*Am J Physiol Endocrinol Metab* 319: E276–E290, 2020.
First published June 23, 2020; doi:10.1152/ajpendo.00529.2019.

**RESEARCH ARTICLE**

# Sugar causes obesity and metabolic syndrome in mice independently of sweet taste

**Ana Andres-Hernando,[1] Masanari Kuwabara,[1] David J. Orlicky,[2] Aurelie Vandenbeuch,[3,4] Christina Cicerchi,[1] Sue C. Kinnamon,[3,4] Thomas E. Finger,[4,5] Richard J. Johnson,[1] and Miguel A. Lanaspa[1]**

[1]*Division of Renal Diseases and Hypertension, University of Colorado School of Medicine, University of Colorado, Aurora, Colorado;* [2]*Department of Pathology, University of Colorado School of Medicine, University of Colorado, Aurora, Colorado;* [3]*Department of Otolaryngology, University of Colorado School of Medicine, University of Colorado, Aurora, Colorado;* [4]*Rocky Mountain Taste & Smell Center, University of Colorado School of Medicine, University of Colorado, Aurora, Colorado; and* [5]*Department of Cell and Developmental Biology, University of Colorado School of Medicine, University of Colorado, Aurora, Colorado*

Submitted 5 December 2019; accepted in final form 16 June 2020

**Andres-Hernando A, Kuwabara M, Orlicky DJ, Vandenbeuch A, Cicerchi C, Kinnamon SC, Finger TE, Johnson RJ, Lanaspa MA.** Sugar causes obesity and metabolic syndrome in mice independently of sweet taste. *Am J Physiol Endocrinol Metab* 319: E276–E290, 2020. First published June 23, 2020; doi:10.1152/ajpendo.00529.2019.—Intake of sugars, especially the fructose component, is strongly associated with the development of obesity and metabolic syndrome, but the relative role of taste versus metabolism in driving preference, intake, and metabolic outcome is not fully understood. We aimed to evaluate the preference for sweet substances and the tendency to develop metabolic syndrome in response to these sugars in mice lacking functional taste signaling [P2X2 (P2X purinoreceptor 2)/P2X3 (P2X purinoreceptor 3) double knockout mice (DKO)] and mice unable to metabolize fructose (fructokinase knockout mice). Of interest, our data indicate that despite their inability to taste sweetness, P2X2/3 DKO mice still prefer caloric sugars (including fructose and glucose) to water in long-term testing, although with diminished preference compared with control mice. Despite reduced intake of caloric sugars by P2X2/3 DKO animals, the DKO mice still show increased levels of the sugar-dependent hormone *FGF21* (fibroblast growth factor 21) in plasma and liver. Despite lower sugar intake, taste-blind mice develop severe features of metabolic syndrome due to reduced sensitivity to leptin, reduced ability to mobilize and oxidize fats, and increased hepatic de novo lipogenesis. In contrast to P2X2/3 DKO and wild-type mice, fructokinase knockout mice, which cannot metabolize fructose and are protected against fructose-induced metabolic syndrome, demonstrate reduced preference and intake for all fructose-containing sugars tested but not for glucose or artificial sweeteners. Based on these observations, we conclude that sugar can induce metabolic syndrome in mice independently of its sweet properties. Furthermore, our data demonstrate that the metabolism of fructose is necessary for sugar to drive intake and preference in mice.

fructokinase; fructose; sugar; sweet taste; metabolic syndrome

## INTRODUCTION

Sucrose (table sugar) and high-fructose corn syrup (HFCS) are the two major sweeteners used in foods today and are often added to beverages and foods to enhance their taste. Intake of caloric sweeteners has skyrocketed over the last several centuries, from an intake (based on sales) of ~4 pounds per capita per year in 1700 to over 150 pounds per capita per year in 2000 (12). Today nearly 70% of processed foods and beverages in US supermarkets contain these sweeteners, including many foods that one might initially not consider to contain such additives (30). The increase in sugar and HFCS intake is concerning, because these sweeteners are associated with increased risk for the development of obesity and metabolic syndrome, resulting in increased risk for diabetes and cardiovascular disease (24, 41). Indeed, previous studies have shown that the administration of sugar and HFCS in the drinking water, or their components glucose and fructose, can induce obesity and metabolic syndrome in mice and rats (11, 18).

A common viewpoint is that one of the reasons for the high intake of sugars is because of its sweet taste. This is consistent with the observation that animals show increased intake of artificial sweeteners that carry no nutritional value (19). Sucrose and HFCS activate sweet taste receptors [the taste receptor type 1 member 2 to 3 (T1R2–T1R3) dimers] and their downstream signaling component (TrpM5), in the tongue, resulting in a dopamine response in the brain that might encourage continued sugar intake. Nevertheless, while knocking down either transient receptor potential cation channel subfamily M member 5 (TrpM5) or T1R3 blocks the sensation of sweetness, the mice continue to prefer sucrose over water (5, 38, 43), although these sweet taste-defective knockout (KO) mice ingest less sucrose than wild-type (WT) mice (9, 36, 43). Furthermore, in a short-term (38 day) study, T1R3 KO mice fed with sucrose showed significantly less weight gain than sucrose fed WT mice (9). In contrast, the TrpM5 KO mice on a sucrose diet had a tendency for greater weight gain than TrpM5 KO mice on a control diet, but less than that of WT mice on a sucrose diet (9). However, interpretation of these studies is difficult, as weight gain was modest due to the short-term nature of the study and because T1R3 (15, 25, 28) and TrpM5 (4) are also expressed in the gut and have additional "non-taste" functions such as modulating glucose absorption and incretin and insulin responses. Thus, additional

Correspondence: M. A. Lanaspa (Miguel.lanaspagarcia@cuanschutz.edu).

  

Table 1. *Sweetener preferences (%, 2-bottle) in WT and P2X2/3 DKO mice during the first 4 days after exposure*

| | Water | Fructose | Sucrose | FG Mixture | Glucose | Sucralose | Saccharin |
|---|---|---|---|---|---|---|---|
| $WT^{P2X}$ | | | | | | | |
| *Day 1* | 50.5 ± 3.5 | 60.5 ± 3.5## | 75.5 ± 3.5## | 85.5 ± 5.5## | 80.0 ± 5.5## | 40.5 ± 3.5## | 62.5 ± 6.5## |
| *Day 2* | 55.5 ± 3.5 | 75.5 ± 5.0## | 85.5 ± 2.5## | 95.5 ± 2.5## | 92.5 ± 3.5## | 60.5 ± 3.5## | 70.5 ± 5.5## |
| *Day 3* | 52.5 ± 4.0 | 73.5 ± 6.5## | 92.5 ± 3.5## | 95.0 ± 3.0## | 90.0 ± 5.0## | 65.5 ± 7.5## | 67.7 ± 3.5## |
| *Day 4* | 50.5 ± 2.5 | 80.5 ± 4.5##** | 93.0 ± 3.5##** | 93.5 ± 3.5##** | 91.5 ± 2.5##* | 63.5 ± 8.0#** | 65.5 ± 2.5## |
| P2X2/3 DKO | | | | | | | |
| *Day 1* | 45.4 ± 6.5 | 65.5 ± 3.5## | 77.5 ± 5.5## | 82.5 ± 6.5## | 80.5 ± 7.5## | 45.5 ± 5.0 | 60.0 ± 5.5# |
| *Day 2* | 44.5 ± 3.5 | 70.5 ± 5.5## | 85.5 ± 7.5## | 88.0 ± 6.5## | 82.5 ± 5.0## | 55.0 ± 5.0 | 55.5 ± 5.5 |
| *Day 3* | 42.5 ± 5.5 | 80.5 ± 4.5## | 88.5 ± 4.5## | 91.5 ± 3.5## | 90.0 ± 4.5## | 61.5 ± 3.5## | 58.5 ± 3.0# |
| *Day 4* | 51.5 ± 6.5 | 83.5 ± 6.5##** | 85.0 ± 3.0##* | 90.5 ± 5.0## | 85.0 ± 3.5## | 60.0 ± 5.0#** | 55.5 ± 5.5 |

Data indicate means ± SD. $n = 6$ mice per group except for the P2X purinoreceptor (P2X)2/3 double-knockout (DKO) sucralose/saccharin groups in which $n = 3$. All sugars were administered as 15% solutions (833 mM for fructose and glucose, 438 mM for sucrose), sucralose at 30 mM and saccharin at 35 mM. FG, fructose glucose mixture consisting of 55% fructose and 45% glucose; WT, wild type. $*P < 0.05$ and $**P < 0.01$ vs. *day 1* for each sugar in each strain. $\#P < 0.05$ and $\#\#P < 0.01$ vs. no difference in preference (50%). Two-tail t test.

studies are needed to understand the role of taste in sugar-induced metabolic syndrome.

One way to test the role of taste in sugar-induced metabolic syndrome is to use a mouse that lacks all taste signaling while leaving intact the sweet receptor functions of the gut. A so-called taste-blind mouse results from the genetic deletion of P2X purinoreceptors 2 and 3 (P2X2 and P2X3) (the P2X2/P2X3 double-knockout mouse, P2X2/3 DKO) necessary for transmission of taste information from taste buds to the taste nerves (8, 31). This mouse has the advantage over the sweet taste receptor knockout in that it leaves intact the sweet receptor signaling in the gut.

In addition, we wanted to compare the P2X2/3 DKO mouse with a mouse that has deficient metabolism of sucrose, specifically the fructokinase A/C knockout mouse (also known as ketohexokinase, KHK A/C KO) (11). Sucrose and HFCS contain glucose and fructose, either combined as a disaccharide (sucrose) or as a mixture of monosaccharides (HFCS). Both glucose and fructose can produce metabolic syndrome when the sugars are added to the drinking water, with glucose inducing metabolic syndrome more rapidly than fructose (11, 18). Nevertheless, the KHK A/C KO mouse, which cannot metabolize fructose, is protected from both glucose or fructose-induced metabolic syndrome (11, 18, 26), because glucose-fed mice have some of the glucose converted to fructose in the liver due to activation of the polyol pathway (18). In contrast, the KHK-A isoform KO mouse (KHK-A KO) that has the KHK-C isoform intact is not protected from fructose-induced metabolic syndrome (18), demonstrating that it is the KHK-C isoform that drives the metabolic response to sugar.

This study tests whether the P2X2/3 DKO mouse is still prone to developing sugar-induced obesity and metabolic syndrome despite being unable to taste the sweetness of sugar.

## MATERIALS AND METHODS

*Animals.* P2X2/3 DKO mice (B6;129-$P2rx2^{tm1Ckn}$/$P2rx3^{tm1Ckn}$) were obtained from a breeding colony at Charles River (Wilmington, MA) under MTA with Afferent Pharmaceuticals. The mixed background matched (B6;129) WT controls ($WT^{P2X}$) were bred and maintained at the University of Colorado but were backcrossed to the DKO mice every two to three generations to minimize genetic drift. KHK-A/C KO (B6;129-$Khk^{tm2Dtb}$) and KHK-A KO (B6;129-$Khk^{tm2.1Dtb}$) mice were originally developed by David Bonthorn at Leeds University (UK) (7) and were bred and maintained at the University of Colorado with pure C57/Bl6 for over 7 generations to ensure the mice were on the B6 genetic background. All experimental mice were maintained in temperature- and humidity-controlled specific pathogen-free condition on a 14-h dark/10-h light cycle and allowed ad libitum access to normal laboratory chow (Harlan Teklad, #2920X). All experiments were conducted with adherence to the NIH Guide for the Care and Use of Laboratory Animals. In all studies, 7- to 10-wk-old male mice ($n = 3$–7) were used. Food consumption was monitored daily, and body weight was recorded.

For 24-h two-bottle preference studies, mice were housed individually and provided with two similar water bottles filled with water for acclimation for a 5-day period. For the experiment, water in one of the bottles was substituted by water containing either fructose (833 mM), glucose (833 mM), sucrose (438 mM), a mixture of fructose and glucose (FG) with similar concentrations as HFCS [prepared in the laboratory and containing a mixture of 55% fructose (458 mM)/45% glucose (375 mM)], saccharin (35 mM), or sucralose (30 mM) at the indicated concentration. Data presented in Table 1 indicate number of

Table 2. *Daily sweetener intake and sugar solute intake (g/day) in 30 wk WT and P2X2/3 DKO mice*

| | Water | Fructose | Sucrose | FG Mixture | Glucose | Sucralose | Saccharin |
|---|---|---|---|---|---|---|---|
| Total fluid intake, mL/day | | | | | | | |
| $WT^{P2X}$ | 3.21 ± 0.4 | 6.52 ± 0.3 | 9.26 ± 2.2 | 13.43 ± 2.3 | 8.30 ± 1.0 | 5.37 ± 0.4 | 6.00 ± 0.4 |
| P2X2/3 DKO | 3.26 ± 0.1 | 5.83 ± 0.3# | 5.72 ± 0.4# | 6.11 ± 0.6# | 5.90 ± 0.4# | 4.75 ± 0.4# | 4.97 ± 0.3# |
| Sugar solute intake, g/day | | | | | | | |
| $WT^{P2X}$ | 0 | 0.98 ± 0.04 | 1.38 ± 0.36 | 2.01 ± 0.32 | 1.24 ± 0.13 | 0.002 ± 0.0003 | 0.004 ± 0.0002 |
| P2X2/3 DKO | 0 | 0.87 ± 0.04# | 0.86 ± 0.05# | 0.91 ± 0.08# | 0.88 ± 0.08# | 0.002 ± 0.0001 | 0.002 ± 0.0001 |

Data indicate means ± SD. $n = 6$ mice per group except for sucralose and saccharin in which $n = 3$. Daily water, sugar, and artificial sweetener intake in wild-type and P2X purinoreceptor (P2X)2/3 double-knockout (DKO) mice. All sugars were administered as 15% solutions [8.25% fructose and 6.75% glucose for fructose/glucose (FG) mixture, 15% for sucrose], sucralose at 30 mM and saccharin at 35 mM. $\#P < 0.05$ one-way ANOVA-Tukey post hoc analysis vs. WTP2X in each sugar.

mice and statistical methods. To avoid conditioning bias, data in Table 1 are obtained from naive mice never exposed before to sugar. One day after exposure, the position of the two bottles (regular and sweet-containing water) was switched to control for side preference. The preference ratio was calculated as the ratio of volume of tastant consumed over the 2-day test period to total volume consumed, i.e., a score of 0.5 or 50% shows no preference.

