The role of T1r3 and Trpm5 in carbohydrate-induced obesity in mice

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Abstract

We examined the role of T1r3 and Trpm5 taste signaling proteins in carbohydrate-induced overeating and obesity. T1r3, encoded by *Tas1r3*, is part of the T1r2+T1r3 sugar taste receptor, while Trpm5 mediates signaling for G protein-coupled receptors in taste cells. It is known that C57BL/6 wild-type (WT) and *Tas1r3* knock-out (KO) mice are attracted to the taste of Polycose (a glucose polymer), but not sucrose. In contrast, *Trpm5* KO mice are not attracted to the taste of sucrose or Polycose. In Experiment 1, we maintained the WT, *Tas1r3* KO and *Trpm5* KO mice on one of three diets for 38 days: lab chow plus water (Control diet); chow, water and 34% Polycose solution (Polycose diet); or chow, water and 34% sucrose solution (Sucrose diet). The WT and *Tas1r3* KO mice overconsumed the Polycose diet and became obese. The WT and *Tas1r3* KO mice also overconsumed the Sucrose diet, but only the WT mice became obese. The *Trpm5* KO mice, in contrast, showed little or no overeating on the Sucrose and Polycose diets, and gained slightly or significantly less weight than WT mice on these diets. In Experiment 2, we asked whether the *Tas1r3* KO mice exhibited impaired weight gain on the Sucrose diet because it was insipid. To test this hypothesis, we maintained the WT and *Tas1r3* KO mice on one of two diets for 38 days: chow, water and a dilute (1%) but highly palatable Intralipid emulsion (Control diet); or chow, water and a 34% sucrose + 1% Intralipid solution (Suc+IL diet). The WT and *Tas1r3* KO mice both gained weight and became obese on the Suc+IL diet. Our results suggest that nutritive solutions must be highly palatable to cause carbohydrate-induced obesity in mice, and that palatability produces this effect in part by enhancing nutrient utilization.

Keywords

palatability; taste; carbohydrate; diet-induced obesity; nutrient utilization

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1. Introduction

There is widespread concern that the superabundance of sugar- and/or fat-rich foods is contributing to the current obesity epidemic in the United States [4,21,28,52]. While the evidence linking the intake of nutrient-rich foods to obesity in humans is largely circumstantial [1,23,44], the evidence in rats [31,39,40,43,54,53] and mice [12,30,65] is incontrovertible. The rodent studies report that unlimited access to foods or solutions rich in sugars, fats or both leads to diet-induced obesity, although there are some exceptions (e.g., see [25]). Several factors contribute to diet-induced obesity, including oral (sweet taste, fatty taste and texture) and post-oral (nutritive) stimulation [37], increased nutrient utilization [72] and reduced energy expenditure [2,27].

Rodents are attracted to both the oral and post-oral effects of sugar and fat, but the relative contribution of each factor in promoting diet-induced obesity is unclear [56]. One way to separate oral from post-oral factors involves a chronic nutrient self-infusion procedure. In one study, separate groups of rats were permitted to self-infuse a high-fat or isocaloric high-carbohydrate liquid diet by licking a saccharin solution [71]. The high-fat group self-infused more liquid diet into their stomach and gained more weight than did the high-carbohydrate group. This demonstrated that with flavor held constant, a high-fat diet promoted more obesity than did a high-carbohydrate diet. A subsequent study demonstrated the importance of flavor palatability to diet-induced obesity [55]. In this case, intragastric infusion of a concentrated maltodextrin solution was paired with the consumption of either a highly palatable (saccharin + maltodextrin) or mildly unpalatable (bitter-tasting sucrose octaacetate) solution. The rats with the palatable solution self-infused more of the concentrated maltodextrin solution and gained more weight than did the rats with the unpalatable solution.

The present study further investigated the contribution of oral and post-oral factors to diet-induced obesity, using genetically modified mice. In mammals, the oral attraction to sugars is mediated by the heterodimeric T1r2+T1r3 sweet taste receptor [17,47] as well as downstream signaling elements including Trpm5, a transient Ca^{2+}-activated cation channel [51,76]. *Tas1r3*, the gene that encodes T1r3, knockout (KO) mice show little or no attraction to the taste of sucrose [68,77,78], but learn to prefer sucrose solutions based on their post-oral nutritional actions [68,78]. Yet, even after acquiring a strong sucrose preference, *Tas1r3* KO mice consume less sucrose at high concentrations than do C57BL/6 (B6) wild-type (WT) mice [78]. In contrast, *Tas1r3* KO (and *Tas1r2* KO) mice are strongly attracted to the taste of Polycose [68,78], a starch-derived maltodextrin that is rapidly absorbed as glucose, indicating that Polycose taste is not mediated by the sweet receptor. Despite this strong oral attraction, *Tas1r3* KO mice ingest fewer calories from concentrated (16–32%) Polycose solutions than do WT controls in 24-hr tests [78]. This may be because, in addition to serving as a sweet taste receptor in the mouth, *Tas1r3* is expressed in enteroendocrine and pancreatic beta cells [29,42,45]. The absorption of glucose from the gut of *Tas1r3* KO mice may be compromised because T1r3 mediates the up-regulation of the glucose transporter, SGLT1, on high-carbohydrates diets [36,42]. Because T1r3 also contributes to the release of incretin hormones [33,64] and insulin [32,34,45], *Tas1r3* KO mice may also have impaired post-absorptive processing of carbohydrates.

Like *Tas1r3*, *Trpm5* is necessary for the taste response to sugars and other sweeteners [19,58,75]. *Trpm5* KO mice are also impaired in their taste response to Polycose [58], which indicates that Trpm5 serves as a downstream signaling element in Polycose taste detection. *Trpm5* KO mice can learn to prefer sugar and Polycose solutions based on post-oral nutritive feedback, but they still underconsume these solutions compared with WT mice [20,60]. The attenuated intake of carbohydrate solution by *Trpm5* KO mice may reflect their

impaired taste response to these carbohydrates, but post-oral metabolic deficits may also contribute. Like *Tas1r3*, *Trpm5* is expressed in both enteroendocrine [8] and pancreatic beta [15] cells of mice. The function of Trpm5 in intestinal cells is uncertain, but *Trpm5* KO mice display impaired insulin release in response to circulating glucose and fructose [10,15,34].

