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Fat preference deficits and experience-induced recovery in global taste-deficient *Trpm5* and *Calhm1* knockout mice

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Abstract

There is much evidence that gustation mediates the preference for dietary fat in rodents. Several studies indicate that mice have fat taste receptors that activate downstream signaling elements, including TRPM5 and CALHM1 ion channels and P2X2/P2X3 purinergic gustatory nerve receptors. Experiment 1 further documented the involvement of TRPM5 in fat appetite by giving *Trpm5* knockout (KO) mice, which show global taste deficits, 24-h two-bottle choice tests with ascending concentrations of soybean oil (0.1 – 10% Intralipid) vs. water. Unlike wildtype (WT) mice, naive *Trpm5* KO mice were indifferent to 0.5 – 2.5% fat. They preferred 5–10% fat but consumed much less than WT mice. The same KO mice preferred all fat concentrations in a second test series, which is attributed to a postoral fat conditioned attraction to the non-taste flavor qualities of the Intralipid, although they consumed less fat than the WT mice. The fat preference deficits of the *Trpm5* KO mice were as great or greater than those observed in *Calhm1* KO mice, another KO line with global taste deficits. Experiment 2 examined experience-enhanced fat preferences in *Trpm5* KO and *Calhm1* KO mice by giving them one-bottle training with 1%, 2.5%, and 5% fat prior to two-bottle fat vs. water tests. The KO mice displayed increased two-bottle preferences for all concentrations, although they still consumed less 1% and 2.5% fat than WT mice. Thus, the postoral actions of fat induce robust preferences for fat in taste-deficient mice, but do not stimulate the high fat intakes observed in WT mice with normal fat taste signaling.

Keywords

Fat taste; soybean oil; one-bottle exposure; two-bottle preference; postoral fat appetition

1. Introduction

Like many humans, laboratory rodents are attracted to fat-rich foods, and when given unlimited access are prone to overeat and become obese [5,17]. There is now considerable evidence that dietary fat elicits a specific taste in rodents and modulates their fat preference. In 2005, Laugerette et al. [18] confirmed an earlier study [14] that CD36, a fatty acid

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transporter, is expressed in mouse taste cells and reported behavioral findings implicating CD36 as a fatty acid taste receptor. That is, *Cd36* knockout (KO) mice missing the CD36 transporter, unlike normal wild-type (WT) mice, did not prefer a fatty acid solution (linoleic acid) to a control solution in short- or long-term two-bottle tests. In a subsequent study we confirmed this finding and further observed that *Cd36* KO mice had reduced preferences for soybean oil emulsions in 24-h two-bottle tests [35]. Other investigators proposed that fatty acid G-protein-coupled receptors (GPR40, GPR120, GPR84) may also function as fat taste receptors [8,20]. In particular, fatty acid preference deficits were reported in *Gpr40* KO and *Gpr120* KO mice [8]. However, the expression of GPR40 in taste cells is questionable [6,8,23,24] and there are inconsistent reports on the role of lingual GPR120 in fatty acid detection and preference [3,16,45]. In addition, *Gpr40* KO, *Gpr120* KO, and *Gpr40/120* double KO (DKO) mice display normal preferences for triglyceride oils (soybean oil, rapeseed oil) in long-term tests (Sclafani, et al., 2013, Ancel, et al., 2015). Recent findings indicate that GPR84 functions as a medium chain fatty acid taste receptor, but its role in fat preference has not been established [20].

Additional evidence for the gustatory mediation of fat preference is provided by studies of mice missing critical taste signaling components (TRPM5 sodium channel, CALHM1 calcium channel, P2X2/P2X3 purinergic receptor) which mediate the preference or aversion to prototypical sweet, umami, and bitter tastants [9,13,43,47]. In an early experiment, *Trpm5* KO mice, unlike WT mice, failed to prefer soybean oil emulsions (0.313 – 2.5%) to a vehicle control in 24-h two-bottle tests [40]. Soybean oil preference deficits were also displayed by knockout mice missing the CALHM1 channel that mediates taste cell release of the ATP neurotransmitter, as well as *P2x2/P2x3* double KO (*P2x2/3* DKO) mice missing the gustatory neuron ATP receptor [32,40]. In these experiments the mice were given 24-h two-bottle tests with Intralipid (a commercial soybean oil emulsion) vs. water over a range of ascending fat concentrations. In contrast to WT controls, the *Calhm1* KO mice did not prefer 0.1 – 1% Intralipid to water but did prefer the fat at 2.5 – 5% concentrations. The *P2x2/3* DKO mice failed to prefer 0.313 – 2.5% fat, but preferred the 5 – 20% fat solutions. Note that after completing the initial series of 24-h tests, the global taste KO mice, like WT mice, displayed significant preferences for all Intralipid concentrations (0.1 – 20%) in a second test series [30,32,40]. This was attributed to an acquired preference for the residual flavor properties (odor, texture) of the fat solutions conditioned by the postoral actions of the fat. Postoral fat conditioning is well documented in rodents by the significant preferences and increased intake (acceptance) for arbitrary flavored solutions (e.g., cherry saccharin) produced by intragastric fat infusions in short- or long-term test sessions [2,21,26,35,36]. This conditioned preference/acceptance process is referred to as “appetition” to distinguish it from the postoral satiation actions of nutrients that inhibit food intake [4,29].