*Biochemical analysis.* Blood was collected in microtainer tubes (BD) from cardiac puncture of mice under isoflurane, and serum was obtained after centrifugation at 13,000 rpm for 2 min at room temperature. Serum parameters were performed biochemically following manufacturer's instruction (uric acid: Bioassay systems, DIUA-250; FGF21: R&D, MF2100, AST: Bioassay Systems, EASTR-100, ALT: Bioassay Systems, EALT-100, insulin: Crystal Chem, 90080, leptin: R&D, MOB00). Determination of parameters in tissue was performed in freeze-clamped tissues and measured biochemically following manufacturer's protocol [triglycerides (liver): Bioassay Systems, ETGA-200; uric acid: Bioassay Systems DIUA-250].

*Histopathology.* Formalin-fixed paraffin-embedded kidney sections were stained with hematoxylin and eosin (H&E). Histological examination was performed through an entire cross section of liver from each mouse. Images were captured on an Olympus BX51 microscope equipped with a four-megapixel Macrofire digital camera (Optronics; Goleta, CA) using the PictureFrame Application 2.3 (Optronics). Composite images were assembled with the use of Adobe Photoshop. All images in each composite were handled identically.

*Western blotting.* Protein lysates were prepared from mouse tissue employing MAP kinase lysis buffer as previously described (16). Protein content was determined by the BCA protein assay (Pierce). Total protein (50 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% w/v) and transferred to PVDF membranes (BioRad). Membranes were first blocked for 1 h at 25°C in 4% (w/v) instant milk dissolved in 0.1% Tween-20 Tris-buffered saline (TTBS), incubated with primary rabbit or mouse-raised antibodies (1:1,000 dilution in TTBS) pSTAT3/STAT3 (Cell Signaling 12640/9131; RRID:AB_2629499, RRID:AB_331586), fosb (Cell signaling 2251; RRID:AB_2106903), GluR2 (Cell Signaling 13607; RRID:AB_2650557), KHK (Sigma HPA007040; RRID:AB_1079185), and GAPDH (Santa Cruz Biotechnologies sc-365062; RRID:AB_10847862) and visualized using an anti-rabbit (7074;

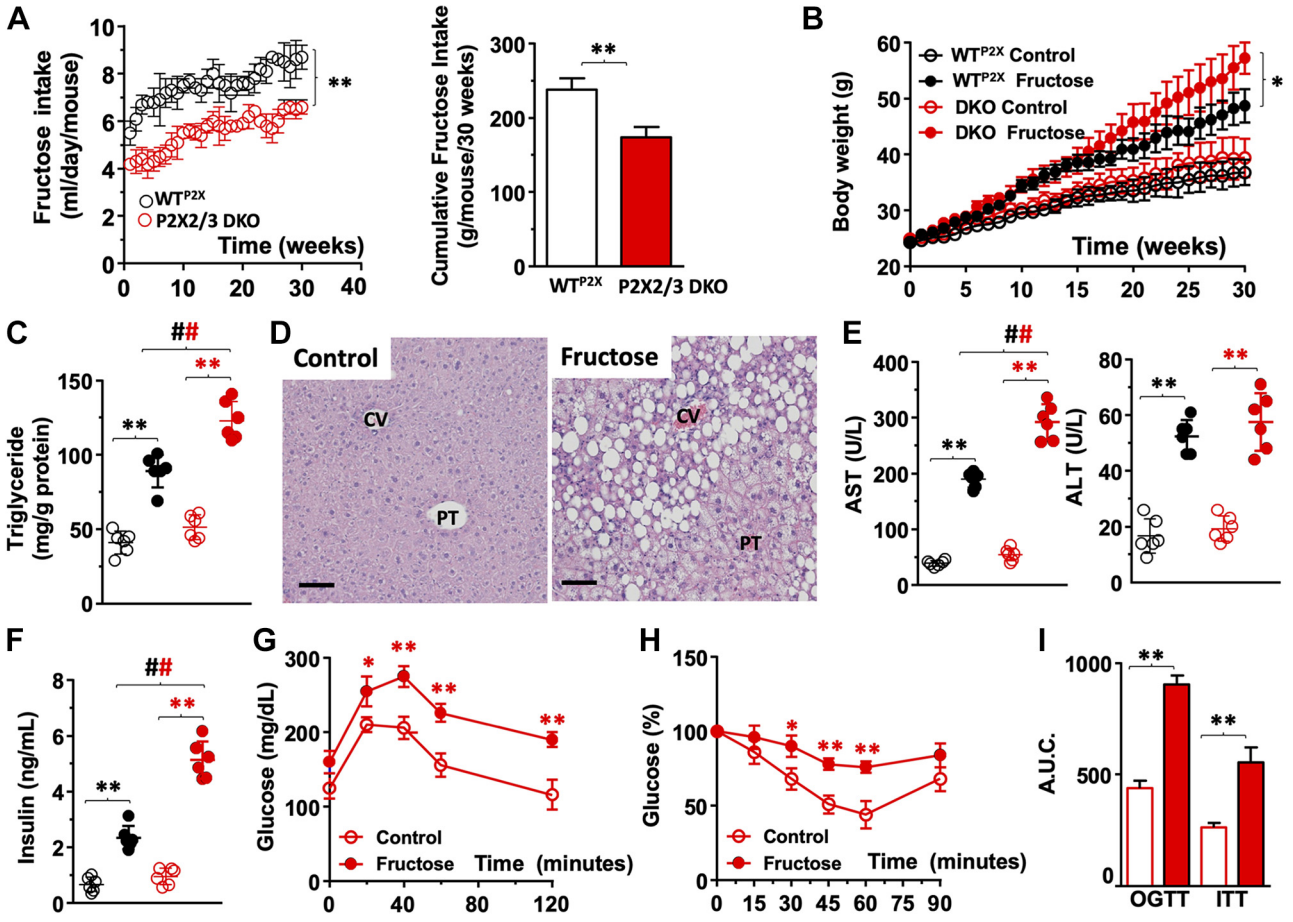


Fig. 1. Sugar consumption and development of metabolic syndrome in P2X purinoreceptor (P2X)2/3 double-knockout (DKO) mice. *A*: daily intake of 15% fructose over a 30-wk period demonstrating that P2X2/3 DKO (red) consistently drank less fructose than wild-type (black) and that both strains had greater intake at week 30 than at the outset of the experiment. $n = 6$ mice/group. *Right*: total accumulated fructose solution intake in wild-type (white) and P2X2/3 DKO mice (red) over a 30-wk period. *B*: body weight gain in wild-type (black) and P2X2/3 DKO mice (red) exposed to regular water (open symbols) or 15% fructose (solid symbols) for 30 wk. *C*: liver triglyceride levels at time of death (*week 30*) in wild-type (black) and P2X2/3 DKO (red) mice exposed to fructose compared with mice drinking regular water. *D*: representative H&E liver images from P2X2/3 DKO control or on fructose and demonstrating micro- and macrosteatotic areas induced by fructose. Size bars: 50 μM, PT: portal triad; CV: central vein. *E*: serum transaminases AST (*left*) and ALT (*right*) levels in the same mice as in *C*. *F*: plasma insulin levels in the same mice as in *C*. *G* and *H*: plasma glucose levels in P2X2/3 DKO mice drinking regular water (control, open symbols) or fructose solution (solid symbols) for 25 wk and undergoing an oral glucose tolerance test (OGTT; *left*) or an insulin tolerance test (ITT; *right*). $n = 4$ mice per group. *I*: area under the curve (AUC) for oral glucose and insulin tolerance tests in the same mice as in *G* and *H*. $^*P < 0.05$, $^{**}P < 0.01$, one-way ANOVA Tukey post hoc analysis.

RRID:AB_2099233) or anti-mouse IgG (7076; RRID:AB_330924) horseradish-peroxidase conjugated secondary antibody (1:2000, Cell Signaling) using the HRP Immunstar detection kit (BioRad, Hercules, CA). Chemiluminescence was recorded with an Image Station 440CF, and results were analyzed with the 1D Image Software (Kodak Digital Science, Rochester, NY).

*Determination of ChREBP expression, acetylation, and activity.* Acetylated ChREBP was determined as previously described (21). Briefly, liver tissues were homogenized in buffer containing 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 50 mM Tris pH7.5, and protease inhibitor cocktail and precleared with protein A/G agarose (Invitrogen) for 30 min at 4°C, lysates were then quantified and 500 μg incubated for 4 h with 25 μL of goat polyclonal ChREBP antibody (Santa Cruz Biotechnologies, sc21189, RRID:AB_2146396). After 4 h, 40 μL of protein A/G agarose (Invitrogen) were added to the tubes, and incubations were continued overnight at 4°C. Immunoprecipitates were then washed extensively and collected by centrifugation and acetylated ChREBP levels were determined by Western blot using a mouse monoclonal acetylated lysine antibody (Cell Signaling, 9441S, RRID:AB_331805).

Real-time PCR cytosolic RNA was isolated from liver using the RNeasy kit (Qiagen, Valencia, CA). Before real-time PCR, RNA was converted to cDNA using the iScript reverse transcriptase kit (Bio-Rad) as described by the manufacturer. Specific RT-PCR primers were designed using Beacon Designer 5.0 software (Premier Biosoft International, Palo Alto, CA) and are available upon request. RT-PCR was performed using 70 nM primers and the SYBR Green JumpStart *Taq* Readymix QPCR kit (Sigma) on a BioRad I-Cycler. RT-PCR runs were analyzed by agarose gel electrophoresis and melt curve to verify that the correct amplicon was produced. β-Actin RNA was used as an internal control, and the amount of RNA was calculated by the comparative $C_T$ method.

*KHK activity in liver lysates.* KHK activity was determined as we previously described (17) and is based on the determination of %ATP consumed by liver extracts upon exposure of fructose.

*Leptin and insulin tolerance tests.* Insulin sensitivity was determined by both oral glucose and insulin tolerance tests as we previously described (19). For leptin sensitivity, mice are habituated to injection by receiving IP saline on fasting condition for 3 consecutive days. At *day 4*, food is removed and animals are injected with either saline (control) or leptin (0.6 mg/kg body wt, Peprotech) and the amount of food consumed afterward is recorded for 7 h. For hypothalamic activation of STAT3, mice are injected with leptin and euthanized 1 h postinjection. The hypothalamic area of the brain is then dissected out, lysed, and total and phosphorylated (S727) STAT3 levels analyzed by Western blot.

*Nerve recording of tastants.* Chorda tympani nerve recordings were performed as described by Vandenbeuch et al. (39). In brief, the chorda tympani nerve was exposed using a ventral approach and placed on a platinum electrode. The neural activity was amplified (P511; Grass Instrument), integrated, and recorded using AcqKnowledge software (Biopac). The anterior part of the tongue was stimulated with various tastants (30 s) and rinsed with water (40 s). Responses were normalized to $NH_4Cl$ 100 mM.

*Statistical analysis.* All numerical data are presented as means ± SE. Independent replicates for each data point ($n$) are identified in figure legends. Data graphics and statistical analyses were performed using Prism 5 (GraphPad). Data without indications were analyzed by one-way ANOVA and Tukey post hoc test. A value of $P < 0.05$ was regarded as statistically significant. Animals were randomly allocated in each group using randomizer (https://www.randomizer.org). No animals were excluded from the study.

*Study approval.* All animal experiments were conducted with adherence to the NIH Guide for the Care and Use of Laboratory Animals (29). The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Colorado (Aurora, CO).

## RESULTS

*Role of taste in preference of added sugars in taste-blind mice.* We first evaluated whether a lack of sweet taste affected the mouse's preference for caloric sugars (sucrose, fructose, glucose, and FG mixture) and artificial sweeteners (saccharin and sucralose) compared with water. To this end, we employed P2X2/3 DKO mice, which lack transmission of sweet taste from taste buds to nerves. Preference was evaluated over a 24-h period using a two-bottle system in which one bottle contained water and the other a 15% sugar solution or, in the case of noncaloric sugars, a solution of 35 mM (saccharin) or 30 mM (sucralose). As shown in Table 1, wild-type (WT) mice

Table 3. *Daily food and total caloric intake in wild-type and P2X2/3 DKO mice*

| | Water | Fructose | Sucrose | FG Mixture | Glucose | Sucralose | Saccharin |
|---|---|---|---|---|---|---|---|
| 30 week chow intake, g | | | | | | | |
| $WT^{P2X}$ | 682 ± 56 | 541 ± 33 | 525 ± 25 | 441 ± 33 | 553 ± 41 | 594 ± 58 | 583 ± 44 |
| P2X2/3 DKO | 898 ± 47* | 871 ± 61* | 835 ± 61* | 798 ± 55*# | 865 ± 56* | 888 ± 52* | 898 ± 52* |
| 30 week caloric intake, kcal | | | | | | | |
| $WT^{P2X}$ | 2252 ± 72 | 2448 ± 77## | 2743 ± 131## | 3016 ± 138## | 2703 ± 131## | 1978 ± 72## | 1928 ± 14## |
| P2X2/3 DKO | 2968 ± 141* | 3350 ± 116*## | 3280 ± 110*## | 3190 ± 124# | 3391 ± 278*# | 2946 ± 21* | 2968 ± 14* |
| 30 week total by nutrient sugar water, kcal | | | | | | | |
| $WT^{P2X}$ | 0.00 | 822.60 ± 20## | 1168.00 ± 73## | 1693.00 ± 46## | 1046.10 ± 31## | 0.00 | 0.00 |
| P2X2/3 DKO | 0.00 | 735.50 ± 21*## | 772.60 ± 67*## | 796.00 ± 58*## | 795.40 ± 90*## | 0.00 | 0.00 |
| Chow protein, kcal | | | | | | | |
| $WT^{P2X}$ | 540.48 ± 16 | 390.10 ± 21## | 378.00 ± 16## | 317.52 ± 23## | 397.66 ± 49## | 474.72 ± 25## | 462.72 ± 51## |
| P2X2/3 DKO | 712.32 ± 46* | 627.48 ± 51*# | 601.78 ± 16*## | 574.56 ± 23*## | 622.94 ± 36*## | 707.04 ± 6* | 712.32 ± 70* |
| Chow fat, kcal | | | | | | | |
| $WT^{P2X}$ | 405.36 ± 16 | 292.57 ± 12## | 283.50 ± 12## | 238.14 ± 16## | 298.24 ± 50## | 356.04 ± 13## | 347.04 ± 23## |
| P2X2/3 DKO | 534.24 ± 23* | 470.61 ± 20*## | 451.33 ± 13*## | 430.92 ± 23*## | 467.21 ± 31*## | 530.28 ± 9* | 534.24 ± 33* |
| Chow carbs, kcal | | | | | | | |
| $WT^{P2X}$ | 1306.16 ± 40 | 942.73 ± 24## | 913.50 ± 30## | 767.34 ± 53## | 961.00 ± 51## | 1147.2 ± 34## | 1118.24 ± 70## |
| P2X2/3 DKO | 1721.44 ± 72* | 1516.4 ± 25*## | 1454.3 ± 14*## | 1388.5 ± 23*## | 1505.4 ± 79*## | 1708.68 ± 6* | 1721.44 ± 37* |

Data indicate means ± SD. $n = 6$ mice per group except for the P2X2/3 DKO sucralose/saccharin groups in which $n = 3$. *Top*: daily food intake (g/day) in wild-type and P2X2/3 DKO mice. *Bottom*: 30 wk total caloric intake and itemized by protein/fat/carbohydrate in chow and sugary water in wild-type and P2X2/3 DKO mice. All sugars were administered as 15% solutions (8.25% fructose and 6.75% glucose for FG mixture, 15% for sucrose), sucralose at 30 mM, and saccharin at 35 mM. *$P < 0.05$ one-way ANOVA-Tukey post hoc analysis vs. respective treatment. #$P < 0.05$ and ##$P < 0.01$ vs. water in each strain.

showed a preference for both caloric sugars and artificial sweeteners that steadily increased over the first 4 days after initial exposure. Similarly, taste-blind mice (P2X2/3 DKO) also showed marked preference for FG mixture, sucrose, fructose, and glucose. However, their preference for noncaloric sugars (particularly saccharin) is substantially lower than wild-type counterparts. Of note, the P2X2/3 DKO mice showed some preference for sucralose, which suggests that some artificial sweeteners can still stimulate preference independently of its sweet taste. It is important to note that, unlike sweet aguesic T1R3 KO and Trpm5 KO mice in which their preference for caloric sugars (32% sucrose) was not evident on the first 24 h postexposure but rather on later days (2–4) postexposure (9), P2X2/3 DKO mice displayed preferences for sucrose solutions (15%), as well as other sugars, from day 1 and thereafter, indicating that P2X2/3 DKO mice develop a more rapid preference for caloric sugars than sweet receptor knockout mice. This difference in the timing of preference could be due to the intact sugar metabolism and gut sweet receptor signaling present in P2X2/3 DKO mice.