While the taste deficits of *Tas1r3* and *Trpm5* KO mice are well-documented, little is known about their long-term ingestive and weight gain responses to carbohydrate solutions. Given the differential involvement of *Tas1r3* in sucrose and Polycose taste, we predicted that *Tas1r3* KO mice would consume fewer calories and gain less weight on a sucrose-supplemented diet than on a Polycose-supplemented diet. The Polycose-fed *Tas1r3* KO mice, in turn, should gain less weight than WT mice because their post-oral absorption and metabolism of carbohydrates are impaired. The importance of SGLT1-mediated glucose absorption to carbohydrate-induced obesity is indicated by the recent finding that the addition of gum arabic to a glucose solution reduced SGLT1 expression and weight gain, but not energy intake in mice, relative to that observed in control mice [46]. These findings indicate that alterations in SGLT1-mediated glucose absorption attenuate weight gain by influencing carbohydrate utilization rather than intake. In contrast, we predicted that *Trpm5* KO mice would show similar reductions in carbohydrate intake and weight gain on the sucrose- and Polycose-supplemented diets because of their attenuated taste response to and post-oral processing of both carbohydrates.

In Experiment 1, we tested the aforementioned predictions by supplementing the diet of *Tas1r3* KO, *Trpm5* KO and WT mice with a carbohydrate solution (34.2% sucrose or 34.2% Polycose) for 38 days, and measuring caloric intake, weight gain, and adiposity. In Experiment 2, we asked whether enhancing the palatability of the sucrose solution to *Tas1r3* KO mice would increase daily caloric intake, weight gain and adiposity. This was accomplished by adding a calorically insignificant concentration (i.e., 1%) of Intralipid, a soybean oil emulsion, to the 34.2% sucrose solution. Both the *Tas1r3* KO and WT mice are attracted to the orosensory attributes of 1% Intralipid [24,59]. In addition to enhancing the palatability of the sucrose solution, the oral and post-oral effects of the Intralipid could alter post-oral processing of the sucrose by stimulating GLP-1 [3] and insulin [14] release and increasing insulin sensitivity [70]. *Trpm5* KO mice were excluded from Experiment 2 because they are not attracted to the taste of dilute Intralipid [58].

2. Methods

2.1 Animals and housing conditions

Tas1r3 KO and *Trpm5* KO mice were derived from parental stock produced by homologous recombination in C57BL/6J embryonic stem cells and maintained on this background [19,18]. The C57BL/6 WT mice were derived from parental stock obtained from the Jackson Laboratories (Bar Harbor, ME). Because mice from all three strains had virtually identical genetic backgrounds, we treated the responses of the WT strain as "normal" and inferred how deletion of *Tas1r3* or *Trpm5* altered these responses. None of the mice had prior exposure to the sapid solutions. All mice were housed and tested individually in standard polycarbonate cages (27.5 × 17 × 12.5 cm) with Bed-O'Cobs™ bedding (Andersons; Maumee, OH) and Nestlets™ cotton pads (Ancare; Bellmore, NY). Mice obtained water and test solutions through sipper spouts (with a 1.5 mm hole; Ancare, Belmore, NY) attached to bottles that were placed on the wire cage-top. The housing facilities had automatically controlled temperature, humidity and lighting (12 h:12 h light:dark cycle). All mice were offered laboratory chow (Rat Diet 5012; PMI Nutrition, Brentwood, MO) and tap water *ad libitum* throughout the experiments. According to the manufacturer, the chow had a physiological fuel value of 3.43 kcal/g. All experimental protocols were approved by the

Institutional Animal Care and Use Committees at Columbia University and Brooklyn College, and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Approximately equal numbers of adult males and females from each strain were assigned randomly to each treatment group, beginning at 7–9 weeks of age (see Tables S1 and S4 for sample sizes). To confirm that the mice did not differ systematically in initial body weight across strain or dietary treatment, we ran a two-way ANOVA, separately for each experiment. For Experiment 1, there was no significant main effect of strain ($F_{2,115} = 1.8$, $P > 0.05$) or diet treatment ($F_{2,115} = 1.5$, $P > 0.05$); further the interaction of strain $\times$ diet treatment was non-significant ($F_{4,115} = 1.4$, $P > 0.05$). The mean ($\pm$ S.E.) initial weights (in g) on the control, sucrose and Polycose diets were, in respective order: 21.0 ± 0.6, 21.9 ± 0.8 and 21.9 ± 1.0 for the WT mice; 21.5 ± 0.5, 22.6 ± 1.0 and 21.7 ± 0.8 for the *Tas1r3* KO mice; and 20.1 ± 1.5, 19.3 ± 0.7 and 22.5 ± 0.9 for the *Trpm5* KO mice. Likewise, for Experiment 2, there was no significant main effect of strain ($F_{1,36} = 0.6$, $P > 0.05$) or diet treatment ($F_{1,36} = 0.4$, $P > 0.05$); further the interaction of strain $\times$ diet treatment was also non-significant ($F_{1,36} = 0.7$, $P > 0.05$). The mean ($\pm$ S.E.) initial weights (in g) on the Suc +IL and IL diets were, in respective order: 21.9 ± 0.7 and 20.8 ± 0.9 for the WT mice; and 21.8 ± 0.8 and 21.8 ± 0.8 for the *Tas1r3* KO mice.

2.2. Test solutions

The test solutions were prepared by dissolving sucrose (Domino Foods, Inc., Yonkers, NY), Polycose (Ross Laboratories, Columbus, OH), soybean oil emulsion (20% Intralipid™; Baxter; Dearfield, IL) or sodium saccharin (Sigma-Aldrich, St. Louis, MO) in deionized water. All solutions were presented at room temperature, and were replaced with fresh solutions every two days.

For the phenotypic screening, the test solution was 10 mM saccharin. We selected the 10 mM concentration because WT mice, but not *Tas1r3* KO or *Trpm5* KO mice, strongly prefer it over water [19,18,58,78]. For Experiment 1, we used 34.2% (1.0 M) sucrose and 34.2% (0.34 M) Polycose; both solutions had a caloric density of 1.19 kcal/g. We chose these solutions because (a) *ad libitum* access to 32–34.2% sucrose or Polycose causes diet-induced obesity in rodents [25,54]; (b) 32–34.2% sucrose solution elicits vigorous taste-mediated licking responses in WT, but not *Tas1r3* KO or *Trpm5* KO mice [19,68,78]; and (c) 32% Polycose solutions cause vigorous taste-mediated licking responses in WT and *Tas1r3* KO mice [68,78]. For Experiment 2, we used 1% Intralipid (IL, 0.105 kcal/g) and a mixture of 34.2% sucrose plus 1% IL (Suc+IL, 1.295 kcal/g). The 1% IL emulsion was prepared by diluting the 20% IL with deionized water. The caloric density of the 1% IL solution was 0.1 kcal/g, while that of the sucrose + IL solution was 1.295 kcal/g. We selected these solutions because the *Tas1r3* KO and WT mice avidly consume IL [24,59].