In Experiment 1 of the present study we further characterized the soybean oil preference and intake deficits of *Trpm5* KO mice which show global taste deficits. In a second experiment experience-induced enhancement in fat preference and acceptance in *Trpm5* KO were compared to *Calhm1* KO mice, which also have global taste deficits, using a modified test procedure sensitive to postoral nutrient appetition.

2. Experiment 1: Fat preferences in Trpm5 KO and WT mice

In our original study of fat appetite in *Trpm5* KO mice, naive KO and WT were initially tested with a limited range of soybean oil concentrations (0.313 – 2.5%) which did not establish what higher concentrations, if any, might be preferred by the *Trpm5* KO mice. After experience with other tastants (non-nutritive fat substitute (sefa soyate oil), corn oil, maltodextrin, corn starch) the *Trpm5* KO mice subsequently preferred Intralipid at 2.5 – 20% concentrations but these preferences may have been influenced by their prior soybean oil and/or other tastant experiences. For example, whereas naive *Trpm5* KO are indifferent to fructose solutions, glucose-experienced *Trpm5* KO display significant fructose preferences [49]. Similarly, naive *Cd36* KO mice are indifferent to linoleic acid solutions, but *Cd36* KO mice experienced with soybean oil and other tastants display significant linoleic acid preferences [35]. Therefore in the current study, naïve *Trpm5* KO and WT mice were given 24-h two-bottle tests with 0.1 – 10% Intralipid as in our studies of *Cd36* KO, *Calhm1* KO, and *P2x2/3* DKO mice [30,32,39]. The same mice were then given a second test series with these Intralipid solutions to determine the extent of their experience-induced enhancement in fat preference and intake.

2.1. Materials and methods

2.1.1. Animals—Naïve *Trpm5* KO mice (4 males, 6 females) were bred from mice produced by homologous recombination in C57BL/6J (B6) embryonic stem cells and maintained on this background [9]. Naïve B6 WT mice (4 males, 6 females) mice were bred from stock obtained from the Jackson Laboratories (Bar Harbor, ME). The mice were 11 wk old at the start of the experiment; the WT and *Trpm5* KO mice did not differ significantly in mean body weight (22.3 vs. 20.3 g). The animals were singly housed in plastic tub cages in a room maintained at 22°C with a 12:12-h light:dark cycle and given ad libitum access to a standard, low-fat chow diet (5001; PMI Nutrition International) and water. Experimental protocols were approved by the institutional animal care and use committee at Brooklyn College and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.1.2. Test solutions—Intralipid solutions were prepared using 20% Intralipid (Baxter, Deerfield, IL), a stable fat emulsion containing 20% soybean oil, 2.25% glycerol, and 1.2% egg yolk phospholipids. The 20% Intralipid was diluted with deionized water to produce emulsions that contained 0.1, 0.25, 0.5, 0.75, 1, 5 and 10% soybean oil. The Intralipid solution and deionized water control were presented in two 50-ml plastic tubes with stainless steel sipper spouts. The tubes were placed on the tub cage tops to the right of the chow feeder and were fixed in place with clips. Fluid intakes were measured to the nearest 0.1 g with an electronic balance interfaced to a laptop computer. Fluid spillage was estimated by recording the change in weights of two drinking tubes placed on an empty cage.

2.1.3. Procedure—The mice were adapted to being singly housed with two water bottles for one week. They were then given a series of two-bottle Intralipid vs. water tests with 0.1 – 10% fat concentrations (Test 1). They were next given 4 days of water only followed by a second series of Intralipid vs. water tests at the same concentrations (Test 2). In these

tests, each concentration was presented for 2 days in an ascending order and the left-right positions of the fluids were alternated daily to control for a position preference. In addition, to eliminate the possibility that the mice might acquire a preference for a sipper spout associated with the fat solution, the sipper spouts were assigned to a side rather than a fluid [11].

2.1.4. Data analysis—Fluid intakes were averaged over the 2 days at each concentration. Fat preferences were expressed as percent intakes (Intralipid intake/total intake × 100). Group differences in Intralipid intakes and preferences were evaluated using separate mixed model analyses of variance (ANOVAs) with group (genotype) and concentration as between-group and within-group factors, respectively. Significant interaction effects were evaluated using simple main effects tests. Within group differences in Intralipid and water intakes were evaluated using ANOVAs with fluid and concentration as within-group factors.

