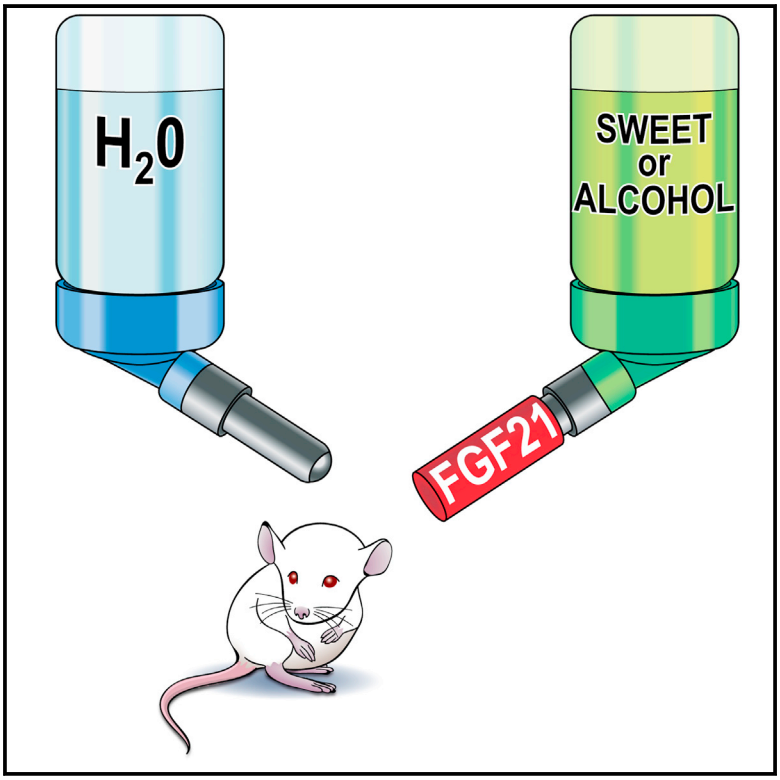
Short Article

Cell Metabolism

# FGF21 Regulates Sweet and Alcohol Preference

## Graphical Abstract




## Highlights

- FGF21 suppresses sweet and alcohol preference but not bitter taste
- The effects of FGF21 on taste preference require its action in the CNS
- FGF21 decreases dopamine levels in the nucleus accumbens
- FGF21 regulation of sweet preference also occurs in primates

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## In Brief

FGF21 has well-established beneficial metabolic effects. Talukdar et al. now extend this repertoire and reveal that FGF21 also suppresses sweet and alcohol preference in mice, and sweet preference in monkeys, by acting on the CNS. These effects are associated with decreased dopamine, a key neurotransmitter used in reward pathways.

Talukdar et al., 2016, Cell Metabolism *23*, 344–349
February 9, 2016 
http://dx.doi.org/10.1016/j.cmet.2015.12.008

CellPress

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# FGF21 Regulates Sweet and Alcohol Preference

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http://dx.doi.org/10.1016/j.cmet.2015.12.008

## SUMMARY

**Fibroblast growth factor 21 (FGF21) is a hormone induced by various metabolic stresses, including ketogenic and high-carbohydrate diets, that regulates energy homeostasis. In humans, SNPs in and around the *FGF21* gene have been associated with macronutrient preference, including carbohydrate, fat, and protein intake. Here we show that FGF21 administration markedly reduces sweet and alcohol preference in mice and sweet preference in cynomolgus monkeys. In mice, these effects require the FGF21 co-receptor β-Klotho in the central nervous system and correlate with reductions in dopamine concentrations in the nucleus accumbens. Since analogs of FGF21 are currently undergoing clinical evaluation for the treatment of obesity and type 2 diabetes, our findings raise the possibility that FGF21 administration could affect nutrient preference and other reward behaviors in humans.**

## INTRODUCTION

FGF21 is induced in liver in response to fasting and other forms of metabolic stress including ketogenic and high-carbohydrate diets (Badman et al., 2007; Dushay et al., 2015; Inagaki et al., 2007; Sánchez et al., 2009; Zhao et al., 2015). FGF21 in turn regulates diverse aspects of energy homeostasis, including hepatic fatty acid oxidation and ketogenesis, circadian behavior, growth, and female reproduction (Owen et al., 2015). Pharmacologically, FGF21 causes weight loss and improves insulin sensitivity in obese mice, monkeys, and humans (Gimeno and Moller, 2014). Long-acting analogs of FGF21 are currently in clinical trials for the treatment of obesity and type 2 diabetes.