2.3. Phenotypic screen for saccharin preference

We subjected mice from the two experiments to a 24-h two-bottle test with 10 mM saccharin vs. water; the test was conducted for two days with the position of the bottles alternated each day. Saccharin solution percent preferences were calculated as follows: $100 \times$ [saccharin intake / (saccharin + water intake)]. A preference approaching 100% indicates that the mice strongly preferred the saccharin solution; a preference approaching 50% indicates that they were indifferent to it; and a preference approaching 0% indicates that they strongly avoided it.

We analyzed saccharin preferences (following arcsine transformation) in two ways. First, we compared preference scores across strains, using one-way ANOVA and Tukey post hoc

tests. In this and all subsequent statistical tests, we set the alpha level at 0.05. Second, we asked whether any strain was attracted to (i.e., exhibited a preference significantly > 50%) or repelled by (i.e., exhibited a preference significantly < 50%) the saccharin solution, using one-sample t-tests.

2.4. Experiment 1: Do carbohydrates induce dietary obesity in WT, Tas1r3 KO and Trpm5 KO mice?

This experiment asked how deletion of *Tas1r3* or *Trpm5* influenced caloric intake, weight gain and adiposity on chow diets supplemented with a 34.2% sucrose or Polycose solution. To this end, male and female mice from the WT, *Tas1r3* KO and *Trpm5* KO strains were each assigned randomly to one of three treatment groups. All mice were given ad libitum access to lab chow and two sipper tubes. For the Control diet, both sipper tubes contained water. For the Sucrose diet, one sipper contained water and the other 34.2% sucrose. For the Polycose diet, one sipper contained water and the other 34.2% Polycose. The sipper tubes were positioned approximately 5 cm apart. To control for side preferences, the left-right position of each sipper tubes was alternated each day.

In this and the next experiment, body weight was measured on alternate days (to the nearest 0.1 g). Daily fluid intakes were measured (to the nearest 0.1 g) by recording the change in weight of the drinking bottles, using an electronic balance interfaced to a computer. Daily fluid spillage was estimated by recording the change in weight of bottles representing each fluid treatment in an empty cage. To correct for fluid spillage, we subtracted the estimated spill from the quantity consumed over the 24 hr test.

Testing was conducted at two sites: Barnard College and Brooklyn College. While we standardized the testing conditions at both sites in most respects (e.g., diet, caging, bedding, temperature and day-length), we used different systems for presenting lab chow and measuring its consumption. At Brooklyn College, we presented chow in a customized food hopper, which captured the vast majority of spilled diet in a trough. To estimate chow consumption, we weighed the food hoppers every two days, and assumed that the loss in weight reflected intake. At Barnard College, we presented chow in the wire cage-top. Chow consumption was measured one day a week (i.e., 5 times over the 38-day experiment). On each measurement day, a mouse was given approximately 7 g of chow pellets, and the change in weight of the pellets over the subsequent 24 hr was measured. To recover any diet that fell into the cage as the mice ate, we sifted the cage bedding each day by passing it through two sieves; the first had a grid size of 2.3×2.3 mm and the second a grid size of 1.5×1.5 mm. The diet recovered from the sifting was subtracted from the daily intake for each mouse (range = 0.1 to 1.6 g/day). To determine whether the two systems for presenting and measuring intake of chow influenced the results, we ran redundant experiments with control and some experimental treatments at both sites. Because the mean weight gains and chow intakes of analogous control and experimental groups at the two sites were statistically indistinguishable (P > 0.05), we pooled results across both sites.

At the end of this and the next experiment, we euthanized each mouse, measured its weight and body length (nose-anus; to nearest 1 mm), excised the gonadal, retroperitoneal, omental and mesenteric fat depots, and measured the wet weight of each (to the nearest 0.001 g). We focused on these fat depots because they together constitute a large proportion of total body fat, can be removed easily, and respond to high-calorie diets in mice [72]. The retroperitoneal depot (in males and females) consisted of fat associated with the perirenal capsule and the dorsal body wall near the kidneys. In cases where an adrenal gland was embedded in the fat tissue, it was removed. The gonadal depot consisted of fat associated with the epididymis and vesicular gland in males, and the ovary, fallopian tubes and uterus in females. The mesenteric depot consisted of fat associated with the omental membrane and

the mesentery along the duodenum, jejunum, and ileum. The mesentery was carefully stripped off the small intestine as a continuous piece; no attempt was made to dissociate fat from the mesenteric tissue. We combined the weights of the omental and mesenteric fat depots, and henceforth refer to them collectively as the "mesenteric" fat depot. We standardized the fat depot extractions across mice by having the same person remove the fat depots from all mice, under a blind protocol.

2.4.1. Data Analyses—We used ANOVAs to examine effect of diet, strain and sex on each of three dependent measures: daily caloric intake averaged over 38 days, weight gain over 38 days, and terminal adiposity. We examined adiposity by measuring the final wet weight of each of three depots: gonadal, retroperitoneal and mesenteric. Finally, we examined percent preference for each carbohydrate solution over water during the initial four days of the experiment. To this end, we tested for an effect of strain and time, using a mixed-model ANOVA and one-sample t-tests; all preference scores were subjected to arcsine transformation. All statistics in this and the next experiment were conducted with IBM SPSS Statistics (version 20). We set the alpha level for all tests at 0.05.

2.5. Experiment 2: Does increasing the palatability of the Sucrose diet cause obesity in Tas1r3 KO mice?

In Experiment 1, the *Tas1r3* KO mice did not develop diet-induced obesity on the Sucrose diet. Here, we asked whether the addition of a highly palatable (but dilute) concentration of Intralipid to the 34.2% sucrose solution would cause the *Tas1r3* KO mice to develop diet-induced obesity.

We tested both *Tas1r3* KO and WT mice. *Trpm5* KO mice were not tested because they are indifferent to dilute oil emulsions [58]. Mice from both strains were assigned randomly to one of two treatment diets. All mice were given daily access to lab chow and two sipper tubes. For the Control diet (i.e., IL diet), one sipper tube contained water and the other contained 1% IL. For the experimental diet (i.e., Suc+IL diet), one sipper tube contained water and the other contained a mixture of 34.2% sucrose plus 1% IL. Caloric intake, body weight gain, and adiposity were recorded as in Experiment 1.