*Role of taste in sugar intake and sugar-induced metabolic syndrome in taste-blind mice.* After characterizing the preference responses to sugar in P2X2/3 DKO mice, we then evaluated their metabolic response to sugar. To this end, animals were exposed to water or different sugar solutions for 30 wk (one single bottle), and metabolic syndrome was characterized. Although P2X2/3 DKO mice demonstrate marked preference for calorie-containing sugars over water, they do not consume the same amount of caloric sugars as WT mice under similar feeding conditions. This observation would suggest that the sweet signal from taste buds is important to enhance intake of caloric sugars. As shown in Table 2, P2X2/3 DKO mice exhibited significantly lower total intake of caloric sweeteners compared with wild-type control mice, with a greater relative reduction for glucose-containing sugars compared with fructose alone, while intake of noncaloric sweeteners was also mildly reduced.

Reduced intake of sugar might suggest that P2X2/3 DKO mice would be protected against sugar-induced metabolic syndrome, as their sugar-derived caloric intake is markedly lower than that of wild-type mice. To address whether the taste-blind (P2X2/3 DKO) mice are protected from metabolic syndrome, we placed WT mice or P2X2/3 DKO on fructose (15%—830 mM—in the drinking water) or other sugars for 30 wk, which is the usual time period used to induce metabolic syndrome in WT mice (11). Control groups consisted of P2X2/3 DKO and

Table 4. *Effect of fructose intake on metabolic syndrome in the P2X2/3 DKO mouse compared with WT*

| | Wild Type | | P2X2/3 DKO | | Wild-Type Fructose vs. P2X2/3 DKO Fructose (ANOVA) |
|---|---|---|---|---|---|
| | Water ($n$ = 6) | Fructose ($n$ = 6) | Water ($n$ = 6) | Fructose ($n$ = 6) | |
| Body weight and composition | | | | | |
| Body weight, g; 0 wk | 24.2 ± 0.4 | 24.3 ± 0.2 | 24.8 ± 0.4 | 25.0 ± 0.4 | NS |
| Body weight, g; 30 wk | 36.8 ± 2.3 | 48.7 ± 3.0* | 39.4 ± 3.4 | 57.2 ± 2.8* | $P < 0.01$ |
| ΔBody weight, g; 30 wk | 12.6 ± 1.3 | 24.4 ± 1.6* | 14.6 ± 1.6 | 32.2 ± 1.6* | $P < 0.01$ |
| Average food intake, g·mouse$^{-1}$·day$^{-1}$ | 3.25 ± 0.1 | 2.60 ± 0.1** | 4.28 ± 0.2 | 4.15 ± 0.2 | $P < 0.01$ |
| Liver weight, g; 30 wk | 1.56 ± 0.25 | 2.56 ± 0.46** | 1.67 ± 0.12 | 4.10 ± 0.62** | $P < 0.01$ |
| Liver/body weight ratio | 0.042 ± 0.01 | 0.052 ± 0.02* | 0.042 ± 0.02 | 0.071 ± 0.03** | $P < 0.01$ |
| Kidney weight, g; 30 wk | 0.41 ± 0.06 | 0.50 ± 0.04 | 0.40 ± 0.07 | 0.53 ± 0.06* | NS |
| Kidney/body weight ratio | 0.011 ± 0.02 | 0.010 ± 0.03 | 0.010 ± 0.02 | 0.010 ± 0.02 | NS |
| Heart weight, g; 30 wk | 0.14 ± 0.07 | 0.22 ± 0.04 | 0.16 ± 0.04 | 0.27 ± 0.03** | NS |
| Heart/body weight ratio | 0.004 ± 0.002 | 0.005 ± 0.001 | 0.004 ± 0.001 | 0.005 ± 0.002 | NS |
| Epididymal fat weight, g; 30 wk | 0.75 ± 0.28 | 2.46 ± 0.51** | 0.81 ± 0.23 | 3.25 ± 0.46** | $P < 0.05$ |
| Epididymal fat/body weight ratio | 0.02 ± 0.008 | 0.05 ± 0.015** | 0.02 ± 0.001 | 0.06 ± 0.011** | NS |
| Biochemical blood analysis | | | | | |
| AST, IU/L | 39.1 ± 4.1 | 189.5 ± 13.7** | 54.6 ± 11.1 | 292.7 ± 31.9** | $P < 0.01$ |
| ALT, IU/L | 16.7 ± 6.1 | 52.5 ± 8.2** | 19.3 ± 4.5 | 57.5 ± 10.4** | NS |
| Serum triglycerides, mg/dL | 65.7 ± 14.8 | 84.8 ± 12.6 | 71.7 ± 8.9 | 90.2 ± 15.5 | NS |
| Serum uric acid, mg/dL | 2.56 ± 0.4 | 3.89 ± 0.4** | 3.18 ± 0.3 | 4.95 ± 0.3** | $P < 0.01$ |
| Fasting serum glucose, mg/dL | 116 ± 8.1 | 216 ± 14.8** | 153 ± 12.5 | 248 ± 11.1** | $P < 0.01$ |
| Fasting insulin, ng/mL | 0.67 ± 0.25 | 2.34 ± 0.42** | 0.95 ± 0.29 | 5.12 ± 0.66** | $P < 0.01$ |
| HOMA-IR | 5.5 ± 1.4 | 35.9 ± 8.8** | 10.4 ± 2.2 | 90.1 ± 8.6** | $P < 0.01$ |
| OGTT (AUC) | 376 ± 75 | 678 ± 35** | 483 ± 26 | 880 ± 36** | $P < 0.01$ |
| ITT (AUC) | 310 ± 55 | 535 ± 40** | 226 ± 38 | 515 ± 25** | NS |
| Fasting leptin, ng/mL | 3.96 ± 1.42 | 16.6 ± 2.66** | 6.26 ± 1.36 | 23.87 ± 5.46** | $P < 0.05$ |
| Adipose *leptin* mRNA, AU | 0.78 ± 0.36 | 2.25 ± 0.56** | 1.18 ± 0.28 | 3.85 ± 0.51** | $P < 0.05$ |
| Fructose, mM | 0.02 ± 0.01 | 0.14 ± 0.04** | 0.02 ± 0.01 | 0.10 ± 0.03** | NS |
| Serum creatinine, mg/dL | 0.10 ± 0.004 | 0.12 ± 0.03 | 0.16 ± 0.005 | 0.23 ± 0.004 | $P < 0.01$ |
| Blood urea nitrogen, mg/dL | 21.0 ± 7.0 | 16 ± 12.0 | 28.2 ± 8.5 | 35.8 ± 6.8 | $P < 0.05$ |
| FGF21, pg/mL | 428.7 ± 360.1 | 1724.0 ± 394.6* | 1074.0 ± 539.6 | 3751 ± 1160** | $P < 0.01$ |
| Liver analysis | | | | | |
| Glycogen, mg/g | 8.89 ± 0.08 | 39.16 ± 11.51** | 14.58 ± 5.56 | 46.23 ± 10.22** | NS |
| Uric acid, μg/mg | 0.63 ± 0.06 | 2.51 ± 0.15** | 1.27 ± 0.08 | 9.12 ± 2.74** | $P < 0.01$ |
| ATP, μmol/g | 4.46 ± 0.5 | 3.81 ± 0.3* | 3.55 ± 0.3 | 3.38 ± 0.2 | $P < 0.05$ |
| β-Hydroxybutyrate, nmol/g | 0.024 ± 0.008 | 0.31 ± 0.006 | 0.044 ± 0.006 | 0.023 ± 0.007** | NS |
| *Fgf21* mRNA, AU | 0.63 ± 0.25 | 3.26 ± 0.73** | 1.23 ± 0.32 | 6.31 ± 0.69** | $P < 0.01$ |

Data indicate means ± SD. $n$ = 6 mice per group. Fructose was administered as a 15% solution (833 mM). AUC, area under the curve; FGF21, fibroblast growth factor 21; HOMA-IR, homeostatic model assessment for insulin resistance; ITT, insulin tolerance test; KO, knockout; NS, not significant; OGTT, oral glucose tolerance test; P2X2, P2X purinoreceptor 2; P2X3, P2X purinoreceptor 3; P2X2/3 DKO, P2X purinoreceptor 2 and 3 double knockout; WT, wild type. *$P < 0.05$ and **$P < 0.01$ vs. respective water controls. One-way ANOVA with post hoc Tukey. Two-tail $t$ test.

WT mice only receiving water. All mice were provided chow ad libitum during this time.

As expected, over the 30-wk period, P2X2/3 DKO mice consumed less fructose and caloric sugars (Fig. 1*A* and Tables 4–7) than wild-type controls. However, although sugar-exposed WT mice demonstrated a significant reduction (~20%) in food intake compared with control mice on water, possibly to compensate for the calories received from the sugar water, P2X2/3 DKO mice did not reduce their chow intake despite being exposed to sugars. As a consequence, P2X2/3 DKO mice had higher total caloric intake compared with WT animals for each specific drink except for the FG mixture (Table 3).

Of note, we observed substantial metabolic differences between wild-type and P2X2/3 DKO mice at baseline. As shown in Tables 4–7, P2X2/3 DKO mice on water demonstrated greater fasting insulin and leptin levels as well as reduced insulin sensitivity as denoted by higher homeostatic model assessment for insulin resistance. However, despite this and the observation that P2X2/3 DKO mice had higher total energy intake compared with wild-type animals, we observed no substantial markers of metabolic syndrome in water-exposed P2X2/3 DKO mice (Tables 4–7). The absence of metabolic syndrome in P2X2/3 DKO mice on water, despite getting significantly more calories than wild-type animals on water would suggest the presence of mechanisms to efficiently regulate energy expenditure to counterbalance the excess in energy intake in P2X2/3 DKO mice, thus preventing them from gaining more weight than WT counterparts. In this regard, hepatic levels of β-hydroxybutyrate are markedly higher in control P2X2/3 DKO mice (Tables 4–7) and would suggest enhanced fat oxidation in these animals compared with wild-type controls. Of interest, these protective mechanisms seem to be impaired by sugar as the P2X2/3 DKO mice developed marked features of the metabolic syndrome when exposed to fructose or other caloric sweeteners with parallel reduction in β-hydroxybutyrate levels in liver. As shown in Fig. 1 and Table 4, despite lower intake of fructose or other caloric sugars, P2X2/3 DKO mice demonstrated markedly worse features of obesity and metabolic syndrome compared with WT controls. For example, and as shown Fig. 1*B* and Table 4, body weight gain was significantly higher in P2X2/3 DKO mice drinking fructose compared with wild-type mice on fructose at *week 20* postexposure to fructose with final weight gains of 24.4 g on average in WT and 32.2 g in P2X2/3 DKO mice.