FGF21 acts through a cell-surface receptor composed of a conventional FGF receptor in complex with β-Klotho, a single-pass transmembrane protein (Owen et al., 2015). FGF21 crosses the blood-brain barrier (Hsuchou et al., 2007) and exerts many of its actions, including its effects on growth, female reproduction, and weight loss, by acting on its cognate receptor in the CNS (Bookout et al., 2013; Douris et al., 2015; Liang et al., 2014; Owen et al., 2013, 2014; Sarruf et al., 2010). Among its central actions, FGF21 induces corticotropin-releasing factor and suppresses arginine vasopressin expression in the hypothalamus (Bookout et al., 2013; Liang et al., 2014; Owen et al., 2013, 2014).

In humans, SNPs in and around the *FGF21* gene are associated with changes in macronutrient preference, including increases in carbohydrate consumption and decreases in fat and protein intake (Chu et al., 2013; Tanaka et al., 2013). These findings raise the possibility of additional effects of FGF21 on the brain. In this report, we examine the effect of FGF21 on sweet and alcohol preference in mice and monkeys.

## RESULTS AND DISCUSSION

Since FGF21 is induced by carbohydrates in rodents and humans (Dushay et al., 2015; Sánchez et al., 2009), and SNPs in the *FGF21* gene are associated with carbohydrate intake in humans (Chu et al., 2013; Tanaka et al., 2013), we investigated whether chronic FGF21 exposure affects sweet preference. Two-bottle preference assays with water and either 3% sucrose or 0.2% saccharin (Krishnan et al., 2007; Tordoff and Bachmanov, 2003) were performed using wild-type (WT) and *Fgf21*-transgenic (Tg) mice expressing supraphysiological concentrations of FGF21 (Inagaki et al., 2007). Saccharin was included to eliminate the potentially confounding effect of caloric content. As expected, WT mice showed a strong preference for drinking water sweetened with either sucrose or saccharin (Figures 1A and 1B, Table S1). Notably, the Tg(*Fgf21*) mice had significant decreases in both sucrose and saccharin preference (Figures 1A and 1B).

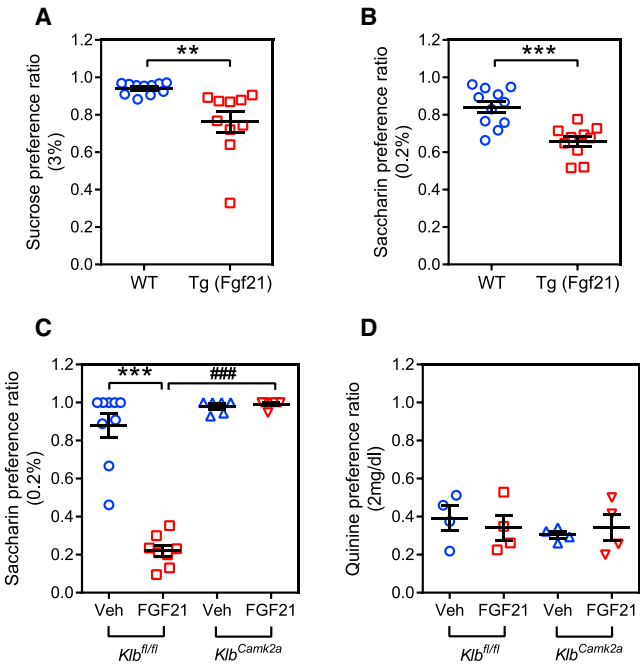


**Figure 1. FGF21 Decreases Sweet Preference Ratio in Mice by Acting on the CNS**

(A) Two-bottle preference assay in wild-type (WT) and Tg(*Fgf21*) mice administered water versus 3% sucrose. Representative 24 hr data from day 2 after initiating the assay are shown as the sucrose preference ratio (sucrose intake volume/total fluid intake volume). n = 10–11/group.