3. Results

3.1. Saccharin phenotypic screen

The mean saccharin preferences differed significantly across strains ($F_{5,327} = 611.9$, $P < 0.05$), with the following relative magnitudes: WT (93.2%) > *Trpm5* KO (45.3%) > *Tas1r3* KO (33.5%). A one-sample t-test revealed that the saccharin solution was preferred by WT mice (t = 42.4, df = 74, $P < 0.05$), avoided by sweet-insensitive *Tas1r3* KO mice (t = 8.9, df = 59, $P < 0.05$), and treated indifferently by sweet- and bitter-insensitive *Trpm5* KO mice (t = 1.6, df = 28, $P > 0.05$). These results establish that the deletion of *Tas1r3* and *Trpm5* eliminated the preference for saccharin; and that the two KO strains had divergent responses to the taste of 10 mM saccharin.

3.2. Experiment 1: Impact of deleting Tas1r3 or Trpm5 on responses to the carbohydrate diets

There were significant strain differences in sucrose and Polycose preferences across the initial four days of the experiment (Figure 1). With respect to sucrose, there was a strain × day interaction ($F_{6,105} = 24.6$, $P < 0.05$). Individual one-sample t-tests indicated that *Tas1r3* KO and *Trpm5* KO mice were indifferent to the sucrose solution on day 1 (i.e., they did not prefer the sucrose solution over water) and displayed lower ($P < 0.05$) preferences than did WT mice. On days 2–4, sucrose preference in *Tas1r3* KO mice was comparable to that in

WT mice and approached 100%; in contrast, *Trpm5* KO mice displayed a reduced ($P < 0.05$) sucrose preference, relative to WT mice, on days 2 and 3, but not day 4. The strains also differed in their Polycose preference (strain × days interaction: $F_{6,78} = 13,2, P < 0.05$), although in this case *Trpm5* KO mice were indifferent to Polycose exclusively on day 1. All strains displayed Polycose preferences approaching 100% on days 2–4. On days 5–38 of the experiment, the KO and WT mice all continued to exhibit strong preferences for the sucrose and Polycose solutions.

We used 3-way ANOVAs to examine the effects of diet, mouse strain and sex on daily caloric intake and weight gain. For daily caloric intake, there was a significant main effect of diet ($F_{2,123} = 50.5, P < 0.001$) and strain ($F_{2,123} = 17.0, P < 0.001$), but not of sex ($F_{1,123} = 2.9, P > 0.05$); the only significant interaction was diet × strain ($F_{4,123} = 7.1, P < 0.001$). These results reflect three notable findings. First, when offered the Sucrose or Polycose diet, all strains obtained the majority of their calories from the carbohydrate solution (Figure 2, top row of panels). Second, the WT and *Tas1r3* KO strains obtained significantly more calories overall from the Sucrose and Polycose diets than from the Control diet. The *Trpm5* KO mice, on the other hand, obtained significantly more calories from the Polycose than the Control diet, but the same number of calories from the Sucrose and Control diets. Third, across-strain analyses indicated that the three strains responded similarly to the Control diet (i.e., consumed similar quantities of chow), but differently to the carbohydrate diets (Table S1). On the Sucrose diet, WT mice consumed more sucrose solution than did *Tas1r3* KO mice, which, in turn, consumed more than *Trpm5* KO mice; chow intakes did not differ across strains. The total daily caloric intake of WT and *Tas1r3* KO mice did not differ, but exceeded that of *Trpm5* KO mice. On the Polycose diet, intakes of the Polycose solution by WT and *Tas1r3* KO mice were similar, and exceeded those of *Trpm5* KO mice; chow intakes did not differ across strains. The daily caloric intake of WT and *Tas1r3* KO mice on the Polycose diet was the same, but exceeded that of *Trpm5* KO mice. Taken together, these findings indicate that genetic deletion of *Trpm5*, but not *Tas1r3* diminished daily caloric intake from the Sucrose and Polycose diets.

For weight gain, there were significant main effects of diet ($F_{2,123} > 50.5, P < 0.001$), strain ($F_{2,123} > 17.0, P < 0.001$) and sex ($F_{1,123} > 8.0, P > 0.05$); furthermore, the two-way interactions were all significant ($P < 0.05$), but the three-way interaction was not. In WT mice, the Sucrose diet induced greater weight gain than did the Control diet, and the Polycose diet, in turn, induced greater weight gain than did the Sucrose diet (Figure 2, middle row of panels). In *Tas1r3* KO mice, the Sucrose diet failed to induce greater weight gain than the Control diet, even though it stimulated greater caloric intake. However, the Polycose diet induced dramatically higher weight gain than did both the Control and Sucrose diets. In *Trpm5* KO mice, there was a trend for the Sucrose diet to induce greater weight gain than the Control diet, but the difference was not significant. The Polycose diet, however, induced significantly more weight gain than did the Control diet. Between strain analyses indicated that WT and *Trpm5* KO mice gained more weight than the *Tas1r3* KO mice on the Control and Sucrose diets (Table S1). In contrast, the WT and *Tas1r3* KO mice gained substantially more weight on the Polycose diet than did the *Trpm5* KO mice.

The carbohydrate-induced weight gains covaried with the final fat depot weights across the three diets (Figure 2, bottom row of panels), indicating that the weight gains were associated with increased fat accumulation. Between-strain comparisons, however, revealed a dissociation between weight gain and fat depot weight. In particular, the *Trpm5* KO mice gained more weight on the Control and Sucrose diets than did the *Tas1r3* KO mice, but did not have heavier fat depots (Table S1). There were no significant effects of diet treatment on body length in any of the mouse strains (in all one-way ANOVAs, $P > 0.05$).

Although the three fat depots were generally larger in the mice on the carbohydrate diets, there were some notable strain differences (Table S2). For WT mice, the relative size of the gonadal fat depot across diets (Polycose diet > Sucrose diet > Control diet) differed from that of the mesenteric and retroperitoneal fat depots (Polycose diet = Sucrose diet > Control diet). For *Tas1r3* KO mice, the relative size of all three fat depots across diets was uniform (i.e., Polycose diet > Sucrose diet = Control diet). For *Trpm5* KO mice, consumption of the carbohydrate diets increased the relative size of the gonadal and retroperitoneal fat depots, but not of the mesenteric fat depot.

To explore the specific contribution of sex to daily caloric intake and weight gain, we compared male and females, separately for each strain and diet (Table S3). However, we observed only two significant sex differences: (a) WT males exhibited higher weight gain than WT females on the Sucrose and Polycose diets; and (b) *Tas1r3* KO males displayed higher weight gains than *Tas1r3* KO females on the Polycose diet. Because sex differences were the exception rather than the rule, we pooled the sexes for the statistical analyses described previously.