Table 5. *Effect of sucrose intake on metabolic syndrome in the P2X2/3 DKO mouse compared with WT*

| | Wild Type | | P2X2/3 DKO | | Wild-Type Sucrose vs. P2X2/3 DKO Sucrose (ANOVA) |
|---|---|---|---|---|---|
| | Water ($n$ = 6) | Sucrose ($n$ = 5) | Water ($n$ = 6) | Sucrose ($n$ = 5) | |
| Body weight and composition | | | | | |
| Body weight, g; 0 wk | 24.2 ± 0.4 | 24.6 ± 0.5 | 24.8 ± 0.4 | 25.2 ± 0.6 | NS |
| Body weight, g; 30 wk | 36.8 ± 2.3 | 46.6 ± 2.9** | 39.4 ± 3.4 | 58.1 ± 3.6** | $P < 0.01$ |
| ΔBody weight, g; 30 wk | 12.6 ± 1.3 | 22.0 ± 2.6** | 14.6 ± 1.6 | 32.9 ± 2.1** | $P < 0.01$ |
| Average food intake, g·mouse$^{-1}$·day$^{-1}$ | 3.25 ± 0.1 | 2.50 ± 0.2** | 4.28 ± 0.2 | 3.98 ± 0.1 | $P < 0.01$ |
| Liver weight, g; 30 wk | 1.56 ± 0.25 | 2.36 ± 0.41** | 1.67 ± 0.12 | 4.23 ± 0.32** | $P < 0.01$ |
| Liver/body weight ratio | 0.042 ± 0.01 | 0.052 ± 0.01 | 0.042 ± 0.02 | 0.072 ± 0.04** | $P < 0.01$ |
| Kidney weight, g; 30 wk | 0.41 ± 0.06 | 0.56 ± 0.08 | 0.40 ± 0.07 | 0.55 ± 0.03* | NS |
| Kidney/body weight ratio | 0.011 ± 0.02 | 0.012 ± 0.04 | 0.010 ± 0.02 | 0.010 ± 0.01 | NS |
| Heart weight, g; 30 wk | 0.14 ± 0.07 | 0.24 ± 0.07 | 0.16 ± 0.04 | 0.34 ± 0.05** | NS |
| Heart/body weight ratio | 0.004 ± 0.002 | 0.005 ± 0.001 | 0.004 ± 0.001 | 0.006 ± 0.002 | NS |
| Epididymal fat weight, g; 30 wk | 0.75 ± 0.28 | 2.43 ± 0.31** | 0.81 ± 0.23 | 3.60 ± 0.36** | $P < 0.05$ |
| Epididymal fat/body weight ratio | 0.02 ± 0.008 | 0.05 ± 0.008** | 0.02 ± 0.001 | 0.06 ± 0.013** | NS |
| Biochemical blood analysis | | | | | |
| AST, IU/L | 39.1 ± 4.1 | 183.5 ± 20.7** | 54.6 ± 11.1 | 313.3 ± 23.7** | $P < 0.01$ |
| ALT, IU/L | 16.7 ± 6.1 | 47.1 ± 3.6** | 19.3 ± 4.5 | 66.85 ± 13.9** | NS |
| Serum triglycerides, mg/dL | 65.7 ± 14.8 | 90.3 ± 6.6* | 71.7 ± 8.9 | 98.7 ± 9.3* | NS |
| Serum uric acid, mg/dL | 2.56 ± 0.4 | 3.67 ± 0.6* | 3.18 ± 0.3 | 4.80 ± 0.4** | $P < 0.01$ |
| Fasting serum glucose, mg/dL | 116 ± 8.1 | 198 ± 12.2** | 153 ± 12.5 | 236 ± 9.11** | $P < 0.01$ |
| Fasting insulin, ng/mL | 0.67 ± 0.25 | 2.10 ± 0.22** | 0.95 ± 0.29 | 4.89 ± 0.36** | $P < 0.01$ |
| HOMA-IR | 5.5 ± 1.4 | 31.1 ± 5.8** | 10.4 ± 2.2 | 82.3 ± 9.1** | $P < 0.01$ |
| OGTT, AUC | 376 ± 75 | 736 ± 26** | 483 ± 26 | 955 ± 45** | $P < 0.01$ |
| ITT, AUC | 310 ± 55 | 587 ± 42** | 226 ± 38 | 616 ± 36** | NS |
| Fasting leptin, ng/mL | 3.96 ± 1.42 | 17.81 ± 3.45** | 6.26 ± 1.36 | 27.78 ± 2.31** | $P < 0.05$ |
| Adipose *leptin* mRNA, AU | 0.78 ± 0.36 | 2.08 ± 0.31** | 1.18 ± 0.28 | 4.55 ± 0.27** | $P < 0.05$ |
| Fructose, mM | 0.02 ± 0.01 | 0.09 ± 0.02** | 0.02 ± 0.01 | 0.07 ± 0.04* | NS |
| Serum creatinine, mg/dL | 0.10 ± 0.004 | 0.17 ± 0.02 | 0.16 ± 0.005 | 0.27 ± 0.01* | $P < 0.05$ |
| Blood urea nitrogen, mg/dL | 21.0 ± 7.0 | 25 ± 8.0 | 28.2 ± 8.5 | 40.1 ± 9.8 | NS |
| FGF21, pg/mL | 428.7 ± 360.1 | 2106.0 ± 337.8** | 1074.0 ± 539.6 | 3209 ± 778** | NS |
| Liver analysis | | | | | |
| Glycogen, mg/g | 8.89 ± 0.08 | 27.8 ± 8.5** | 14.58 ± 5.56 | 53.35 ± 6.67** | $P < 0.01$ |
| Uric acid, μg/mg | 0.63 ± 0.06 | 3.1 ± 0.08** | 1.27 ± 0.08 | 13.34 ± 4.41** | $P < 0.01$ |
| ATP, μmol/g | 4.46 ± 0.5 | 3.76 ± 0.2* | 3.55 ± 0.3 | 3.71 ± 0.3 | NS |
| β-Hydroxybutyrate, nmol/g | 0.020 ± 0.007 | 0.026 ± 0.004 | 0.039 ± 0.005 | 0.022 ± 0.006** | NS |
| *Fgf21* mRNA, AU | 0.63 ± 0.25 | 3.88 ± 0.11** | 1.23 ± 0.32 | 5.66 ± 0.41** | $P < 0.01$ |

Data indicate means ± SD. $n$ = 5 or 6 mice per group. AUC, area under the curve; FGF21, fibroblast growth factor 21; HOMA-IR, homeostatic model assessment for insulin resistance; ITT, insulin tolerance test; KO, knockout; NS, not significant; OGTT, oral glucose tolerance test; P2X2, P2X purinoreceptor 2; P2X3, P2X purinoreceptor 3; P2X2/3 DKO, P2X purinoreceptor 2 and 3 double knockout; WT, wild type. Sucrose was administered as a 15% solution (430 mM) *$P < 0.05$ and **$P < 0.01$ vs. respective water controls. One-way ANOVA with post hoc Tukey. Two-tail $t$ test.

Table 6. *Effect of FG Intake on metabolic syndrome in the P2X2/3 DKO mouse compared with WT*

| | Wild Type | | P2X2/3 DKO | | Wild-Type FG vs. P2X2/3 DKO FG (ANOVA) |
|---|---|---|---|---|---|
| | Water ($n$ = 6) | FG ($n$ = 5) | Water ($n$ = 6) | FG ($n$ = 4) | |
| Body weight and composition | | | | | |
| Body weight, g; 0 wk | 24.2 ± 0.4 | 24.1 ± 0.3 | 24.8 ± 0.4 | 25.7 ± 0.5 | NS |
| Body weight, g; 30 wk | 36.8 ± 2.3 | 49.1 ± 1.6** | 39.4 ± 3.4 | 64.6 ± 5.1** | $P < 0.01$ |
| ΔBody weight, g; 30 wk | 12.6 ± 1.3 | 25.0 ± 1.1** | 14.6 ± 1.6 | 38.9 ± 3.1** | $P < 0.01$ |
| Average food intake, g·mouse$^{-1}$·day$^{-1}$ | 3.25 ± 0.1 | 2.10 ± 0.2** | 4.28 ± 0.2 | 3.80 ± 0.1* | $P < 0.01$ |
| Liver weight, g; 30 wk | 1.56 ± 0.25 | 2.68 ± 0.36** | 1.67 ± 0.12 | 4.70 ± 0.12** | $P < 0.01$ |
| Liver/body weight ratio | 0.042 ± 0.01 | 0.054 ± 0.03* | 0.042 ± 0.02 | 0.072 ± 0.06** | $P < 0.01$ |
| Kidney weight, g; 30 wk | 0.41 ± 0.06 | 0.61 ± 0.08* | 0.40 ± 0.07 | 0.65 ± 0.03** | NS |
| Kidney/body weight ratio | 0.011 ± 0.02 | 0.012 ± 0.03 | 0.010 ± 0.02 | 0.010 ± 0.01 | NS |
| Heart weight, g; 30 wk | 0.14 ± 0.07 | 0.26 ± 0.04 | 0.16 ± 0.04 | 0.36 ± 0.02** | NS |
| Heart/body weight ratio | 0.004 ± 0.002 | 0.005 ± 0.002 | 0.004 ± 0.001 | 0.005 ± 0.002 | NS |
| Epididymal fat weight, g; 30 wk | 0.75 ± 0.28 | 2.61 ± 0.21** | 0.81 ± 0.23 | 3.88 ± 0.23** | $P < 0.05$ |
| Epididymal fat/body weight ratio | 0.02 ± 0.008 | 0.05 ± 0.004** | 0.02 ± 0.001 | 0.06 ± 0.015** | NS |
| Biochemical blood analysis | | | | | |
| AST, IU/L | 39.1 ± 4.1 | 193.6 ± 13.3** | 54.6 ± 11.1 | 298 ± 33.1** | $P < 0.01$ |
| ALT, IU/L | 16.7 ± 6.1 | 56.6 ± 6.8** | 19.3 ± 4.5 | 65.5 ± 8.6** | NS |
| Serum triglycerides, mg/dL | 65.7 ± 14.8 | 82.3 ± 4.9 | 71.7 ± 8.9 | 86.5 ± 7.9 | NS |
| Serum uric acid, mg/dL | 2.56 ± 0.4 | 3.23 ± 0.3 | 3.18 ± 0.3 | 4.46 ± 0.5* | $P < 0.01$ |
| Fasting serum glucose, mg/dL | 116 ± 8.1 | 186 ± 9.3** | 153 ± 12.5 | 230 ± 14.21** | $P < 0.01$ |
| Fasting insulin, ng/mL | 0.67 ± 0.25 | 2.15 ± 0.17** | 0.95 ± 0.29 | 5.16 ± 0.12** | $P < 0.01$ |
| HOMA-IR | 5.5 ± 1.4 | 33.7 ± 4.3** | 10.4 ± 2.2 | 86.9 ± 4.7** | $P < 0.01$ |
| OGTT, AUC | 376 ± 75 | 856 ± 65** | 483 ± 26 | 914 ± 34** | $P < 0.01$ |
| ITT, AUC | 310 ± 55 | 623 ± 21** | 226 ± 38 | 705 ± 45** | $P < 0.05$ |
| Fasting leptin, ng/mL | 3.96 ± 1.42 | 19.21 ± 2.23** | 6.26 ± 1.36 | 24.49 ± 4.16** | NS |
| Adipose *leptin* mRNA, AU | 0.78 ± 0.36 | 2.36 ± 0.44** | 1.18 ± 0.28 | 4.80 ± 0.16** | $P < 0.05$ |
| Fructose, mM | 0.02 ± 0.01 | 0.06 ± 0.02* | 0.02 ± 0.01 | 0.04 ± 0.01 | NS |
| Serum creatinine, mg/dL | 0.10 ± 0.004 | 0.22 ± 0.05* | 0.16 ± 0.005 | 0.36 ± 0.08* | $P < 0.05$ |
| Blood urea nitrogen, mg/dL | 21.0 ± 7.0 | 26 ± 4.0 | 28.2 ± 8.5 | 36.71 ± 6.7 | NS |
| FGF21, pg/mL | 428.7 ± 360.1 | 2381.0 ± 196.4** | 1074.0 ± 539.6 | 3056 ± 420** | $P < 0.05$ |
| Liver analysis | | | | | |
| Glycogen, mg/g | 8.89 ± 0.08 | 19.1 ± 10.5 | 14.58 ± 5.56 | 46.23 ± 7.78** | $P < 0.01$ |
| Uric acid, μg/mg | 0.63 ± 0.06 | 3.7 ± 0.13** | 1.27 ± 0.08 | 10.86 ± 2.26** | $P < 0.01$ |
| ATP, μmol/g | 4.46 ± 0.5 | 2.89 ± 0.62* | 3.55 ± 0.3 | 2,71 ± 0.5* | NS |
| β-Hydroxybutyrate, nmol/g | 0.022 ± 0.004 | 0.032 ± 0.006 | 0.049 ± 0.01 | 0.026 ± 0.003** | NS |
| *Fgf21* mRNA, AU | 0.63 ± 0.25 | 3.66 ± 0.51** | 1.23 ± 0.32 | 4.86 ± 0.22** | $P < 0.01$ |

Data indicate means ± SD. $n$ = 4–6 mice per group. FG was administered as a 15% solution (430 mM). AUC, area under the curve; FGF21, fibroblast growth factor 21; HOMA-IR, homeostatic model assessment for insulin resistance; ITT, insulin tolerance test; KO, knockout; NS, not significant; OGTT, oral glucose tolerance test; P2X2, P2X purinoreceptor 2; P2X3, P2X purinoreceptor 3; P2X2/3 DKO, P2X purinoreceptor 2 and 3 double knockout; WT, wild type. *$P <$ 0.05 and **$P < 0.01$ vs. respective water controls. One-way ANOVA with post hoc Tukey. Two-tail $t$ test.

Similar differences in body weight gain was observed between P2X2/3 DKO and wild-type mice exposed to other caloric sugars (Tables 4–7). Similarly, compared with WT mice drinking fructose, P2X2/3 DKO mice developed more severe obesity, epididymal fat deposition, insulin resistance [including greater serum glucose excursions with an oral glucose tolerance test (OGTT)], worse fatty liver, and higher serum transaminase level (Fig. 1, *C–I*, and Table 4). Furthermore, specific histologic measurements of the liver using the Brunt Scoring Index showed marked changes of fatty liver with injury and inflammation in the fructose-fed P2X2/3 DKO mouse (Supplemental Table S1; all Supplemental material is available at https://doi.org/10.6084/m9.figshare.12449687.v1). Of interest and similar to our findings on fructose-exposed mice, taste-blind animals also demonstrated marked body weight gain and features of metabolic syndrome in response to other fructose-containing sugars (FG mixture and sucrose) as well as to glucose (Tables 4–7). Despite the observation that all caloric sugars induced obesity and metabolic syndrome in P2X2/3 DKO mice, FG evoked significantly higher final body weight and body weight change than the other sugars (64.6 g with a 30-wk change of 38.9 g in FG vs. 57.2/32.2 g in fructose, 58.1/32.9 g in sucrose, and 57.8/33.0 g in glucose fed mice, respectively, $P < 0.01$). Similarly, other parameters analyzed, including liver weight and adipose deposition, were substantially higher in FG fed mice compared with any other sugar.

