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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) mice are attracted to the tastes of both Polycose (a glucose polymer) and sucrose, whereas *Tas1r3* KO mice are attracted to the taste of Polycose 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 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 exhibited little or no overeating but 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.

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,

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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-h tests [78]. This may be because, in addition to serving as a sweet taste receptor in the mouth, T1r3 is present 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-carbohydrate 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 T1r3, 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. Trpm5 is present 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 [18,19]. 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 ($27.5 \times 17 \times 12.5$ 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, Bellmore, 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 Figs. 2 and 3 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 were no significant main effects 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 × diet treatment was non-significant ($F_{4,115} = 1.4$, $P > 0.05$). The mean (±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 were no significant main effects 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 × diet treatment was also non-significant ($F_{1,36} = 0.7$, $P > 0.05$). The mean (±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%