Taken together, these results indicate that genetic deletion of *Tas1r3* impaired weight gain on the Sucrose but not the Polycose diet; and that genetic deletion of *Trpm5* impaired weight gain on both the Sucrose and Polycose diets.

3.3. Experiment 2: Does increasing the palatability of the Sucrose diet cause obesity in Tas1r3 KO mice?

The WT and *Tas1r3* KO mice both preferred the Suc+IL solution over water (> 80%) from the first day of the experiment (data not shown). Likewise, the WT and *Tas1r3* KO mice also preferred the IL solution over water (> 95%) from the first experimental day (data not shown). These results establish that the addition of 1% IL to the sucrose solution or water alone rendered the solutions highly palatable to *Tas1r3* KO and WT mice.

We used 3-way ANOVAs to examine the effects of diet, mouse strain and sex on each of the dependent measures. For daily caloric intake, there was a significant main effect of diet ($F_{1,39} = 6.8$, $P < 0.02$) and strain ($F_{1,39} = 32.4$, $P < 0.001$), but not of sex ($F_{1,39} = 3.3$, $P > 0.05$); none of the interactions were significant (in all cases, $P > 0.05$). For weight gain, there was a significant main effect of diet (in both cases, $F_{1,39} > 57.4$, $P < 0.001$) and sex (in both cases, $F_{1,39} > 8.1$, $P < 0.001$), but not of strain (in both cases, $F_{1,39} < 0.5$, $P > 0.05$); the only three-way interactions was significant (in both cases, $F_{1,39} > 8.3$, $P < 0.01$), but the 2-way interactions were not (in all cases, $P > 0.05$).

There were three notable findings for daily caloric intake (Figure 3, top row of panels). First, when offered the Suc+IL diet, both strains obtained the majority of their calories from the caloric solution (Figure 3, top row of panels). Second, the WT (but not the *Tas1r3* KO) mice ingested significantly more calories overall from the Suc+IL diet than from the IL diet. Third, the *Tas1r3* KO mice consumed significantly more calories from both the IL and Suc+IL diets than did the WT mice from the corresponding diets (in both t-test comparisons, $P < 0.05$, Tukey HSD test). The higher caloric intake of the *Tas1r3* KO mice reflected higher intake from the chow (not the caloric solution) in both diets (Table S4).

Both strains exhibited similarly robust weight gains (i.e., 4–5 g) on the Suc+IL diet (Figure 3, middle row of panels). In contrast, neither strain gained weight on the IL diet (i.e., weight gains were not significantly greater than 0). In WT mice, the lack of weight gain could be due in part to the fact that they consumed fewer calories from the IL diet than did the WT mice from the Control diet in Experiment 1 (10.8 vs. 11.7 kcal/day; t-value = 3.82, df = 36, $P < 0.001$). In *Tas1r3* KO mice, the lack of weight gain cannot be attributed to low caloric

intake. This is because they consumed more calories from the IL diet than from the Control diet in Experiment 1 (12.5 vs. 11.3 kcal/day, respectively, t-value = 2.62, df = 28, P < 0.02), but nevertheless gained less weight. Both the WT and *Tas1r3* KO and mice also consumed more total fluid (IL + water) from the IL diet than did either strain from the Control diet in Experiment 1 (for WT mice, 11.1 vs. 4.5 g/day; t-value = 6.6, df = 36, P < 0.001; for *Tas1r3* KO mice: 11.3 vs. 4.2 g/day; t-value = 5.9, df = 28, P < 0.001). Taken together, these results reveal that (a) weight gain (in both strains) was impaired by the IL diet, but not by the Suc +IL diet; and (b) that addition of 1% IL to the 34.2% sucrose solution not only rendered it highly palatable to the *Tas1r3* KO mice, but it also permitted them to experience normal diet-induced obesity relative to WT mice.

The weight of the fat depots in *Tas1r3* KO mice did not differ significantly from those of the WT mice on the Suc+IL or IL diets (Figure 3, bottom row of panels), even though they consumed more calories from both diets. Further, there were no significant strain differences in total weight gain or final fat mass (Table S4).

In Figure 3 (bottom row of panels) we distinguish the final wet weights of three fat depots (gonadal, mesenteric and retroperitoneal). We ran a three-way mixed-model ANOVA on these data, using fat depot as a within factor and strain and diet as between factors. There was a significant main effect of fat depot ($F_{2,36} = 62.5$, P < 0.001), diet ($F_{1,36} = 25.0$, P < 0.001), but not of strain ($F_{1,36} = 0.7$, P > 0.05); further the interaction of diet × strain was non-significant ($F_{1,36} = 1.1$, P > 0.05). These ANOVA results, together with the results in Figure 3, reveal that all three fat depots were heavier in mice offered the Suc+IL than the IL diet, and that both strains had statistically indistinguishable amounts of fat on each of the corresponding diets.

To explore the specific effect of sex on daily caloric intake and weight gain, we compared male and females, separately for each strain and diet (Table S5). We observed only one significant sex difference: *Tas1r3* KO males accumulated more weight than *Tas1r3* KO females on the Suc+IL diet. As in Experiment 1, because sex differences were the exception rather than the rule, we pooled the sexes for the statistical analyses described above.

4. Discussion

4.1. Preferences for saccharin, carbohydrate and Intralipid in Tas1r3 KO and Trpm5 KO mice

Previous studies have established that genetic deletion of *Tas1r3* or *Trpm5* eliminates the preference for saccharin in mice [19,18,58,77] and our data corroborate this finding. In fact, the *Tas1r3* KO mice avoided 10 mM saccharin. This observation is consistent with prior results and is attributed to their aversion to the bitter taste component of saccharin [9,79]. The *Trpm5* KO mice, on the other hand, were indifferent to saccharin, which is explained by the fact that *Trpm5* deletion impairs both sweet and bitter taste signaling [19,75],

Although *Trpm5* KO mice were initially indifferent to the 34.2% sucrose and Polycose solutions on day 1 of testing, they rapidly developed a strong preference for them in Experiment 1. This agrees with earlier findings, showing that *Trpm5* KO mice have an impaired taste response to sucrose and Polycose, but learn to prefer these carbohydrates based on post-oral nutritional feedback [19,20,60,58,75]. In contrast, *Tas1r3* KO mice were indifferent to sucrose, but strongly preferred Polycose on day 1, and then preferred both carbohydrates thereafter. The initial Polycose preference of *Tas1r3* KO mice is consistent with recent findings that the taste of Polycose is not mediated by the T1r3 (or T1r2) receptor [68,69,78]. It is notable, however, that even after the *Tas1r3* and *Trpm5* KO mice acquired a preference for sucrose, and the *Trpm5* KO mice acquired a preference for Polycose, the

feeding and weight gain responses of these mice to the these carbohydrates remained substantially impaired as discussed below.