*Molecular mechanisms associated with sugar-induced metabolic syndrome in taste-blind mice.* We previously reported that excessive fructose consumption can induce leptin resistance in rats (37). In this regard, an enhanced reduction in leptin sensitivity in P2X2/3 DKO mice compared with wild-type counterparts could explain the metabolic differences between the strains. To test this hypothesis, we evaluated leptin levels and leptin sensitivity in water and sugar-exposed wild-type and P2X2/3 DKO mice. As shown in Fig. 2*A* and Tables 4–7, mice on fructose and other caloric sugars demonstrated significantly higher plasma leptin levels than animals on water. Of interest, plasma leptin levels were also significantly higher in P2X2/3 DKO mice compared with WT counterparts, suggestive of reduced leptin sensitivity. To determine whether fructose-exposed P2X2/3 DKO mice have reduced leptin sensitivity, a leptin sensitivity assay was performed at 30 wk. As shown in Fig. 2*B*, P2X2/3 DKO mice on water had normal

Table 7. *Effect of glucose intake on metabolic syndrome in the P2X2/3 DKO mouse compared with WT*

| | Wild Type | | P2X2/3 DKO | | Wild-Type Glucose vs. P2X2/3 DKO Glucose (ANOVA) |
|---|---|---|---|---|---|
| | Water ($n$ = 6) | glucose ($n$ = 5) | Water ($n$ = 6) | glucose ($n$ = 4) | |
| Body Weight and composition | | | | | |
| Body weight, g; 0 wk | 24.2 ± 0.4 | 23.7 ± 0.5 | 24.8 ± 0.4 | 24.8 ± 0.6 | NS |
| Body weight, g; 30 wk | 36.8 ± 2.3 | 45.6 ± 2.2** | 39.4 ± 3.4 | 57.8 ± 5.5** | $P < 0.01$ |
| ΔBody weight, g; 30 wk | 12.6 ± 1.3 | 21.9 ± 1.6** | 14.6 ± 1.6 | 33.0 ± 4.0** | $P < 0.01$ |
| Average food intake, g·mouse$^{-1}$·day$^{-1}$ | 3.25 ± 0.1 | 2.63 ± 0.1** | 4.28 ± 0.2 | 4.12 ± 0.4 | $P < 0.01$ |
| Liver weight, g; 30 wk | 1.56 ± 0.25 | 2.23 ± 0.33** | 1.67 ± 0.12 | 3.97 ± 0.36** | $P < 0.01$ |
| Liver/body weight ratio | 0.042 ± 0.01 | 0.048 ± 0.05 | 0.042 ± 0.02 | 0.068 ± 0.03** | $P < 0.01$ |
| Kidney weight, g; 30 wk | 0.41 ± 0.06 | 0.57 ± 0.11 | 0.40 ± 0.07 | 0.59 ± 0.08* | NS |
| Kidney/body weight ratio | 0.011 ± 0.02 | 0.013 ± 0.03 | 0.010 ± 0.02 | 0.010 ± 0.02 | NS |
| Heart weight, g; 30 wk | 0.14 ± 0.07 | 0.22 ± 0.05 | 0.16 ± 0.04 | 0.38 ± 0.04** | $P < 0.01$ |
| Heart/body weight ratio | 0.004 ± 0.002 | 0.004 ± 0.002 | 0.004 ± 0.001 | 0.006 ± 0.002 | NS |
| Epididymal fat weight, g; 30 wk | 0.75 ± 0.28 | 2.77 ± 0.44** | 0.81 ± 0.23 | 3.27 ± 0.43** | NS |
| Epididymal fat/body weight ratio | 0.02 ± 0.008 | 0.06 ± 0.012** | 0.02 ± 0.001 | 0.056 ± 0.009** | NS |
| Biochemical blood analysis | | | | | |
| AST, IU/L | 39.1 ± 4.1 | 141.1 ± 17.1** | 54.6 ± 11.1 | 148.2 ± 21.1** | NS |
| ALT, IU/L | 16.7 ± 6.1 | 36.6 ± 2.9** | 19.3 ± 4.5 | 46.6 ± 10.2** | NS |
| Serum triglycerides, mg/dL | 65.7 ± 14.8 | 116.6 ± 5.1** | 71.7 ± 8.9 | 123.4 ± 10.1** | NS |
| Serum uric acid, mg/dL | 2.56 ± 0.4 | 2.26 ± 0.3 | 3.18 ± 0.3 | 3.82 ± 0.3* | $P < 0.01$ |
| Fasting serum glucose, mg/dL | 116 ± 8.1 | 135.2 ± 7.7* | 153 ± 12.5 | 189.3 ± 9.87** | $P < 0.01$ |
| Fasting insulin, ng/mL | 0.67 ± 0.25 | 1.33 ± 0.23** | 0.95 ± 0.29 | 2.27 ± 0.21** | $P < 0.01$ |
| HOMA-IR | 5.5 ± 1.4 | 16.8 ± 6.1** | 10.4 ± 2.2 | 27.7 ± 8.2** | $P < 0.01$ |
| OGTT, AUC | 376 ± 75 | 665 ± 40** | 483 ± 26 | 717 ± 51** | NS |
| ITT, AUC | 310 ± 55 | 443 ± 16* | 226 ± 38 | 512 ± 36** | $P < 0.01$ |
| Fasting leptin, ng/mL | 3.96 ± 1.42 | 12.62 ± 5.12** | 6.26 ± 1.36 | 16.67 ± 5.12** | NS |
| Adipose *leptin* mRNA, AU | 0.78 ± 0.36 | 2.02 ± 0.23** | 1.18 ± 0.28 | 2.76 ± 0.30** | NS |
| Fructose, mM | 0.02 ± 0.01 | 0.04 ± 0.02 | 0.02 ± 0.01 | 0.03 ± 0.02 | NS |
| Serum creatinine, mg/dL | 0.10 ± 0.004 | 0.13 ± 0.06 | 0.16 ± 0.005 | 0.22 ± 0.07 | NS |
| Blood urea nitrogen, mg/dL | 21.0 ± 7.0 | 16 ± 2.5 | 28.2 ± 8.5 | 26.6 ± 3.3 | NS |
| FGF21, pg/mL | 428.7 ± 360.1 | 2558.0 ± 225.5** | 1074.0 ± 539.6 | 2200 ± 195** | NS |
| Liver analysis | | | | | |
| Glycogen, mg/g | 8.89 ± 0.08 | 28.8 ± 4.5** | 14.58 ± 5.56 | 23.3 ± 5.56 | NS |
| Uric acid, μg/mg | 0.63 ± 0.06 | 2.35 ± 0.21** | 1.27 ± 0.08 | 3.88 ± 1.90** | $P < 0.01$ |
| ATP, μmol/g | 4.46 ± 0.5 | 5.85 ± 0.12* | 3.55 ± 0.3 | 3.36 ± 0.26 | $P < 0.01$ |
| β-Hydroxybutyrate, nmol/g | 0.024 ± 0.005 | 0.033 ± 0.007 | 0.045 ± 0.004 | 0.026 ± 0.009** | NS |
| *Fgf21* mRNA, AU | 0.63 ± 0.25 | 4.56 ± 0.22** | 1.23 ± 0.32 | 4.05 ± 0.15** | $P < 0.05$ |

Data indicate means ± SD. $n$ = 5 or 6 mice per group. Glucose was administered as a 15% solution (833 mM). AUC, area under the curve; FGF21, fibroblast growth factor 21; HOMA-IR, homeostatic model assessment for insulin resistance; ITT, insulin tolerance test; KO, knockout; NS, not significant; OGTT, oral glucose tolerance test; P2X2, P2X purinoreceptor 2; P2X3, P2X purinoreceptor 3; P2X2/3 DKO, P2X purinoreceptor 2 and 3 double knockout; WT, wild type. *$P < 0.05$ and **$P < 0.01$ vs. respective water controls. One-way ANOVA with post hoc Tukey. Two-tail $t$ test.

fasting levels of leptin and also demonstrated sensitivity to leptin by reducing their food intake in response to leptin injection, similar to WT mice on water (Fig. 2, *A* and *B*). In contrast, P2X2/3 DKO mice ingesting fructose had higher fasting serum and leptin levels and adipose leptin mRNA compared with P2X2/3 DKO mice on water, and unlike water controls, P2X2/3 DKO mice did not reduce their food intake in response to leptin injection (Fig. 2, *A* and *B*, Table 4). Although similar findings in terms of leptin sensitivity were observed in WT mice on fructose compared with water-receiving controls, the consumption of food following leptin injection was markedly higher in fructose-fed P2X2/3 DKO mice than in fructose-fed WT animals (2.5 ± 0.4 vs. 1.5 ± 0.2 g, $P < 0.01$), indicating that proper signaling via P2X2/3 receptors is important to regulate leptin sensitivity in mice. Consistently, Western analysis of hypothalamic extracts from these P2X2/3 DKO mice on fructose showed reduced phosphorylated STAT3 expression, consistent with central leptin resistance in these mice (Fig. 2*C*).

In addition, we noted that over the 30 wk, fructose intake progressively increased in the P2X2/3 DKO mice (see Fig. 1*A*). Indeed, despite having no gustatory perception to sugar, P2X2/3 DKO mice demonstrated induction and activation of the transcription factor associated with reward-related behavior delta fosb (Δfosb) as well as its target gene, the AMPA receptor GluA2 (GluR2) in the nucleus accumbens (NAcc) (Fig. 2, *D* and *E*). These studies suggest P2X2/3 DKO mice show neural responses in the NAcc consistent with their preference for fructose despite lack of taste.

Another mechanism that can explain the observed increased body weight gain in P2X2/3 DKO mice exposed to sugars relates to their regulation of fat metabolism. In this regard, and as shown in Tables 4–7, P2X2/3 DKO on sugar have significantly greater adipose deposition than wild-type counterparts. This could be due to reduced fat oxidation and/or increased de novo lipogenesis. As explained above, P2X2/3 DKO on water have greater levels of ketone bodies in plasma, suggestive of increased fat oxidation. However, and as shown in Fig. 3*A* and Tables 4–7, sugar-exposed mice demonstrate markedly lower levels of β-hydroxybutyrate in the liver, a marker of fat oxidation, indicating that, despite higher fat content, P2X2/3 DKO mice have an impaired ability to mobilize and oxidize fat when exposed to sugar. Conversely, the expression of lipogenic genes *fas, acc*, and *acl* in the liver is markedly upregu-

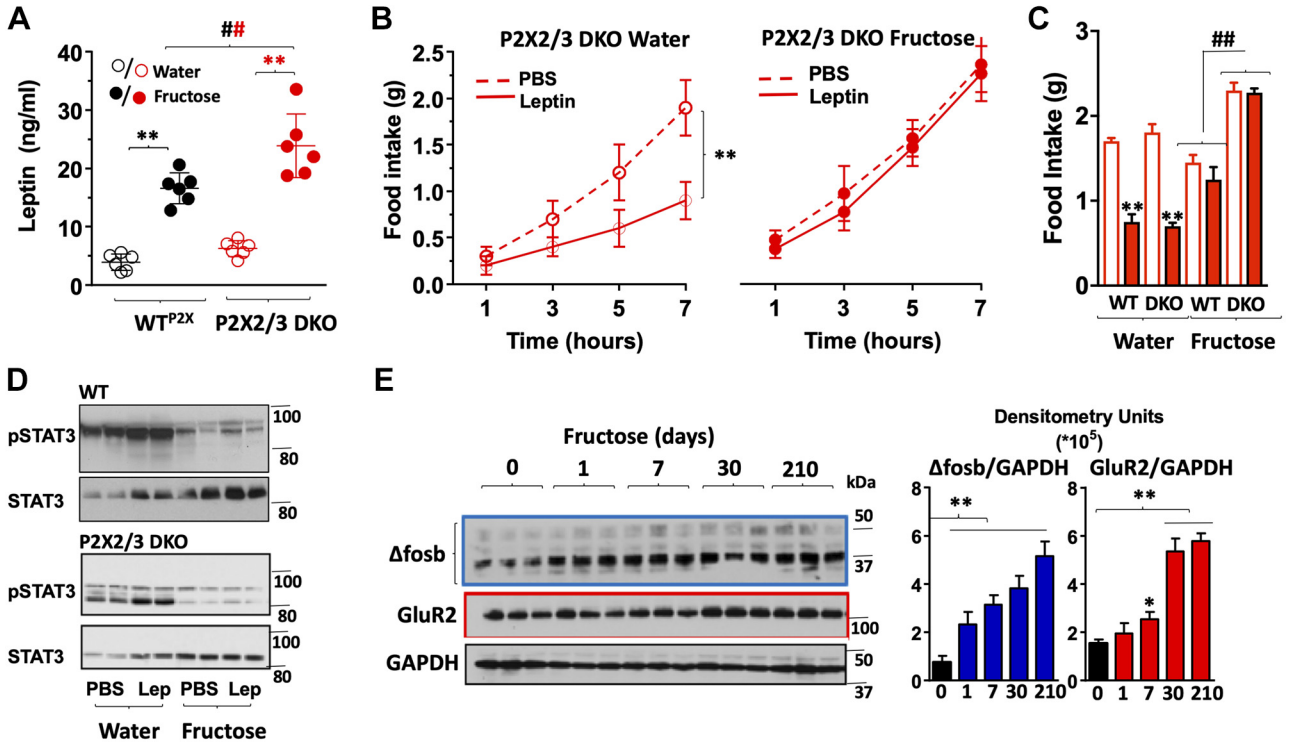


Fig. 2. Molecular mechanisms associated with cerebral leptin sensitivity and sugar preference in P2X purinoreceptor (P2X)2/3 double-knockout (DKO) mice. *A*: plasma leptin levels in wild-type (black) and P2X2/3 DKO (red) receiving either water (open symbols) or a 15% fructose solution (solid symbols) for 30 wk. *B*: food consumption (in g) over a 7-h period following an injection of either saline (PBS, dashed lines) or leptin (solid lines) in P2X2/3 DKO mice drinking either control water (*left*) or 15% fructose solution (*right*) for 25 wk. $n = 4$ mice per group. *C*: 7-h food intake in wild-type and P2X2/3 DKO mice on water or fructose after receiving saline (white columns) or leptin (red solid columns). ***D*:** representative Western blot for hypothalamic total signal transducer and activator of transcription 3 (active pSTAT3) in control or fructose-fed P2X2/3 DKO mice 1 h after being injected with either saline (PBS) or leptin (Lep). Marker size bars (kDa) indicated on *right*. *E*: representative Western blot for ΔfosB, GluR2, and GAPDH in the nucleus accumbens of P2X2/3 DKO mice exposed to 15% fructose solution for 0 to 210 days. *Right*, densitometry values for ΔfosB and GluR2—normalized to GAPDH—in the same animals. Marker size bars (kDa) indicated on the right side. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control, $\#\#P < 0.01$, one-way ANOVA Tukey post hoc analysis.

lated in sugar-exposed P2X2/3 DKO mice compared with water and sugar-exposed wild-type animals (Fig. 3*B* and Tables 4–7), indicating increased de novo lipogenesis in these mice. Consistently, liver weight (both total and after correcting for body weight) and hepatic triglyceride accumulation were significantly higher in P2X2/3 DKO mice on sugar. ChREBP is a carbohydrate-dependent transcription factor stimulated by both glucose and fructose whose activation drives sugar-dependent lipogenesis (14, 22, 23). Of interest and as shown in Fig. 3, *C* and *D*, although total ChREBP expression did not substantially change between strains, P2X2/3 DKO demonstrated increased activity of this transcription factor as denoted by higher acetylation rate and mRNA expression levels of well-known target genes *pklr* and *g6pc*.

