum* choice between regular chow (Brogaarden, Denmark, 1310) and HSD. Mice were individually housed in metabolic cages that measured food intake. The treatment group received a daily injection of 1 µg/mouse for five days, and the control group received the same number of treatments of artificial cerebrospinal fluid (aCSF).

Single choice high-sucrose diet feeding with concurrent FGF21-treatment

Wild-type C57Bl/6 mice were individually caged and provided access to a two choice diet of HSD and regular chow for 3 days. On the 4th day, chow was removed and only HSD was available. Three days later, daily vehicle or FGF21 injections (1 mg/kg) were administered via s.c. injection for 7 consecutive days while food consumption was recorded daily.

c-Fos immunohistochemistry

c-Fos immunostaining was performed as described previously (Fernandes-Santos et al., 2013). Briefly, mice were handled and trained for intraperitoneal (i.p.) injections daily for 1 week. Two hours after i.p. injection of FGF21 (1 mg/kg) or vehicle, mice were anesthetized with ketamine (91 mg/kg) and xylazine (9.1 mg/kg) followed by transcardial perfusion with saline then fixation solution containing 4% paraformaldehyde. Dissected brains were post-fixed in 4% paraformaldehyde solution at 4 °C overnight before they were incubated in a 30% sucrose solution at 4 °C.

Each brain was cut into 30 μm, and free-floating sections (with sections from vehicle- and FGF21-treated mice incubated together in the same vial) were washed to remove the sucrose then treated with 0.3% H_2O_2 in water for 30 seconds to block endogenous peroxidase activity. After washing, sections were blocked for 1 hour with 3% normal goat serum followed by incubation with anti-c-Fos antibody (1:5,000, Santa Cruz sc-253) diluted in 1% normal goat serum at 4 °C overnight. Sections were washed in PBS, incubated 1 hour at room temperature with a biotinylated goat anti-rabbit IgG (1:400 dilution) for 1 hour, washed and then incubated in ABC solution (Vectastain® Elite ABC Kit, Vector Laboratories, PK-6100) for 1 hour at room temperature. Sections were rinsed in PBS, incubated in DAB (Vector Laboratories, SK-4100) for 2 min, rinsed again, mounted onto cleaned slides, air-dried and coverslipped using mounting media (Permount, Fisher Scientific, SP15-500).

Stereotactic injections of AAV virus

Stereotactic surgery was performed as previously described (Yang et al., 2006). Briefly, 9 week old male KLB$^{fl/fl}$ mice were anesthetized using ketamine/xylazine (100:10 mg/kg, i.p.) and placed on a stereotaxic

apparatus (Kopf Instruments, Tujunga, CA). Coordinates for targeted delivery of virus to the paraventricular nucleus (PVN) of hypothalamus were A/P -0.8mm; M/L +0.8mm; D/V -4.6mm, 8 degree angle, and for the suprachiasmatic nucleus (SCN), the coordinates were A/P -0.7mm; M/L +0.9mm; D/V -5.7mm, 8 degree angle. Using hamilton microsyringe with small hub removable needle, 1 µl virus (~1.4E+13 vg/ml) was delivered bilaterally to achieve coverage of targeted regions. The needle was placed at targeted coordinates and inserted to the indicated depth, and virus was slowly infused by pressing the plunger with 0.05 µl/min rate. After 10 minutes of waiting to ensure a full penetration of AAV (AAV2/5-GFP or AAV2/5-Cre) into targeted area, the needle was removed and the incision was closed by wound clips. Mice were then kept on a warming pad until awake. Three weeks after surgery, fluid or food preference experiments were performed. For the fluid preference assessment, all mice were administered saline via i.p. injection for 7 days for acclimation to injections and drinking tubes. After the 7th day, mice were given a two-bottle choice of 10% sucrose and water while being i.p. injected with vehicle for 3 days to obtain a baseline sucrose preference for each mouse. After day 3 of vehicle injection, all mice were administered FGF21 (1 mg/kg) via i.p. injection for 3 days and fluid preference was measured throughout this time. Percent change of sucrose intake by FGF21 was then calculated as follows: $[[Intake_{FGF21} - Intake_{veh}]/Intake_{veh}]*100$. Following the experiment, mice were euthanized and brains harvested, frozen in 2-methylbutane, and stored at -80°C. For food preference studies, a separate cohort of mice were individually caged and provided access to a two choice diet of HSD and regular chow.