We also found that the WT and *Tas1r3* KO mice strongly preferred both the IL and the Suc +IL solutions over water on day 1 and across all subsequent days of Experiment 2. This confirms earlier reports that the taste of 1% IL is not attenuated in mice missing the T1r3 component of the sweet taste receptor [59].

4.2. How did knocking out Tas1r3 impact overeating and obesity?

Tas1r3 KO mice exhibited reduced weight gain and adiposity (relative to WT mice) on both the Control and Sucrose diets. Yet, *Tas1r3* KO strongly preferred sucrose to water (after day 1), and obtained more calories from the Sucrose diet than from the Control diet. Because the *Tas1r3* KO mice did not differ from WT mice in their intake of the Control and Sucrose diets, the most parsimonious explanation for their reduced weight gain on these diets is reduced nutrient utilization. This could have stemmed from the reduced expression of SGLT1 protein in *Tas1r3* KO mice [42]. For instance, a recent study found that dietary gum arabic diminished SGLT1 expression, glucose absorption and glucose-induced weight gain, but not glucose solution intake in B6 mice [46]. Likewise, cats experience reduced glucose absorption because they do not express T1r3 [11,38]. However, the fact that *Tas1r3* KO mice gained weight normally on the Polycose diet argues against an important contribution of glucose malabsorption to their low weight gain on the Sucrose diet. This is because Polycose is digested and absorbed as free glucose.

The observation that the *Tas1r3* KO mice did not differ from WT mice in their obesity response to the Polycose diet was unexpected for two reasons. First, in an earlier study, *Tas1r3* KO mice consumed fewer calories from a 32% Polycose solution than did WT mice during a two-day test [78]. However, while the 38-day Polycose intake of the *Tas1r3* KO mice was similar to that of the WT mice, during the first two study days they consumed significantly less Polycose calories than did the WT mice (7.2 vs. 8.1 kcal/day, respectively; $t = 2.7$, $df = 17$, $P < 0.05$). This establishes that, with sufficient time, the *Tas1r3* KO mice were able to overcome their initial Polycose intake deficit. The second reason we expected *Tas1r3* KO mice to underconsume the Polycose diet (relative to WT mice) is that T1r3 is necessary for (i) sugar-activated up-regulation of SGLT1 glucose transporters in the small intestine [41,42,62], (ii) GLP-1 release from duodenal L cells [33], and (iii) normal glucose tolerance [45]. To explain the apparent contradiction between carbohydrate processing by *Tas1r3* KO mice in the present study and prior studies, it is important to keep in mind the significant methodological differences. In particular, prior studies were based on acute experiments with either intact mice or extracted tissue samples. In contrast, the mice in the present study were allowed to ingest the carbohydrates ad libitum over a 38-day period. This long-time period may have allowed the animals to adapt to any post-oral deficit in carbohydrate processing.

We can propose three non-mutually exclusive explanations for why *Tas1r3* KO mice became obese on the Polycose diet, but not on the Sucrose diet. One is based on the fact that Polycose is a glucose polymer while sucrose is a disaccharide of glucose and fructose. There is evidence that glucose-stimulated insulin release in beta cells is potentiated by fructose, and that this potentiation is mediated by the T1r sweet taste receptor [34]. Accordingly, the absence of T1r3 protein may have disrupted post-absorptive processing of sucrose.

A second explanation stems from the observation that *Tas1r3* KO mice show impaired taste responses to sucrose, but not to Polycose [18,68,77,78]. This was confirmed by their indifference to sucrose and strong preference for Polycose on the first day of Experiment 1. Yet, *Tas1r3* KO mice quickly overcame their sweet taste deficit and by day 2 strongly

preferred sucrose to water. This latter observation can be attributed to a learned preference for *Tas1r3*-independent flavor components (e.g., odor or texture) of the sucrose solution, reinforced by its post-oral nutritive effects [59,77–79]. While the post-oral effects of sugars can condition preferences for hedonically neutral or even aversive solutions [55], they stimulate more robust consumption when the flavored solution is highly palatable [57]. This may explain why, despite conditioning a preference for the sucrose solution, the *Tas1r3* KO mice nevertheless consumed significantly fewer calories from the sucrose diet than the Polycose diet. Further, the percentage of total daily calories obtained from the sucrose solution (65%) was less than that obtained form the Polycose solution (76%).

The third explanation for why *Tas1r3* KO mice became obese on the Polycose diet is based on the findings that oral simulation with a variety of palatable sweeteners—fructose [5,67], glucose [6,26,61,67], sucrose [67], maltose [5] and saccharin [6,66]—elicits cephalic phase insulin release (CPIR) in rats, and that an increase in the magnitude of the CPIR has been reported to promote diet-induced obesity in rats [7]. Accordingly, the highly palatable Polycose solution could have induced a CPIR in *Tas1r3* KO mice, and thereby promoted post-absorptive processing of the ingested carbohydrates and fats [50]. In contrast, because severely attenuating the peripheral taste response to sugars prevents a CPIR [61], the post-absorptive processing of sucrose may have been impaired in *Tas1r3* KO mice, leading to impaired nutrient utilization. This CPIR hypothesis could explain the higher weight gain of *Tas1r3* KO mice on the Polycose diet, and the lower weight gain on the Sucrose diet. We should also not that, in addition to insulin, oral exposure to palatable stimuli can trigger the release of saliva, gastric acid, pancreatic exocrine enzymes, and glucagon [63], all of which might have contributed to the observed findings.

In Experiment 2, we tested a logical prediction of the sweet aguesia hypothesis. If *Tas1r3* KO mice consumed less sucrose solution and gained less weight than WT mice on the Sucrose diet because of their sweet ageusia, then adding a calorically insignificant but highly palatable concentration of Intralipid to the sucrose solution should increase the efficiency of utilization of ingested sucrose, and promote obesity. We found strong support for this prediction. When offered the Suc+IL diet, the *Tas1r3* KO mice consumed the same amount of Suc+IL solution and accumulated the same amounts of weight (and fat) as the WT mice (Table S4). We believe that this effect was mediated by an interaction between IL and sucrose because *Tas1r3* KO mice on the IL or Sucrose diets gained almost no weight. Given that fats bind to taste receptors other than T1r3 [13,35], the addition of 1% IL to the sucrose solution could have activated cephalic-phase responses and thereby enhanced utilization of the sucrose calories. In support of this possibility, oral stimulation with fatty acids has been reported to elicit insulin release in humans ([14]; but see [16]), and a cephalic-phase increase in the protein content of pancreatobiliary secretions in B6 mice [35].