One important finding was that P2X2/3 DKO mice, while showing a clear preference for sugar, demonstrated reduced intake of caloric sweeteners compared with WT mice (Tables 1 and 2). This led us to characterize the expression of *FGF21*, a sugar-specific hormone secreted by the liver that has been reported to block the craving for sugar (40). As shown in Fig. 4 and Table 4, the deletion of the P2X2/3 purinergic receptors did not abrogate the production of *FGF21* secondary to sugar exposure. Of interest, in fructose-fed mice, *FGF21* levels in P2X2/3 DKO animals were significantly higher than in wild-type counterparts (Fig. 4, *A* and *B*), and these values were markedly higher when normalized to overall fructose intake (Fig. 4*D*). Furthermore, levels of this hormone in NAcc were significantly higher in P2X2/3 DKO mice than WT animals despite lower overall consumption of fructose by P2X2/3 DKO mice (Fig. 4*C*). These studies suggest that mice lacking taste reduce their intake of fructose (despite still preferring it over water), probably as a consequence of elevated *FGF21* levels.

*Role of fructose metabolism in preference and sugar intake.* The observation that sugar-exposed P2X2/3 DKO mice developed worse metabolic syndrome and relatively greater leptin resistance compared with wild-type mice led us to investigate if it involved the activation of the fructokinase pathway. In this regard, we previously reported that the deficiency of fructokinase, the first enzyme involved in fructose metabolism, markedly protects against the deleterious effects of fructose (11) and glucose (18) in metabolic syndrome in mice. Similarly, our groups also documented that fructose-dependent leptin resistance is mediated by fructokinase (19). Fructose intake upregulates hepatic fructokinase (KHK) levels and increases hepatic uric acid, a metabolic by-product of KHK-mediated fructose metabolism (21). Of interest, P2X2/3 DKO mice administered fructose demonstrated upregulation of KHK and uric acid (Fig. 5, *A–C*) that was substantially greater than that observed in wild-type mice (5.4-fold increase in P2X2/3 DKO vs. 2.8-fold in wild-type mice, $P < 0.01$). More importantly, KHK activity

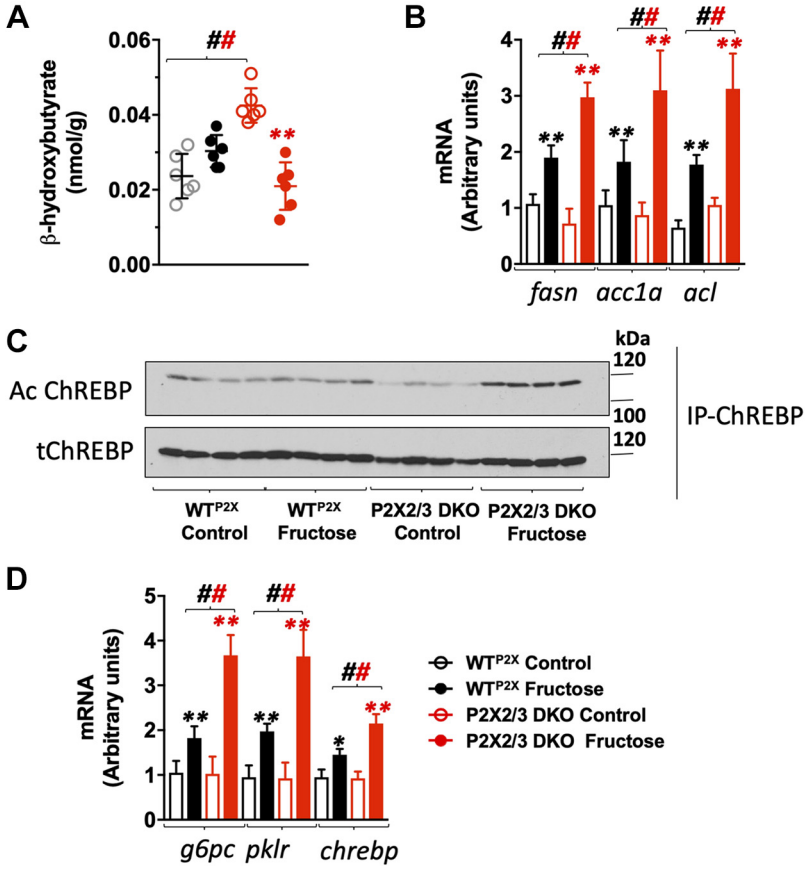


Fig. 3. Fat regulation in liver of control and fructose-exposed P2X purinoreceptor (P2X)2/3 double-knockout (DKO) DKO mice. *A*: liver β-hydroxybutyrate levels—normalized to soluble protein—in wild-type (black) and P2X2/3 DKO receiving either control water (open symbols) or a 15% fructose solution (solid symbols) for 30 wk. *B*: liver mRNA levels of lipogenic enzymes fatty acid synthase (*fasn*), acetyl-Coa Carboxylase (*acc1a*), and ATP-citrate lyase (*acl*) in the same animals as in *A*. *C*: total and acetylated ChreBP levels in the livers of the same mice as in *A*. Marker size bars (kDa) indicated on the *right*. *D*: liver mRNA levels of ChREBP target genes glucose-6-phosphatase (*g6pc*), liver-specific pyruvate kinase (*plkr*), and ChREBP (*chrebp*) in the same animals as in *A*. $*P < 0.05$, $**P < 0.01$, $\#\#P < 0.01$, one-way ANOVA Tukey post hoc analysis.

in the liver was markedly greater in P2X2/3 DKO mice on fructose compared with the rest of the groups (Fig. 5*D*), indicating that, despite similar KHK expression between wild-type and P2X2/3 DKO mice, there are posttranslational modifications occurring that enhance KHK activity in P2X2/3 DKO mice, which may underlie the mechanism whereby P2X2/3 DKO mice have an exacerbated phenotype in response to sugar. Indeed, the relative increase in fructokinase levels correlated with the higher fasting leptin levels in the same mice (Fig. 5*B*, $r = 0.83$, $P < 0.01$), suggesting that fructose metabolism modulates sugar-induced metabolic syndrome in P2X2/3 DKO mice.

To further evaluate the specific role of fructose metabolism in regulating preference and intake of sugar, we employed mice deficient for both isoforms of fructokinase (namely KHK-A/C KO mice). As shown in Table 8 and similar to wild-type mice, KHK-A/C KO mice demonstrate marked preference for glucose and the artificial sweeteners sucralose and saccharin (Table 1), although it is important to note that the preference for glucose in KHK-A/C KO mice is significantly lower than that of wild-type counterparts (72.5 ± 5.0% vs. 93.0 ± 2.5%, $P < 0.01$). However, unlike control and P2X2/3 DKO mice, the deficiency of fructokinase in mice resulted in reduced preference for fructose and relatively decreased preference for FG and sucrose (which both contain fructose). Thus, although KHK-A/C KO mice have decreased preferences for fructose and fructose-containing sugars, they maintain a preference for sweet tastes evoked by artificial sweeteners and glucose. In contrast, KHK-A KO mice who maintain intact KHK-C showed similar preferences for fructose-containing sugars as well as caloric and noncaloric sweeteners than wild-type animals, indicating that the C isoform of KHK is controlling the appetite of mice for fructose-containing sugars (Table 9). Of interest, the lack of appetite for fructose in fructokinase-deficient mice seems to be independent of impaired sweet taste transmission signal in taste buds, as KHK-A/C KO mice demonstrate proper gustatory nerve response to all sugars tested (Supplemental Fig. S1). This would be consistent with the possibility that besides taste, postingestive mechanisms also drive the preference or avoidance for fructose and sugar.

## DISCUSSION

Sucrose and HFCS are currently major staples in the Western diet, with intake varying from 15 to 20% of overall energy, with some individuals ingesting 25% or more in the form of these added sugars (41). Often these added sugars are mixed into foods to increase the overall sweetness to enhance taste and encourage intake. Here we show that animals that lack taste signaling still prefer both glucose- and fructose-containing sugars and will rapidly develop obesity and metabolic syndrome if given access to these sugars. Remarkably, the development of metabolic syndrome not only occurs in P2X2/3 DKO mice ingesting these sugars but is worse than that observed with WT mice despite the latter ingesting more sugar. These studies therefore show that sugars, including both glu-

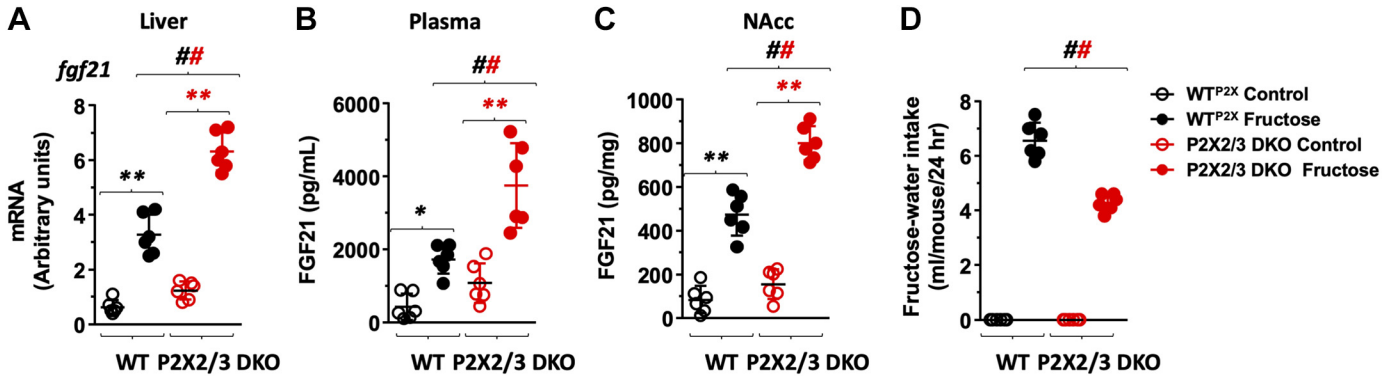


Fig. 4. FGF21 role in fructose-induced metabolic syndrome in P2X purinoreceptor (P2X)2/3 double-knockout (DKO) mice. *A*: liver *fgf21* mRNA levels in wild-type (black) and P2X2/3 DKO receiving either control water (open symbols) or a 15% fructose solution (solid symbols) for 30 wk. *B*: plasma fgf21 levels in the same animals as in *A*. *C*: FGF21 levels in the nucleus accumbens of the same mice as in *A*. *D*: average daily intake of 15% fructose solution in wild-type (black symbols) and P2X2/3 DKO mice in the same mice as in *A*. $*P < 0.05$, $**P < 0.01$, $\#\#P < 0.01$, one-way ANOVA Tukey post hoc analysis.

cose and fructose, can act as "stealth" food ingredients for driving obesity, as one does not have to taste it to suffer its metabolic effects.

Our first finding was that mice lacking taste signaling still showed a preference for caloric-containing sugars, likely due to postingestive reward signals. P2X2/3 DKO mice strongly preferred all caloric sugars (including both fructose and glucose) but not noncaloric sweeteners, suggesting a post-oral mechanism driving preference linked with energy content. Others have performed studies in mice lacking sweet taste receptors that block not only the sweet taste receptors in the tongue but also in the gut. Specifically, mice lacking the sweet receptor (T1R3 KO) or its downstream signaling component (TrpM5) also prefer sucrose and develop a dopamine response following ingestion, whereas this is not observed when these same mice are given artificial sweeteners (5, 9, 32, 36, 43). One candidate for driving the response to caloric sugars is the recently described neuropod cell, which is a gut sensory epithelial cell that resides in the small intestine and signals the brain via the vagus nerve (13). Thus, it appears that much of the preference for sugar involves post-oral mechanisms.

One way to evaluate post-oral mechanisms is to train animals to associate a flavored drink with a gastric infusion of sucrose to test whether a gastric delivery of a sugar can elicit a response to drink more of the flavored substance. Using this approach, Sclafani and colleagues (2, 33, 34) showed that rats develop preference for intragastric infusion of glucose-containing solutions but not fructose-containing solutions. Similarly,

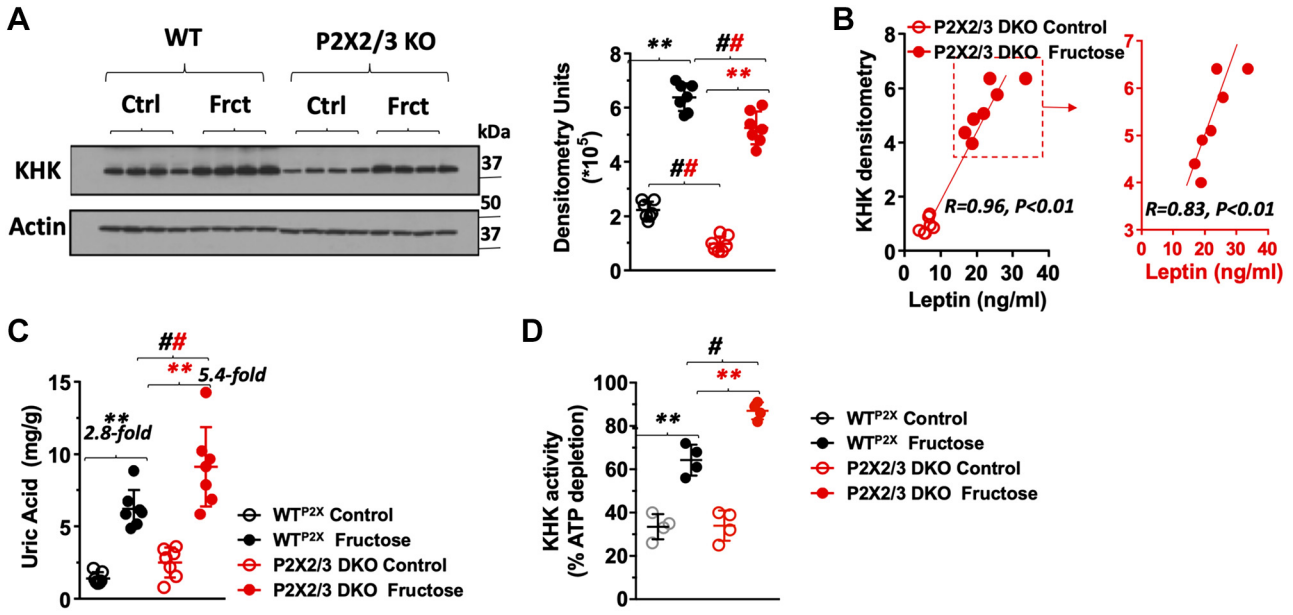


Fig. 5. Hepatic fructose metabolism in P2X purinoreceptor (P2X)2/3 double-knockout (DKO) mice chronically exposed to fructose. *A*: representative Western blot for ketohexokinase (KHK) and actin in liver lysates of wild-type (WT) and P2X2/3 DKO mice exposed to water control (Ctrl) or a 15% fructose solution (frc) for 30 wk. Marker size bars (kDa) indicated on the *right*. *Right*, densitometry values for KHK—normalized to actin—in the same animals. *B*: correlation between liver KHK levels and plasma leptin levels in P2X2/3 DKO mice exposed to water control (open symbols) or 15% fructose (solid symbols) for 30 wk). *C*: liver uric acid levels—a byproduct from fructose metabolism through KHK—in the same animals as in *A*. *D*: KHK activity in liver extracts of the same animals as in *A*. $**P < 0.01$, $\#P < 0.05$, $\#\#P < 0.01$, one-way ANOVA Tukey post hoc analysis.