Nerve Recordings of Tastants

Male C57Bl6 mice (3-5 months old) were injected i.p with FGF21 (1 mg/kg) or vehicle (n = 5/group). After 24 hours, mice were anesthetized with pentobarbital (50mg/kg) and placed in a head holder. The chorda tympani (a branch of the VII cranial nerve innervating the anterior part of the tongue) was exposed using a ventral approach and placed on a platinum electrode. A reference electrode was placed in the surrounding tissue. The electric signal was fed to an amplifier, integrated (time constant 500ms) and

recorded using AcqKnowledge software (Biopac). The anterior part of the tongue was stimulated for 30s with a constant flow of various stimuli including: sucrose 500mM, glucose 500mM, sucralose 50mM, saccharin 50mM, or NaCl 100mM. NH_4Cl 100mM was used as a reference stimulus so recordings from different mice could be compared. The tongue was rinsed with water for 40s between stimulations. The amplitude of the integrated responses was averaged for 30s (using the AcqKnowledge software) and normalized to the NH_4Cl response. Responses were then compared between mice injected with FGF21 or the vehicle using a two-way ANOVA and Bonferroni post hoc tests.

Taste Aversion

Twenty-five C57BL/6 mice were single housed and randomized per body weight. After the habituation period, mice were trained on a water deprivation schedule for 7 days to drink for only 2 hours per day (10-12 am) and the intake was measured to ensure that mice had a stable intake. Mice had access to chow at all times and received a s.c. vehicle injection one hour into the water access period. On the first conditioning day, mice were given access to sucralose for two hours. After the first hour mice received an injection of either saline (n=8), 1 mg/kg FGF21 (n=8) or 0.3M LiCl (n=9), all dosed in a volume of 10 ml/kg. This was repeated for two days and followed by 6 days of water access (2 h) to ensure washout of FGF21. The experimental day followed immediately after the washout period in which mice received one bottle with sucralose for 2 hours.

Gene expression

Total RNA was isolated from Hepg2 cells and mouse liver homogenate with Trizol reagent (Ambion). Two micrograms RNA from each sample were used to generate cDNA and QPCR was conducted using SYBR green (Invitrogen) as described (Markan et al., 2014).

To assess mRNA expression in different brain regions, frozen brains were cut in 50 μm coronal sections and micropunches of the cortex and arcuate were obtained using a 0.75 mm needle. Bilateral punches of the PVN and SCN were taken using a 0.50 mm needle. Tissues from two mice of the same

group were pooled per sample. Total RNA was isolated from all brain regions by TRIzol extraction. QPCR primer sequences are as follows: *Klb* FWD 5'-GATGAAGAATTTCCTAAACCAGGTT-3', REV 5'-AACCAAACACGCGGATTTC-3'; *Cre* FWD 5'-TGTTGCCGCGCCATCTG-3', REV 5'-'TTGCTTCAAAAATCCCTTCCA-3'; *Fgf21* FWD 5'-CCTCTAGGTTTCTTTGCCAACAG-3', REV 5'-AAGCTGCAGGCCTCAGGAT-3'; *ChREBP* FWD 5'- GTGAGCAAAGCAACCACGCTTCA-3', REV 5'- TTGATGGCAGCGTTGAGCTCC-3'; and *FGF21* FWD 5'–AGTGGAGCGATCCATACAGG–3', REV 5'-ACTCCAGTCCTCTCCTGCAA-3'.

SUPPLEMENTAL REFERENCES

Butler, A.A., Marks, D.L., Fan, W., Kuhn, C.M., Bartolome, M., and Cone, R.D. (2001). Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nature neuroscience *4*, 605-611.

Kharitonenkov, A., Wroblewski, V.J., Koester, A., Chen, Y.F., Clutinger, C.K., Tigno, X.T., Hansen, B.C., Shanafelt, A.B., and Etgen, G.J. (2007). The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology *148*, 774-781.

Sclafani, A., Zukerman, S., Glendinning, J.I., and Margolskee, R.F. (2007). Fat and carbohydrate preferences in mice: the contribution of alpha-gustducin and Trpm5 taste-signaling proteins. American journal of physiology. Regulatory, integrative and comparative physiology *293*, R1504-1513.