(B) Two-bottle preference assay in WT and Tg(*Fgf21*) mice administered water versus 0.2% saccharin. Representative 24 hr data from day 2 after initiating the assay are shown. n = 10–11/group.

(C) Two-bottle preference assay with water versus 0.2% saccharin for $Klb^{fl/fl}$ and $Klb^{Camk2a}$ mice administered either FGF21 (1 mg/kg/day) or vehicle. Representative 24 hr data from day 3 after initiating the assay are shown. n = 6–9/group.

(D) Two-bottle preference assay with water versus 2 mg/dl quinine for $Klb^{fl/fl}$ and $Klb^{Camk2a}$ mice administered either FGF21 (1 mg/kg/day) or vehicle. n = 4/group.

Values are means ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{\#\#\#}p < 0.001$ by Student's t test. See also Figure S1 and Tables S1 and S2.

To determine whether FGF21 acts on the CNS to regulate sweet preference, we administered recombinant FGF21 or vehicle by osmotic minipump to groups of control mice with floxed β-Klotho alleles ($Klb^{fl/fl}$) or mice specifically lacking β-Klotho in the CNS ($Klb^{Camk2a}$), and two-bottle saccharin preference tests were performed. FGF21 strongly suppressed saccharin preference in $Klb^{fl/fl}$ mice but had no effect in $Klb^{Camk2a}$ mice (Figure 1C, Table S2). As reported (Camporez et al., 2013), administration of recombinant FGF21 increased total fluid intake, and this effect required β-Klotho in the CNS (Table S2). In contrast, total fluid intake was unchanged in Tg(*Fgf21*) compared to control mice (Table S1). The reason for this difference between Tg FGF21 overexpression and recombinant FGF21 administration on fluid intake is not known. Nevertheless, FGF21 decreased sweet preference in both contexts.

In additional two-bottle preference tests, FGF21 had no effect on preference for 1% sunflower oil (data not shown) or quinine (Figure 1D), indicating that FGF21 does not affect responses to fatty acids or bitter taste. FGF21 administration also had no effect on either tail-suspension or forced-swim tests, both standard measures of behavioral despair (Figures S1A and S1B). We conclude that FGF21 acts directly on the brain to regulate sweet preference without causing despair.

To determine whether FGF21 also affects sweet preference in primates, we analyzed saccharin preference in obese cynomolgus monkeys administered PF-05231023, a long-acting FGF21 analog consisting of two molecules of modified FGF21 linked by an antibody scaffold (Dong et al., 2015; Giragossian et al., 2015; Weng et al., 2015). We first tested this analog in mice. PF-05231023 administration decreased saccharin preference to a degree similar to native FGF21, with maximal efficacy observed 3–5 days after dosing (Figure 2A). For the monkey study, PF-05231023 or vehicle was administered on days 1, 4, and 7 of the 3-week experiment. Notably, PF-05231023 administration significantly decreased saccharin preference in the monkeys (Figure 2B). The effect on saccharin intake in the monkeys was striking even within 1 day of receiving a single dose of the FGF21 analog, and the effect was sustained for several days after receiving the last dose. Thus, FGF21 also affects sweet preference in primates.

The neurotransmitter dopamine has a central role in regulating reward behavior, including sucrose and saccharin preference (Fernstrom et al., 2012). To examine whether FGF21 affects dopamine signaling, including the mesolimbic pathway, we first measured β-Klotho expression in the ventral tegmental area (VTA), nucleus accumbens (NAc), medial prefrontal cortex (PFC), and caudate putamen (CP) of $Klb^{+/-}$ and $Klb^{-/-}$ mice, with the latter mice serving as a negative control. As expected (Bookout et al., 2013; Liang et al., 2014), *Klb* mRNA was detected in the suprachiasmatic nucleus/paraventricular (SCN/PVN) nucleus region of the hypothalamus in $Klb^{+/-}$ mice by quantitative PCR (qPCR) (Figure 3A). *Klb* mRNA was also detected in VTA and NAc in $Klb^{+/-}$ mice, albeit at relatively low levels, but not the PFC or CP (Figure 3A). As expected, *Klb* mRNA was not detected in any of the regions in the $Klb^{-/-}$ control mice (Figure 3A). Consistent with the qPCR data, *Klb* mRNA was detected by in situ hybridization in SCN and a small subset of cells in the VTA and NAc (Figure S2A). Expression of FGF receptor 1, which partners with β-Klotho to form the FGF21 receptor, was detected by qPCR in all of these brain regions (Figure S2B).