Table 8. *Sweetener preferences (%, 2-bottle) in WT KHK-A/C KO and KHK-A KO mice during the first 4 days after exposure*

| $WT^{C57}$ | Water | Fructose | Sucrose | FG Mixture | Glucose | Sucralose | Saccharin |
|---|---|---|---|---|---|---|---|
| *Day 1* | 56.5 ± 4.5 | 65.0 ± 4.0 | 77.5 ± 5.0## | 83.0 ± 6.0## | 82.0 ± 6.5## | 45.5 ± 5.5# | 56.5 ± 5.5 |
| *Day 2* | 53.0 ± 5.5 | 78.5 ± 4.0## | 88.0 ± 5.5## | 92.0 ± 4.5## | 90.5 ± 4.0## | 57.5 ± 5.5 | 63.5 ± 3.0## |
| *Day 3* | 51.5 ± 3.5 | 82.5 ± 3.5## | 94.5 ± 2.5## | 96.0 ± 3.0## | 92.0 ± 4.0## | 63.5 ± 4.5## | 65.0 ± 3.5## |
| *Day 4* | 52.5 ± 3.5 | 85.5 ± 3.5##** | 95.0 ± 5.0##** | 97.5 ± 2.5##** | 93.0 ± 2.5##* | 65.5 ± 3.5##** | 66.5 ± 5.5## |
| KHK-A/C KO | | | | | | | |
| *Day 1* | 55.5 ± 3.5 | 55.0 ± 8.5 | 62.5 ± 5.0 | 56.5 ± 5.5 | 70.5 ± 5.0## | 55.0 ± 5.5 | 60.0 ± 5.5 |
| *Day 2* | 60.0 ± 5.5 | 40.5 ± 5.5## | 65.5 ± 7.5 | 65.5 ± 5.0 | 74.5 ± 5.0## | 52.5 ± 3.5 | 65.5 ± 7.5 |
| *Day 3* | 50.0 ± 5.0 | 40.0 ± 3.5## | 60.0 ± 5.0# | 70.0 ± 5.5## | 80.0 ± 5.0## | 60.5 ± 5.0# | 75.5 ± 3.5## |
| *Day 4* | 55.5 ± 3.0 | 40.5 ± 6.5## | 60.0 ± 5.0 | 65.0 ± 8.5# | 72.5 ± 5.0## | 65.5 ± 8.0# | 70.0 ± 3.5## |
| KHK-A KO | | | | | | | |
| *Day 1* | 50.0 ± 5.0 | 65.0 ± 7.5# | 73.5 ± 7.5## | 80.0 ± 5.0## | 72.5 ± 5.0## | 62.0 ± 3.5## | 70.5 ± 4.5## |
| *Day 2* | 47.5 ± 2.5 | 75.0 ± 5.5## | 80.0 ± 5.0## | 92.5 ± 2.5## | 80.5 ± 8.0## | 68.5 ± 5.0## | 68.5 ± 5.0## |
| *Day 3* | 55.5 ± 3.0 | 82.5 ± 6.0## | 82.5 ± 6.5## | 88.5 ± 5.0## | 90.0 ± 5.0## | 66.0 ± 3.0## | 73.5 ± 7.5## |
| *Day 4* | 54.0 ± 4.0 | 80.0 ± 7.0##* | 85.0 ± 3.0##* | 93.5 ± 3.0##** | 92.5 ± 4.5##** | 70.0 ± 5.5## | 70.5 ± 5.0## |

Data indicate means ± SD. All sugars were administered as 15% solutions (833 mM for fructose and glucose, 438 mM for Sucrose), sucralose at 30 mM and saccharin at 35 mM. KHK-A/C, ketohexokinase A and C; KO, knockout; WT, wild type. *$P < 0.05$ and **$P < 0.01$ vs. *day 1* for each sugar in each strain. #$P < 0.05$ and ##$P < 0.01$ vs. water in each strain 2-tail *t* test.

sweet ageusic mice, i.e., those lacking sweet receptors (T1R3 and TrpM5 knockout) in both tongue and gastrointestinal tract, also prefer sucrose or glucose-based solutions administered by gastric infusion, whereas the preference for fructose is minimal (35, 42). Collectively, these data suggest sweet ageusic mice prefer glucose and sucrose over fructose due to postingestive cues. However, in our studies, both the WT and taste-blind (P2X2/3 KO) mice showed preference for fructose in addition to glucose and sucrose. Whether these differences relate to the different receptor functions or are a consequence of differences in underlying strain of mice is not known.

The second finding was that the absence of sweet taste results in reduced intake of the sweeteners in spite of a continued preference for the sugar compared with water. Sclafani et al. (9, 36) also reported that mice lacking T1R3 or TrpM5 reduced their intake of sucrose compared with control mice. In this regard, intake of sugars is known to be regulated by fibroblast growth factor 21 (high FGF21) (40), and we found that taste-blind mice showed a greater FGF21 response than control mice in response to fructose. These studies would be consistent with the hypothesis that, compared with wild-type mice, taste-blind mice show an enhanced FGF21 response that results in a reduced intake of fructose and other sugars.

Our major finding was that, despite lower sugar intake, taste-blind mice developed metabolic syndrome that was worse than that observed in WT mice given the same sugar. We further show that in the fructose-fed taste-blind mice, this extreme metabolic syndrome was probably due to the development of greater than normal leptin resistance. These data may appear discrepant to a study by Glendinning et al. (9), who reported that sucrose-fed T1R3 KO mice develop less weight gain than wild-type mice given sucrose for 40 days, while TrpM5 KO mice showed similar weight gain as sucrose fed WT mice. One possibility is that 40 days is not enough time to induce obesity and metabolic syndrome. In our study, separation of weight between fructose-exposed wild-type and P2X2/3 DKO mice did not occur until 20 wk (140 days), and by the end of the study at 30 wk, sugar-fed animals suffered from severe metabolic syndrome with marked weight gain, adiposity, fatty liver, and insulin resistance. Indeed, the observation in the Glendinning et al. study that caloric intake was higher in the T1R3 KO mouse on sucrose compared with the T1R3 mouse on water despite failure to induce greater weight gain is consistent with our studies that show enhanced energy intake before weight gain (19).

An important aspect of this study is the finding that both glucose and fructose could induce obesity and metabolic syndrome in the taste-blind mouse. Most taste-based studies have suggested that glucose is the primary sugar that is preferred by mice lacking sweet taste, and some studies do not show a

Table 9. *Daily sweetener intake and sugar solute intake in 30 wk WT, P2X2/3 DKO, KHK-A/C KO, and KHK-A KO mice*

| | Water | Fructose | Sucrose | FG MIXTURE | Glucose | Sucralose | Saccharin |
|---|---|---|---|---|---|---|---|
| Total fluid intake, mL/day | | | | | | | |
| $WT^{C57}$ | 3.16 ± 0.2 | 7.46 ± 0.5 | 9.25 ± 0.6 | 14.50 ± 2.25 | 10.92 ± 2.6 | 5.42 ± 0.5 | 5.85 ± 1.25 |
| KHK-A/C KO | 3.22 ± 0.2 | 2.40 ± 0.3## | 5.85 ± 0.5## | 6.05 ± 0.4## | 9.35 ± 0.6 | 5.35 ± 0.4 | 6.11 ± 0.4 |
| KHK-A KO | 3.20 ± 0.3 | 7.25 ± 0.8 | 10.85 ± 0.6## | 15.75 ± 1.4 | 9.65 ± 1.1 | 5.26 ± 0.3 | 5.75 ± 0.2 |
| Sugar Solute Intake (g/day) | | | | | | | |
| | Water | Fructose | Sucrose | FG MIXTURE | Glucose | Sucralose | Saccharin |
| $WT^{C57}$ | 0 | 1.11 ± 0.06 | 1.38 ± 0.08 | 2.17 ± 0.28 | 1.63 ± 0.46 | 0 | 0 |
| KHK-A/C KO | 0 | 0.36 ± 0.04# | 0.87 ± 0.07# | 0.90 ± 0.06# | 1.40 ± 0.07 | 0 | 0 |
| KHK-A KO | 0 | 1.08 ± 0.12 | 1.62 ± 0.08 | 2.36 ± 0.15 | 1.44 ± 0.16 | 0 | 0 |

Data indicate means ± SD. $n = 6$ mice per group. Daily water, sugar, and artificial sweetener intake in wild-type (WT), ketohexokinase (KHK)-A/C knockout (KO) and KHK-A KO mice. All sugars were administered as 15% solutions [8.25% fructose and 6.75% glucose for fructose/glucose (FG) mixture), 15% for sucrose], sucralose at 30 mM, and saccharin at 35 mM. #$P < 0.05$, ##$P < 0.01$. One Way ANOVA-Tukey post hoc analysis versus or WTC57 in each sugar.

preference for fructose in mice lacking T1R3 and TrpM5 (9, 43). However, these latter sweet ageusic mice have lost sweet detection capabilities in the gut, which would still be intact in P2X2/3 DKO mice, and it is possible these gut receptors play a role in fructose preference. The reduced preference for fructose in the KHK-A/C KO mouse could potentially reflect reduced absorption of fructose due to decreased metabolism in the intestines or perhaps be a consequence of decreased systemic metabolism. Interestingly, while glucose may be the preferred sugar in mice lacking sweet taste, the induction of metabolic syndrome in response to glucose in wild-type mice is partially dependent on the conversion of glucose to fructose in the liver (18). Thus, it seems likely that both fructose and glucose could have important roles in the metabolic responses.

An interesting finding was that the FG mixture resulted in greater intake compared with sucrose and other sugars in our study, and this was observed in both the WT and taste-blind mice (Table 2). This was not due to differences in preference for FG over sucrose (Table 1). Likewise, overall weight gain was only modestly higher in the FG mice compared with sucrose-fed mice during the 30 wk study, which was because the mice on the FG diet reduced their intake of chow relative to the increase in calories from the FG mixture (Tables 5 and 6). The reason for this difference might relate in part to the higher osmolality in the FG mixture, as increased osmolarity can result in increased fructose generation in the liver (19). Epidemiological data suggest that countries where HFCS is being used have higher obesity and diabetes rates compared with countries where sucrose is the main sweetener, even after controlling for total sugar intake (10). Clearly more studies are needed to investigate potential mechanisms.

Another finding was that WT mice given artificial sugars (sucralose and saccharin) consumed ~12% less in calories than WT mice on water (Table 3). Nevertheless, we did not observe lower body weights on artificial sugars, and in fact WT mice on saccharin gained more weight than WT mice given water ($17 \pm 3.2$ g on saccharin vs. $12 \pm 1.3$ g in water, $P < 0.05$) while WT mice given sucralose had nonsignificant weight increase ($15.2 \pm 2.4$ g) compared with water. These studies would support the possibility that some artificial sugars may encourage weight gain in normal mice. In contrast, DKO receiving artificial sugars ingested equivalent calories compared with DKO mice on water (Table 3) with no differences in weight ($14.6 \pm 1.6$ g in water, $16.2 \pm 1.8$ g sucralose, and $16.8 \pm 2.1$ g in saccharin), but interpretation of this aspect of our study of DKO mice is confounded by the small numbers of mice ($n = 3$ per group) that were available.

In terms of limitations, a confounding aspect of this study was the fact that taste-blind mice ingested more calories even when only drinking water, despite similar weight gain and leptin sensitivity. We do not know if this is due to differences in basal metabolism, food spillage, or malabsorption. However, the key finding was that when taste-blind mice were given sweeteners and particularly fructose-containing sugars, they developed rapid leptin resistance in association with a relatively greater increase in liver KHK levels and higher hepatic uric acid levels. Intrahepatic uric acid formation during fructose metabolism has been shown to mediate hepatic fat accumulation and features of fructose-induced metabolic syndrome (3, 6, 20, 27). Thus, our data suggest that the lack of purinergic receptors in our mice resulted in some type of enhanced fructokinase expression, resulting in worse leptin resistance and greater susceptibility to metabolic syndrome.

In summary, sugars, including glucose and fructose, can induce features of metabolic syndrome in mice, even in the absence of taste. Whether these findings in KO mice are directly applicable to humans taking any drugs that acutely block P2X purinergic signaling (1) is questionable. Genetic elimination of receptors affords opportunities for developmental compensatory regulation unavailable with pharmacological intervention. Nonetheless, HFCS and sugar are common in foods, often at levels that are subtle and not evident. These data highlight the need to continue investigations on the role of sugars and HFCS in driving the current epidemics of obesity and diabetes.

## ACKNOWLEDGMENTS

The authors appreciate the contribution to this research made by E. Erin Smith, TL(ASCP)CMQIHC; Jenna Van Der Volgen, HT(ASCP)CM; Allison Quador, HTL (ASCP)CM; and Jessica Arnold HTL(ASCP)CM of the University of Colorado Denver Histology Shared Resource.

## GRANTS

This work has been supported by NIH Grants 1RO1DK105364–01A1 (to M.A.L. and R.J.J.), 1R01DK121496 (to M.A. L. and R.J.J.), and 1R01DK108859 (to M.A.L.) and R01DC012555 (to S.C.K.) and a Pilot Award from the Colorado Nutrition and Obesity Research center (NORC) Grant# #25M7387 to A.A.-H. The University of Colorado Denver Histology Shared Resource is supported in part by the Cancer Center Support Grant (P30CA046934) from the National Institutes of Health: R01-HD075285.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

R.J.J. and M.A.L. conceived and designed research; A.A.-H., M.K., A.V., C.C., and M.A.L. performed experiments; A.A.-H., M.K., D.J.O., A.V., C.C., S.C.K., T.E.F., R.J.J., and M.A.L. analyzed data; A.A.-H., M.K., D.J.O., S.C.K., T.E.F., R.J.J., and M.A.L. interpreted results of experiments; A.A.-H., D.J.O., and M.A.L. prepared figures; A.A.-H., C.C., T.E.F., R.J.J., and M.A.L. approved final version of manuscript; S.C.K. and M.A.L. drafted manuscript; S.C.K., T.E.F., R.J.J., and M.A.L. edited and revised manuscript.