There are two CPIR-independent mechanisms by which the Intralipid in the Suc+IL diet could have augmented sucrose utilization in the *Tas1r3* mice. One stems from the observation that *Tas1r3* KO mice have an attenuated incretin response to sugars [33]. Given that both oral and post-oral stimulation by fats or free fatty acids can stimulate incretin release [3,22,49,74], the Intralipid in the Suc+IL diet could have elicited incretin release. Another mechanism is based on the observation that small quantities of ingested fats can increase hepatic insulin sensitivity in rats [70]. If the Intralipid in the Suc+IL diet enhanced insulin sensitivity in *Tas1r3* KO mice, it may have improved their utilization of the sucrose. More work is needed to assess the relative contribution of these mechanisms to the Suc+IL-induced dietary obesity in *Tas1r3* KO mice.

Unexpectedly, the WT and *Tas1r3* KO mice on the IL diet in Experiment 2 gained less weight than did the same strains of mice on the Control diet in Experiment 1. Conceivably,

the dilute soybean oil emulsion may have activated negative feedback mechanisms that inhibited caloric intake and thereby retarded weight gain [56]. Indeed, WT mice on the IL diet consumed fewer calories than did WT mice on the Control diet in Experiment 1. This explanation is contradicted, however, by the observation that the *Tas1r3* KO mice consumed more energy from the IL diet than from the Control diet in Experiment 1, but nevertheless failed to gain weight. A more likely explanation stems from the fact that the *Tas1r3* KO and WT mice on the IL diet consumed more than twice as much total fluid (IL + water) as the same strains of mice on the Control diet in Experiment. This excess fluid intake may have interfered with nutrient absorption.

4.3. How did knocking out Trpm5 impact overeating and obesity?

The effect of knocking out *Trpm5* on diet-induced obesity was quite distinct from that of knocking out *Tas1r3*. Indeed, the *Trpm5* KO mice gained less weight and/or fat than did WT mice on both the Polycose and Sucrose diets. The lower adiposity of *Trpm5* KO mice stems in part from a loss of responsiveness of the mesenteric fat depot to carbohydrate intake (Table S3). It also stems from the fact that *Trpm5* KO mice consumed significantly less of the carbohydrate solutions than WT mice (Table S1), which is consistent with their impaired taste response to sucrose and Polycose [19,58,75]. The attenuated growth of *Trpm5* KO mice on the carbohydrate diets (relative to WT mice) may also reflect inefficient utilization. Indeed, there are two reports of impaired glucose tolerance and insulin release in *Trpm5* KO mice [10,15]. However, two observations point to a limited role of Trpm5 in post-oral processing of carbohydrates: (a) caloric intake and weight (and fat) gain on the Control diet were all statistically indistinguishable between *Trpm5* KO and WT mice (Table S1); and (b) *Trpm5* KO mice show a normal conditioning response to the post-oral nutritive actions of sugar [20,60].

4.4. Sex differences in carbohydrate-induced obesity

In a study of fat-induced obesity in B6 mice, Nishikawa et al. [48] reported that males become more obese than females. Here, we obtained analogous findings with WT mice on high carbohydrate diets—i.e., male mice gained significantly more weight than female mice on the Sucrose and Polycose diets (Table S3). However, the sex difference in diet-induced obesity was not observed on all diets and in all strains. For instance, when WT mice were offered the Suc+IL diet, both sexes became similarly obese (Table S5). This observation indicates that the diet composition contributes to the sexually dimorphic response. Furthermore, *Tas1r3* KO and *Trpm5* KO mice differed in expression of the sexually dimorphic obesity response. For example, male *Tas1r3* KO mice gained significantly more weight than female *Tas1r3* KO mice on the two diets that caused extensive weight gain (i.e., the Polycose and Suc+IL diets). In contrast, male and female *Trpm5* KO mice experienced a similar degree of diet-induced obesity on the Polycose and Sucrose diets.

Sex differences in diet-induced obesity are thought to stem from differential sensitivity of the brain to adiposity hormones (e.g., insulin and leptin) [73]. Additional work is needed to provide a hormonal explanation for why male WT mice became disproportionately obese on the Polycose and Sucrose diets, but not on the Suc+IL diet. Likewise, it would be valuable to explain why *Trpm5* KO mice failed to exhibit any sex differences in diet-induced obesity.

4.5. Conclusion

The present findings indicate that T1r3 and Trpm5 play critical roles in carbohydrate-induced dietary obesity. *Tas1r3* KO and *Trpm5* KO mice, which are not inherently attracted to sucrose, showed little or no excess weight gain when their diet was supplemented with a sucrose solution. Even though the KO mice rapidly learned to prefer the sucrose solution based on its post-oral actions, this learned preference was not sufficient to promote obesity.

In contrast, when *Tas1r3* KO mice were offered diets supplemented with carbohydrate solutions to which they were inherently attracted (i.e., the Polycose and Suc+IL solutions), they became obese.

Unexpectedly, the diet-induced obesity appeared to be due in large part to increases in carbohydrate utilization not caloric intake. This is illustrated most clearly in *Tas1r3* KO mice, which exhibited elevated caloric intake but no excess weight gain on the Sucrose diet compared to WT and *Trpm5* KO mice. However, when offered the highly palatable Suc+IL solution, the *Tas1r3* KO gained a significant amount of weight although their caloric intake was not elevated.

The fact that *Tas1r3* KO mice exhibited high weight gain on the Polycose and Suc+IL diets indicates that T1r3 signaling is not necessary for efficient carbohydrate metabolism. Conceivably, palatability-driven cephalic responses to Polycose or IL in the mouth may promote carbohydrate processing via *Tas1r3*-independent chemosensory pathways. In addition, gut sensors of Polycose or IL may affect carbohydrate absorption via regulation of SGLT1. Development of tissue-specific knock-out mice would greatly enhance our ability to assess the relative contribution of taste signaling proteins in the mouth versus gut to carbohydrate-induced obesity.

Acknowledgments

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Highlights

- T1r3 and Trpm5 play critical roles in carbohydrate-induced dietary obesity.

- T1r3 knock-out (KO) and Trpm5 KO mice, which are not inherently attracted to sucrose, showed little or no excess weight when their diet was supplemented with a sucrose solution.

- When T1r3 KO mice were offered diets supplemented with carbohydrate solutions to which they were inherently attracted (i.e., the Polycose or sucrose +dilute Intralipid solutions), they became obese.