The FGF21 receptor expression data led us to examine whether FGF21 affects the levels of dopamine and its metabolites in NAc, which coordinates reward behaviors. Notably, FGF21 administration for 2 weeks significantly decreased dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) concentrations (Figure 3B). FGF21 administration did not decrease dopamine, DOPAC, HVA, or 3-MT concentrations in the CP (Figure S2C). FGF21 administration also caused changes in the expression of dopamine-related genes, including an increase in the dopamine transporter in the NAc and CP and a decrease in catechol-O-methyl transferase in the VTA and CP (Figures 3C and 3D). FGF21 had little or no effect on the mRNA levels of tyrosine hydroxylase or dopamine receptor 1 in these brain regions (Figures S2D and S2E), nor did it change the levels of total and

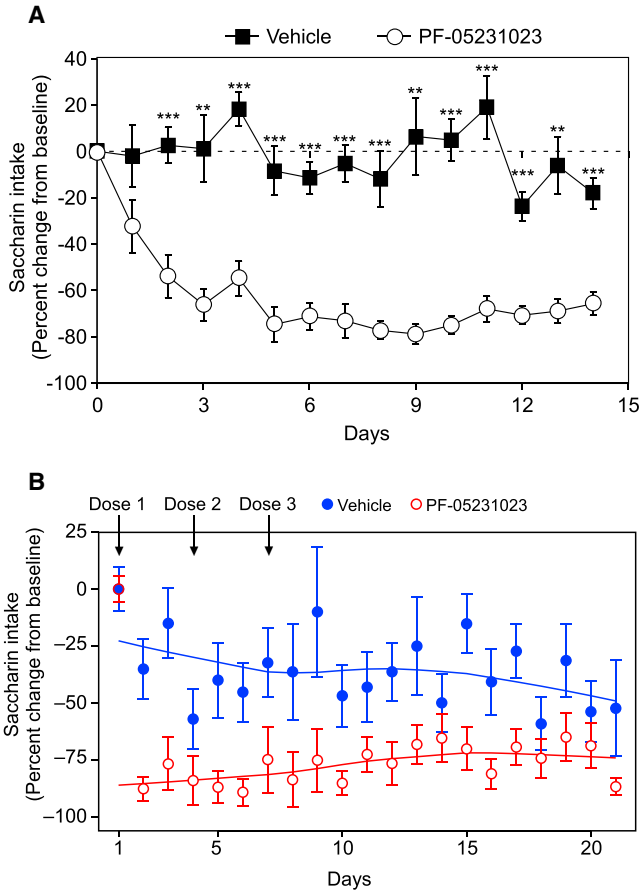


**Figure 2. A Stable FGF21 Analog Decreases Saccharin Preference in Mice and Monkeys**

(A) Two-bottle preference assay with 0.1% saccharin in diet-induced obese mice administered either PF-05231023 (10 mg/kg) or vehicle on days 0, 3, 7, and 10. Data are shown as the mean ± SEM; n = 8/group. **p < 0.01, ***p < 0.001 versus vehicle group.

(B) Two-bottle preference assay with 0.2% saccharin in obese cynomolgus monkeys administered either PF-05231023 (n = 8; 10 mg/kg) or vehicle (n = 7) on days 1, 4, and 7. Data are presented as mean percentage change in saccharin water intake ± SEM for vehicle-treated (closed blue circles) and PF-05231023-treated (open red circles) monkeys. Solid lines are locally weighted scatterplot smoothing fits to the means of percent change. Mixed effect modeling fitted to these longitudinal data using R, version 3.1.2 (Pinheiro et al., 2013), showed a significant difference (p = 0.003) between groups. Number of days after first treatment, treatment type, and the interaction term between treatment groups and time were specified as fixed effects and monkey labels as a random effect.

phosphorylated tyrosine hydroxylase in VTA (Figure S2F). Taken together, these data suggest that FGF21 may affect sweet preference via effects on dopamine signaling. However, additional experiments will be required to confirm this possibility and to determine the mechanism whereby FGF21 reduces dopamine concentrations.