## REFERENCES

1. **Abdulqawi R, Dockry R, Holt K, Layton G, McCarthy BG, Ford AP, Smith JA.** P2X3 receptor antagonist (AF-219) in refractory chronic cough: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet* 385: 1198–1205, 2015. doi:10.1016/S0140-6736(14)61255-1.
2. **Ackroff K, Touzani K, Peets TK, Sclafani A.** Flavor preferences conditioned by intragastric fructose and glucose: differences in reinforcement potency. *Physiol Behav* 72: 691–703, 2001. doi:10.1016/S0031-9384(01)00442-5.
3. **Baldwin W, McRae S, Marek G, Wymer D, Pannu V, Baylis C, Johnson RJ, Sautin YY.** Hyperuricemia as a mediator of the proinflammatory endocrine imbalance in the adipose tissue in a murine model of the metabolic syndrome. *Diabetes* 60: 1258–1269, 2011. doi:10.2337/db10-0916.
4. **Colsoul B, Schraenen A, Lemaire K, Quintens R, Van Lommel L, Segal A, Owsianik G, Talavera K, Voets T, Margolskee RF, Kokrashvili Z, Gilon P, Nilius B, Schuit FC, Vennekens R.** Loss of high-frequency glucose-induced Ca2+ oscillations in pancreatic islets correlates with impaired glucose tolerance in Trpm5-/- mice. *Proc Natl Acad Sci USA* 107: 5208–5213, 2010. doi:10.1073/pnas.0913107107.
5. **de Araujo IE, Oliveira-Maia AJ, Sotnikova TD, Gainetdinov RR, Caron MG, Nicolelis MA, Simon SA.** Food reward in the absence of taste receptor signaling. *Neuron* 57: 930–941, 2008. doi:10.1016/j.neuron.2008.01.032.
6. **DeBosch BJ, Kluth O, Fujiwara H, Schürmann A, Moley K.** Early-onset metabolic syndrome in mice lacking the intestinal uric acid transporter SLC2A9. *Nat Commun* 5: 4642, 2014. doi:10.1038/ncomms5642.

7. **Diggle CP, Shires M, McRae C, Crellin D, Fisher J, Carr IM, Markham AF, Hayward BE, Asipu A, Bonthron DT.** Both isoforms of ketohexokinase are dispensable for normal growth and development. *Physiol Genomics* 42A: 235–243, 2010. doi:10.1152/physiolgenomics.00128.2010.
8. **Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC.** ATP signaling is crucial for communication from taste buds to gustatory nerves. *Science* 310: 1495–1499, 2005. doi:10.1126/science.1118435.
9. **Glendinning JI, Gillman J, Zamer H, Margolskee RF, Sclafani A.** The role of T1r3 and Trpm5 in carbohydrate-induced obesity in mice. *Physiol Behav* 107: 50–58, 2012. doi:10.1016/j.physbeh.2012.05.023.
10. **Goran MI, Ulijaszek SJ, Ventura EE.** High fructose corn syrup and diabetes prevalence: a global perspective. *Glob Public Health* 8: 55–64, 2013. doi:10.1080/17441692.2012.736257.
11. **Ishimoto T, Lanaspa MA, Le MT, Garcia GE, Diggle CP, Maclean PS, Jackman MR, Asipu A, Roncal-Jimenez CA, Kosugi T, Rivard CJ, Maruyama S, Rodriguez-Iturbe B, Sánchez-Lozada LG, Bonthron DT, Sautin YY, Johnson RJ.** Opposing effects of fructokinase C and A isoforms on fructose-induced metabolic syndrome in mice. *Proc Natl Acad Sci USA* 109: 4320–4325, 2012. doi:10.1073/pnas.1119908109.
12. **Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, Gersch MS, Benner S, Sánchez-Lozada LG.** Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr* 86: 899–906, 2007.
13. **Kaelberer MM, Rupprecht LE, Liu WW, Weng P, Bohórquez DV.** Neuropod cells: the emerging biology of gut-brain sensory transduction. *Annu Rev Neurosci* 43: 337–353, 2020. doi:10.1146/annurev-neuro-091619-022657.
14. **Kim MS, Krawczyk SA, Doridot L, Fowler AJ, Wang JX, Trauger SA, Noh HL, Kang HJ, Meissen JK, Blatnik M, Kim JK, Lai M, Herman MA.** ChREBP regulates fructose-induced glucose production independently of insulin signaling. *J Clin Invest* 126: 4372–4386, 2016. doi:10.1172/JCI81993.
15. **Kokrashvili Z, Mosinger B, Margolskee RF.** T1r3 and alpha-gustducin in gut regulate secretion of glucagon-like peptide-1. *Ann N Y Acad Sci* 1170: 91–94, 2009. doi:10.1111/j.1749-6632.2009.04485.x.
16. **Lanaspa MA, Almeida NE, Andres-Hernando A, Rivard CJ, Capasso JM, Berl T.** The tight junction protein, MUPP1, is up-regulated by hypertonicity and is important in the osmotic stress response in kidney cells. *Proc Natl Acad Sci USA* 104: 13672–13677, 2007. doi:10.1073/pnas.0702752104.
17. **Lanaspa MA, Andres-Hernando A, Orlicky DJ, Cicerchi C, Jang C, Li N, Milagres T, Kuwabara M, Wempe MF, Rabinowitz JD, Johnson RJ, Tolan DR.** Ketohexokinase C blockade ameliorates fructose-induced metabolic dysfunction in fructose-sensitive mice. *J Clin Invest* 128: 2226–2238, 2018. doi:10.1172/JCI94427.
18. **Lanaspa MA, Ishimoto T, Li N, Cicerchi C, Orlicky DJ, Ruzycki P, Rivard C, Inaba S, Roncal-Jimenez CA, Bales ES, Diggle CP, Asipu A, Petrash JM, Kosugi T, Maruyama S, Sanchez-Lozada LG, McManaman JL, Bonthron DT, Sautin YY, Johnson RJ.** Endogenous fructose production and metabolism in the liver contributes to the development of metabolic syndrome. [Erratum in *Nat Commun* 4: 2929, 2013]. *Nat Commun* 4: 2434, 2013. doi:10.1038/ncomms3434.
19. **Lanaspa MA, Kuwabara M, Andres-Hernando A, Li N, Cicerchi C, Jensen T, Orlicky DJ, Roncal-Jimenez CA, Ishimoto T, Nakagawa T, Rodriguez-Iturbe B, MacLean PS, Johnson RJ.** High salt intake causes leptin resistance and obesity in mice by stimulating endogenous fructose production and metabolism. *Proc Natl Acad Sci USA* 115: 3138–3143, 2018. doi:10.1073/pnas.1713837115.
20. **Lanaspa MA, Sanchez-Lozada LG, Choi YJ, Cicerchi C, Kanbay M, Roncal-Jimenez CA, Ishimoto T, Li N, Marek G, Duranay M, Schreiner G, Rodriguez-Iturbe B, Nakagawa T, Kang DH, Sautin YY, Johnson RJ.** Uric acid induces hepatic steatosis by generation of mitochondrial oxidative stress: potential role in fructose-dependent and -independent fatty liver. *J Biol Chem* 287: 40732–40744, 2012. doi:10.1074/jbc.M112.399899.
21. **Lanaspa MA, Sanchez-Lozada LG, Cicerchi C, Li N, Roncal-Jimenez CA, Ishimoto T, Le M, Garcia GE, Thomas JB, Rivard CJ, Andres-Hernando A, Hunter B, Schreiner G, Rodriguez-Iturbe B, Sautin YY, Johnson RJ.** Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver. *PLoS One* 7: e47948, 2012. doi:10.1371/journal.pone.0047948.
22. **Ma L, Robinson LN, Towle HC.** ChREBP*Mlx is the principal mediator of glucose-induced gene expression in the liver. *J Biol Chem* 281: 28721–28730, 2006. doi:10.1074/jbc.M601576200.
23. **Ma L, Tsatsos NG, Towle HC.** Direct role of ChREBP.Mlx in regulating hepatic glucose-responsive genes. *J Biol Chem* 280: 12019–12027, 2005. doi:10.1074/jbc.M413063200.
24. **Malik VS, Hu FB.** Fructose and cardiometabolic health: what the evidence from sugar-sweetened beverages tells us. *J Am Coll Cardiol* 66: 1615–1624, 2015. doi:10.1016/j.jacc.2015.08.025.
25. **Margolskee RF, Dyer J, Kokrashvili Z, Salmon KS, Ilegems E, Daly K, Maillet EL, Ninomiya Y, Mosinger B, Shirazi-Beechey SP.** T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci USA* 104: 15075–15080, 2007. doi:10.1073/pnas.0706678104.
26. **Miller CO, Yang X, Lu K, Cao J, Herath K, Rosahl TW, Askew R, Pavlovic G, Zhou G, Li C, Akiyama TE.** Ketohexokinase knockout mice, a model for essential fructosuria, exhibit altered fructose metabolism and are protected from diet-induced metabolic defects. *Am J Physiol Endocrinol Metab* 315: E386–E393, 2018. doi:10.1152/ajpendo.00027.2018.
27. **Nakagawa T, Hu H, Zharikov S, Tuttle KR, Short RA, Glushakova O, Ouyang X, Feig DI, Block ER, Herrera-Acosta J, Patel JM, Johnson RJ.** A causal role for uric acid in fructose-induced metabolic syndrome. *Am J Physiol Renal Physiol* 290: F625–F631, 2006. doi:10.1152/ajprenal.00140.2005.
28. **Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev VO, Lohse MJ, Shigemura N, Ninomiya Y, Kojima I.** Sweet taste receptor expressed in pancreatic beta-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS One* 4: e5106, 2009. doi:10.1371/journal.pone.0005106.
29. **National Research Council (U.S.).** *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academies Press, 2011, p. xxv.
30. **Ng SW, Slining MM, Popkin BM.** Use of caloric and noncaloric sweeteners in US consumer packaged foods, 2005-2009. *J Acad Nutr Diet* 112: 1828–1834, 2012. doi:10.1016/j.jand.2012.07.009.
31. **Ohkuri T, Horio N, Stratford JM, Finger TE, Ninomiya Y.** Residual chemoresponsiveness to acids in the superior laryngeal nerve in "taste-blind" (P2X2/P2X3 double-KO) mice. *Chem Senses* 37: 523–532, 2012. doi:10.1093/chemse/bjs004.
32. **Ren X, Ferreira JG, Zhou L, Shammah-Lagnado SJ, Yeckel CW, de Araujo IE.** Nutrient selection in the absence of taste receptor signaling. *J Neurosci* 30: 8012–8023, 2010. doi:10.1523/JNEUROSCI.5749-09.2010.
33. **Sclafani A, Ackroff K.** Glucose- and fructose-conditioned flavor preferences in rats: taste versus postingestive conditioning. *Physiol Behav* 56: 399–405, 1994. doi:10.1016/0031-9384(94)90213-5.
34. **Sclafani A, Cardieri C, Tucker K, Blusk D, Ackroff K.** Intragastric glucose but not fructose conditions robust flavor preferences in rats. *Am J Physiol Regul Integr Comp Physiol* 265: R320–R325, 1993. doi:10.1152/ajpregu.1993.265.2.R320.
35. **Sclafani A, Glass DS, Margolskee RF, Glendinning JI.** Gut T1R3 sweet taste receptors do not mediate sucrose-conditioned flavor preferences in mice. *Am J Physiol Regul Integr Comp Physiol* 299: R1643–R1650, 2010. doi:10.1152/ajpregu.00495.2010.
36. **Sclafani A, Marambaud P, Ackroff K.** Sucrose-conditioned flavor preferences in sweet ageusic T1r3 and Calhm1 knockout mice. *Physiol Behav* 126: 25–29, 2014. doi:10.1016/j.physbeh.2013.12.003.
37. **Shapiro A, Mu W, Roncal C, Cheng KY, Johnson RJ, Scarpace PJ.** Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. *Am J Physiol Regul Integr Comp Physiol* 295: R1370–R1375, 2008. doi:10.1152/ajpregu.00195.2008.
38. **Treesukosol Y, Blonde GD, Spector AC.** T1R2 and T1R3 subunits are individually unnecessary for normal affective licking responses to Polycose: implications for saccharide taste receptors in mice. *Am J Physiol Regul Integr Comp Physiol* 296: R855–R865, 2009. doi:10.1152/ajpregu.90869.2008.
39. **Vandenbeuch A, Larson ED, Anderson CB, Smith SA, Ford AP, Finger TE, Kinnamon SC.** Postsynaptic P2X3-containing receptors in gustatory nerve fibres mediate responses to all taste qualities in mice. *J Physiol* 593: 1113–1125, 2015. doi:10.1113/jphysiol.2014.281014.
40. **von Holstein-Rathlou S, BonDurant LD, Peltekian L, Naber MC, Yin TC, Claflin KE, Urizar AI, Madsen AN, Ratner C, Holst B, Karstoft K, Vandenbeuch A, Anderson CB, Cassell MD, Thompson AP, Solomon TP, Rahmouni K, Kinnamon SC, Pieper AA, Gillum MP, Pot-**

**thoff MJ.** FGF21 mediates endocrine control of simple sugar intake and sweet taste preference by the liver. *Cell Metab* 23: 335–343, 2016. doi:10.1016/j.cmet.2015.12.003.

41. **Yang Q, Zhang Z, Gregg EW, Flanders WD, Merritt R, Hu FB.** Added sugar intake and cardiovascular diseases mortality among US adults. *JAMA Intern Med* 174: 516–524, 2014. doi:10.1001/jamainternmed.2013.13563.
42. **Zukerman S, Glendinning JI, Margolskee RF, Sclafani A.** Impact of T1r3 and Trpm5 on carbohydrate preference and acceptance in C57BL/6 mice. *Chem Senses* 38: 421–437, 2013. doi:10.1093/chemse/bjt011.
43. **Zukerman S, Glendinning JI, Margolskee RF, Sclafani A.** T1R3 taste receptor is critical for sucrose but not Polycose taste. *Am J Physiol Regul Integr Comp Physiol* 296: R866–R876, 2009. doi:10.1152/ajpregu.90870.2008.