- The diet-induced obesity appeared to be due largely to increases in carbohydrate utilization, not caloric intake.

- The fact that T1r3 KO mice exhibited high weight gain on the Polycose and sucrose+dilute Intralipid diets demonstrates that T1r3 signaling is not necessary for efficient carbohydrate metabolism.
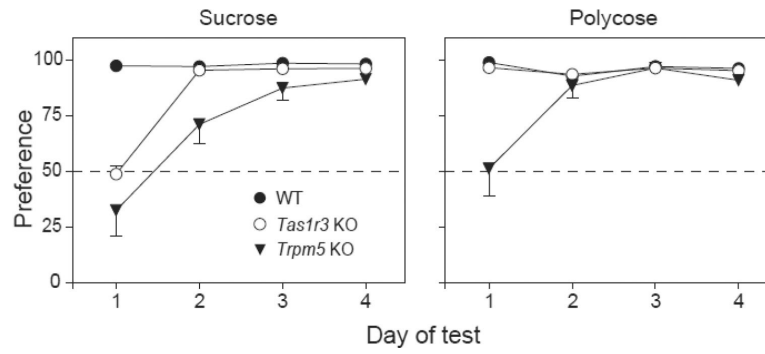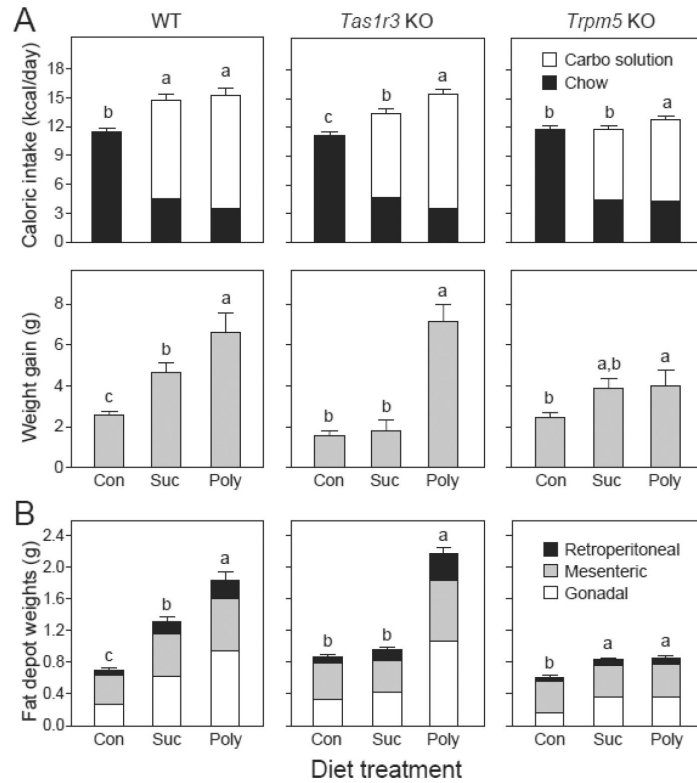
Figure 1.
Preferences of WT, *Tas1r3* KO and *Trpm5* KO mice for (A) 34.2% sucrose or (B) 34.2% Polycose over water during the initial 4 days of Experiment 1. The dashed line in each panel indicates 50% preference (i.e., when the mouse consumed equal quantities of the carbohydrate solution and water). We present mean ± S.E. See text for statistical analyses of these data.

Figure 2.
Comparison of how the WT (left column of panels), *Tas1r3* KO (middle column of panels) and *Trpm5* KO (right column of panels) mice responded to the Control (Con), Sucrose (Suc) and Polycose (Poly) diets in Experiment 1. The composition of each diet was as follows: Con (chow and water), Suc (chow, water and a 34.2% sucrose solution) and Poly (i.e., chow, water and a 34.2% Polycose solution). In *A*, we show daily caloric intake and weight gain, both of which were calculated across the 38-day experiment. For daily caloric intake, we distinguish between calories obtained from the carbohydrate solution and chow. In each panel, we compare responses across diets with a Tukey HSD post hoc test; bars with different letters atop differ significantly from one another (P < 0.05). In *B*, we show wet weights of the gonadal, mesenteric and retroperitoneal fat depots at the end of Experiment 1; the weight weights of each depot are stacked upon each other for a given strain and diet. In each panel, we compare the total weight of all three fat depots diets with a Tukey HSD post hoc test; bars with different letters atop differ significantly from one another (P < 0.05). For the Control, Sucrose and Polycose diets, we tested (in respective order) 28, 18 and 9 WT mice; 20, 10 and 10 *Tas1r3* KO mice; and 9, 10 and 10 *Trpm5* KO mice. We present mean ± S.E.
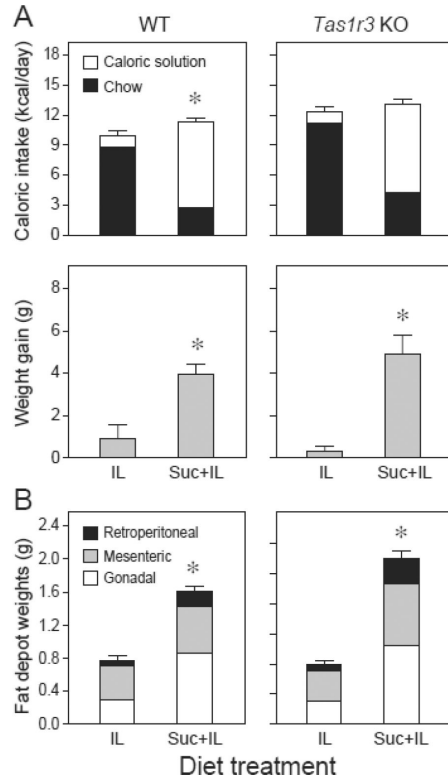
Figure 3.
Comparison of how the WT (left column of panels) and *Tas1r3* KO (right column of panels) mice responded to the IL (chow, water and a 1% IL solution) and Suc+IL (chow, water, and a 1% IL + 34.2% sucrose solution) diets in Experiment 2. In *A*, we show daily caloric intake and weight gain, both of which were calculated across the 38-day experiment. For daily caloric intake, we distinguish between calories obtained from the caloric solution and chow. In each panel, we compare responses across diets with an unpaired t-test (* $P < 0.05$). In *B*, we show wet weights of the gonadal, mesenteric and retroperitoneal fat depots at the end of Experiment 2; the weight weights of each depot are stacked upon each other for a given strain and diet. In each panel, we compare the total weight of all three fat depots diets with an unpaired t-test (* $P < 0.05$). N = 10 per strain and diet treatment. We show mean ± S.E.