Since dopamine signaling impacts ethanol drinking behavior (Gonzales et al., 2004), we examined whether FGF21 also affects alcohol preference. Groups of WT and Tg(*Fgf21*) mice were exposed stepwise to increasing concentrations of ethanol in a two-bottle preference assay. Tg(*Fgf21*) mice had a decreased ethanol preference ratio at the 4%, 8%, 12%, and 16% ethanol concentrations (Figure 4A, Table S3). In an ethanol bioavailability test, there was no difference between WT and Tg(*Fgf21*) mice in plasma ethanol concentrations at 1 and 3 hr after ethanol administration (Figure 4B). Thus, FGF21 suppresses ethanol preference without affecting its bioavailability.

In summary, we show that FGF21 regulates sweet and alcohol preference in mice and sweet preference in monkeys. Since circulating levels of FGF21 increase in response to carbohydrate consumption in rodents and humans (Dushay et al., 2015; Sánchez et al., 2009) and alcohol consumption in rodents (Zhao et al., 2015), this may represent a feedforward regulatory pathway for limiting consumption. In mice, the effects on sweet and alcohol preference correlate with reductions in dopamine concentrations in the NAc, which coordinates reward behavior. These results suggest a mechanistic basis for the association between SNPs in and around the FGF21 gene with macronutrient preference in humans (Chu et al., 2013; Tanaka et al., 2013). Moreover, since FGF21 is currently in clinical trials for treating obesity and type 2 diabetes, these findings suggest that additional studies are warranted to assess the effects of FGF21 on sweet and alcohol preference and other reward behavior in humans.

## EXPERIMENTAL PROCEDURES

### Mouse Experiments with FGF21

All mouse experiments involving native FGF21 were approved by the Internal Animal Care and Use Committee of the University of Texas Southwestern Medical Center. WT and Tg(*Fgf21*) mice were on a C57BL/6J background. $Klb^{fl/fl}$ and $Klb^{Camk2a}$ mice were on a mixed C57BL/6J;129/Sv background as described (Bookout et al., 2013). All experiments were performed with 2- to 4-month-old male mice. Tg(*Fgf21*) and $Klb^{fl/fl}$ mouse experiments were performed with littermates. Mice were housed on a standard 12 hr light/dark cycle and had free access to chow. Recombinant human FGF21 protein was provided by Novo Nordisk and administered by subcutaneous osmotic minipumps (Alzet) at a dose of 1 mg/kg/day. Mice were allowed to recover from minipump surgery for 1 week prior to preference tests. Mice were single-caged following minipump surgery, which was conducted under isoflurane anesthesia and 24 hr buprenorphine analgesia.

### Two-Bottle Preference Assays

For the two-bottle sucrose and saccharin preference assays, mice were acclimated to cages with two bottles of just water for 4 days. Mice were then given access to bottles with water and water containing 3% sucrose or 0.2% saccharin (w/v). For the quinine preference assay, mice were given access to water and water containing 2 mg/dl quinine. In each case, consumption was measured daily for at least 3 days. For ethanol preference assays, mice were given access to two bottles, one containing water and the other containing either 2%, 4%, 8%, 12%, or 16% ethanol (v/v) in water. The same mice were exposed to an ascending concentration of each ethanol concentration for 5 days. The position of the two bottles was changed every 2 days to exclude position effects. Water and ethanol-containing water intake were measured each day.

### Mouse Immobility Assays

Tail-suspension and forced-swim tests were performed as described (Can et al., 2012a, 2012b) on mice administered either FGF21 or vehicle by osmotic minipump for 7–14 days. In both tests, the experimenter was blinded to the treatment group. For the forced-swim test, mice were placed in cylindrical tanks (20 cm in diameter) filled with water (25 ± 2°C). The cylinder was filled to a depth of 12 cm to prevent the mice from using their tails to support

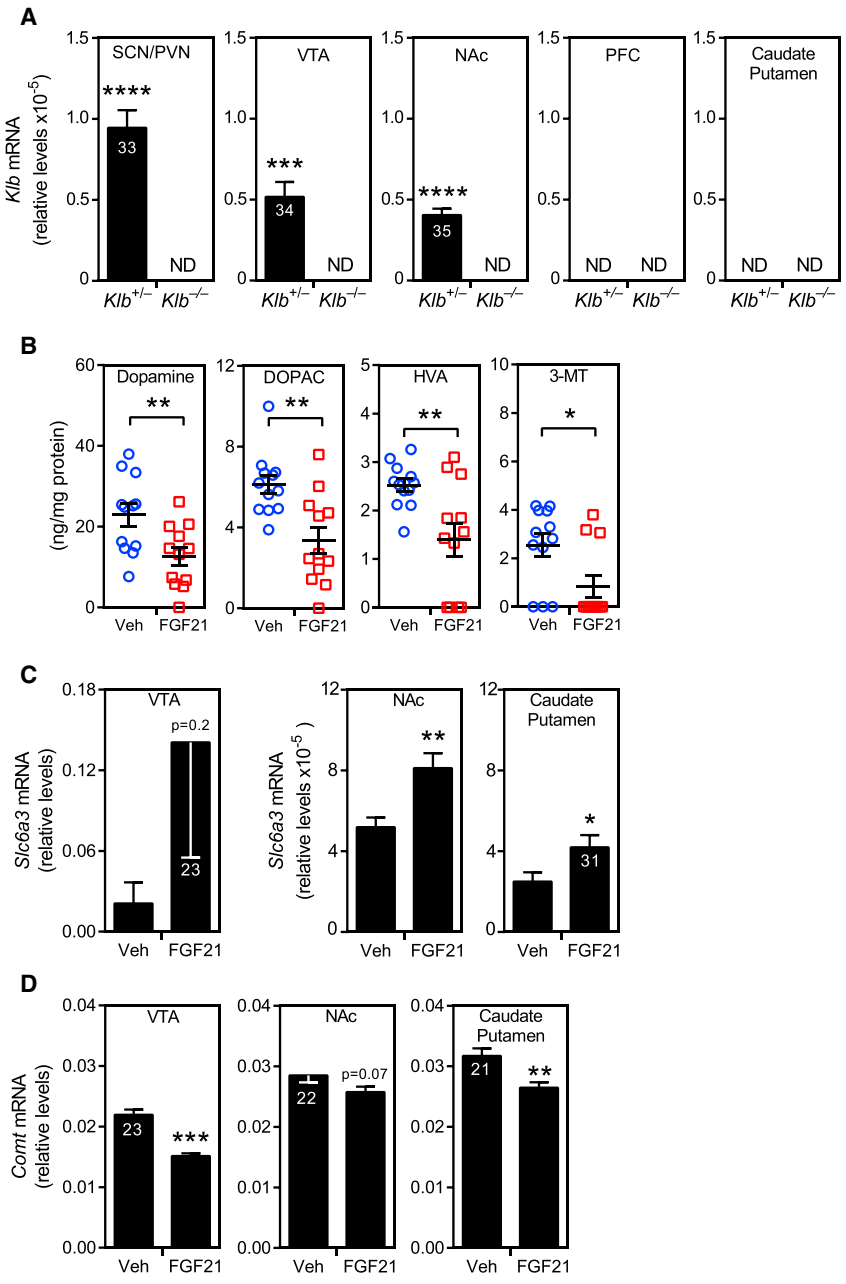


**Figure 3. FGF21 Affects Dopamine Signaling**

(A) β-Klotho (*Klb*) mRNA levels in the suprachiasmatic nucleus/paraventricular nucleus (SCN/PVN) region of the hypothalamus, ventral tegmental area (VTA), nucleus accumbens (NAc), medial prefrontal cortex (PFC), and caudate putamen of $Klb^{+/-}$ and $Klb^{-/-}$ mice (n = 6/group). Ct values are shown in the bars. ND, not detected.

(B) Concentrations of dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxytyramine (3-MT) in the NAc of mice administered either vehicle or FGF21 for 2 weeks by osmotic minipump (n = 12/group).

(C and D) mRNA levels of dopamine transporter (*Slc6a3*) or (D) catechol-O-methyl transferase (*Comt*) in VTA, NAc, and caudate putamen of mice administered either vehicle or FGF21 for 2 weeks by osmotic minipump (n = 7–8/group). Ct values are shown.

Values are means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus control group by Student's t test. See also Figure S2.

themselves in the water. In both experiments, the cumulative time spent immobile was recorded over the course of a 6 min experiment.

### Ethanol Clearance Assays

Mice were intraperitoneally (i.p.) injected with ethanol (4 g/kg) in saline, and tail vein blood was drawn at regular intervals. Plasma ethanol levels were measured using the EnzyChrom Ethanol Assay Kit (BioAssay Systems).

### Microdissection of Brain Regions

Mouse brains were extracted from the skull and kept under dry ice vapor for all dissections. Coronal sections (1-mm thick) were cut using a brain-slicing matrix (Braintree Scientific). Medial prefrontal cortex, whole NAc (shell and core), the hypothalamic SCN/PVN region, CP (striatum), and VTA were identified by gross architectural landmarks (Paxinos and Franklin, 2004). Medial prefrontal cortex and the hypothalamic SCN/PVN region were dissected using a 14G tissue punch. NAc, CPA, and the VTA were dissected using a 16G tissue punch. Tissue was homogenized by passage through a 26.5G syringe in RNA-STAT60.

### Quantitative PCR Analysis

Total RNA was isolated from tissue using RNA-STAT60 reagent, and RNA was reverse-transcribed into cDNA (Invitrogen). Gene expression was measured with an Applied Biosystems 7900HT Sequence Detection System using the $\Delta\Delta C_T$ assay and normalized to GAPDH.

### In Situ Hybridization Analysis

Brains were dissected from male C57BL/6J mice, embedded in OCT compound (Sakura), and flash frozen in cooled isopentane. Coronal sections (14–16 μm) were cut using a cryostat (Leica) followed by fixation for 15 min at 4°C with 10% neutral buffered formalin. In situ hybridization was performed using the RNAscope 2.5 brown chromogenic assay pretreatment and detection kits (Advanced Cell Diagnostics). Probes for *cyclophilin B* (positive control), *dapB* (negative control), and *Klb* were purchased from Advanced Cell Diagnostics. Hybridized sections were counterstained with hematoxylin, dehydrated, cleared, and mounted with Ecomount (Biocare Medical). Images were taken using a Zeiss Axioscan Z1 at 40× magnification. The signal from the mRNA was highlighted using the color threshold function in ImageJ.

### Western Blot Analysis

Western blot analysis was performed using antibodies for total (Cell Signaling, #2792) and Ser40-phosphorylated tyrosine hydroxylase (AbCam, #51206). Data were acquired and quantified using an ImageQuant LAS 4000 and Multi Gauge v3.1 software (Fujifilm).

### Dopamine Measurements

Dopamine and its metabolites were measured by HPLC by the Vanderbilt Neurochemistry Core.

### Mouse and Monkey Experiments with PF-05231023

All animal care and experimental procedures for studies involving PF-05231023 were conducted in compliance with the U.S. Animal Welfare Act

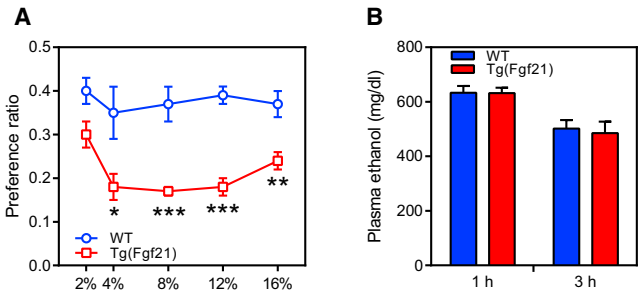


**Figure 4. FGF21 Decreases Alcohol Preference**

(A) Ethanol preference ratio in wild-type (WT) and Tg(*Fgf21*) mice at the indicated ethanol concentrations (n = 9/group).

(B) Plasma ethanol concentrations in groups of WT and Tg(*Fgf21*) mice 1 or 3 hr after i.p. injection of ethanol (4 g/kg) (n = 4–5/group).

Values are means ± SEM. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ versus control group by Student's t test. See also Table S3.

and the ILAR Guide for the Care and Use of Laboratory Animals, 1996. The procedures used in these studies were reviewed and approved by the Pfizer Institutional Animal Care and Use Committee (AUP #GTN-2013-00793).

Male C57BL/6J mice fed a 60% high-fat diet (Research Diets D12492i) for 12–14 weeks were used for the saccharin studies. Mice were single-housed with access to regular water and 0.1% saccharin (S6047, Sigma-Aldrich) water throughout the study. Water intake was monitored using a BioDaq system (Research Diets Inc.). The position of sweetened and unsweetened water bottles was switched each day to eliminate positional preference. Mice were acclimatized to the cage for 10–14 days, after which they were stratified based on body weight and baseline saccharin water intake. PF-05231023 (Huang et al., 2013; Weng et al., 2015) or vehicle was administered subcutaneously twice per week for a total of three doses at 10 mg/kg.

Male *Macaca fascicularis* (cynomolgus monkeys) from Charles River Laboratories were single-housed under a 12 hr light/dark cycle. Monkeys were between 7 and 24 years of age and weighed between 7 and 12 kg. Monkeys were provided standard lab diet 5K91 (LabDiet) supplemented once a day with fruits and peanuts. The two-bottle saccharin preference assay was performed as described (Tordoff and Bachmanov, 2003). The monkeys underwent a training period during which the standard water system was shut off and two bottles, one with water and the other with water containing 0.2% saccharin, were attached side-by-side to the cage. To determine the amount of water consumed, bottles were weighed before and after filling, with the difference in weight equated to the volume consumed. The position of sweetened and unsweetened water bottles was switched each day to eliminate positional preference. Several animals in the original cohort were eliminated from the study due to water bottle damage, failure to adapt to the water bottle, or if one of the allocation endpoints represented an outlier.

Fifteen monkeys were divided into two groups based on body weight, triglyceride and fasted glucose levels, and baseline daily food and water consumption. Following a baseline period, PF-05231023 or vehicle (30 mM lactate [pH 4.8], 9% Trehalose, 0.05 mg/ml EDTA, 0.1 mg/ml L-methionine, 0.5 mg/ml Tween 20) was administered intravenously to eight and seven animals, respectively, on days 1, 4, and 7. Dosing was performed in a blinded manner. Water consumption was monitored at least twice daily.

### Statistical Analyses

All data are expressed as means ± SEM. Statistical analysis between the two groups was performed by unpaired two-tailed Student's t test using Excel or GraphPad Prism (GraphPad Software, Inc.) or by using R software (Fraley et al., 2012; Pinheiro et al., 2013).

## SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2015.12.008.

## AUTHOR CONTRIBUTIONS

S.T., B.M.O., P.S., G.H., and Y. Zhou designed, performed and analyzed experiments; Y. Zhang and W.T.S. performed and analyzed experiments; B.P., T.T., A.S., and B.B. performed experiments; H.T. analyzed experiments; C.P.M. designed and analyzed experiments; B.G., S.A.K., and D.J.M. designed, supervised, and analyzed experiments and wrote the paper. P.S. and G.H. contributed equally. All authors commented and approved the paper.

## CONFLICTS OF INTEREST

S.T. and Y. Zhou completed the work at Pfizer and are now employees of Merck; B.P., T.T., A.S., B.B., and B.G. are employees of Pfizer; D.J.M. is a founder of Metacrine and a member of its scientific advisory board; the other authors have no conflicts of interest to declare.

## ACKNOWLEDGMENTS

We thank Birgitte Andersen (Novo Nordisk) for providing recombinant FGF21, Yang Xie for assistance with the statistical analyses, and David Self for assistance with brain dissection. This work was supported by National Institutes of Health grants R01DK067158 (S.A.K. and D.J.M.), R01CA152301 (H.T.), NNX11AC54G (H.T.), and 5P50 CA70907-16 (H.T.); the Robert A. Welch Foundation (grant I-1558 to S.A.K. and grant I-1275 to D.J.M.); the Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and the Royal Society (Grant Number 105545/Z/14/Z to B.M.O.); the Ford Foundation Fellowship (G.H.); the German National Science Foundation (DFG) grant MU 2789/8-1 and IZKF (Project E13) (C.P.M.); and the Howard Hughes Medical Institute (D.J.M.).

Received: September 18, 2015
Revised: December 8, 2015
Accepted: December 17, 2015
Published: December 24, 2015

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