2.2. Results

In Test 1, the *Trpm5* KO mice consumed less Intralipid than WT mice at all concentrations except 0.1% and 0.25% [Group × Concentration, $F(7,126) = 43.4$, $P < 0.0001$] (Fig. 1). Overall, their mean intake of 0.5 – 10% Intralipid was only 41% of that of the WT mice. The percent fat preferences of the *Trpm5* KO mice were lower than those of the WT mice at all concentrations (Group × Concentration interaction ($F(1,118) = 133.9$, $P < 0.001$) (Fig. 1). Furthermore, whereas the WT consumed more ($P < 0.05$) Intralipid than water at 0.5% and higher concentrations, the *Trpm5* KO mice consumed significantly more ($P<0.05$) Intralipid only at 5% and 10% concentrations.

In Test 2, the *Trpm5* KO mice consumed less Intralipid than the WT mice at the intermediate concentrations of 0.5 – 2.5% (Group × Concentration interaction, $F(7,126) = 30.4$, $P < 0.0001$) (Fig. 2). Overall, their intakes of 0.5 – 10% Intralipid were now 60% that of the WT mice. The percent fat preferences of the *Trpm5* KO mice were similar to those of the WT mice except that they were lower ($P < 0.05$) at 0.10, 0.25, and 1% (Group × Concentration interaction ($F(7,126) = 3.5$, $P < 0.01$) (Fig. 1). The *Trpm5* KO and WT mice consumed more Intralipid than water at all concentrations. Within group comparisons indicated that the KO mice increased their Intralipid absolute and percent intakes from Test 1 to 2 at all concentrations except 10% (Test × Concentration interactions, $F(7,63) = 5.0, 51.4$, $P < 0.0001$). The WT mice increased their absolute fat intakes from Test 1 to 2 at the 0.25 – 1%, 5% concentrations and percent fat preferences at 0.1 – 0.5% concentrations (Test × Concentration interactions, $F(7,63) = 9.4, 25.7$, $P < 0.001$).

2.3 Discussion

The results of the first test series are consistent with our prior findings that naïve *Trpm5* KO mice failed to prefer soybean oil to vehicle at concentrations of 0.313 to 2.5% and demonstrate that the KO mice preferred soybean oil (Intralipid) at 5% and 10% concentrations. Nevertheless, their fat preferences remained below those of the WT mice at all concentrations tested, and the *Trpm5* KO mice consumed substantially less fat than WT mice at 0.5 to 10% concentrations. When given a second series of fat vs. water tests,

the *Trpm5* KO mice preferred all fat concentrations, although their preferences were less than WT mice at some concentrations and their Intralipid intakes were substantially lower at 0.5 – 5% concentrations. This confirms and extends prior findings obtained with *Trpm5* KO mice given repeated tests with a range of Intralipid concentrations [40]. The experience-induced enhancement of fat preference observed in *Trpm5* KO and *Calhm1* KO mice is explored further in the second experiment.

3. Experiment 2: Experience-induced fat preferences in *Trpm5* KO, *Calhm1* KO and WT mice

The fat preference and acceptance deficits displayed by the naive *Trpm5* KO mice in Experiment 1 mirrored those observed in *Calhm1* KO mice but were more pronounced. That is, naive *Calhm1* KO mice significantly preferred 2.5% and higher Intralipid concentrations in Test 1 [32], whereas the naive *Trpm5* KO mice in Experiment 1 were indifferent to 2.5% Intralipid and preferred fat only at 5% and 10% concentrations (see Supplementary Fig. S1). In brief-access two-bottle tests, *Calhm1* KO mice showed no attraction to 2.5% Intralipid, so their preference displayed in the 24-h tests was attributed to an acquired avidity for the non-taste flavor features of the Intralipid (odor, texture) conditioned by the postoral actions of the fat [32]. Consistent with this interpretation, WT mice trained 24 h/day with a flavored solution (the CS+) paired with IG infusions of 5% Intralipid (diluted to 2.5% in the stomach by the ingested CS+) learned to significantly prefer the CS+ flavor [35] over a water-paired CS- flavor. Perhaps the *Trpm5* KO mice failed to acquire a preference for 2.5% Intralipid because they were less sensitive to the postoral appetition actions of fat at this concentration. This possibility is suggested by the findings that TRPM5 is co-localized in gut enteroendocrine cells with the fatty acid receptor GPR120 which, along with GPR40, is implicated in the postoral appetition actions of fat [39,41]. Alternately, slight differences in sensitivity to the non-taste orosensory features of Intralipid might account for the different preference thresholds displayed by *Trpm5* KO and *Calhm1* KO mice.

The present experiment directly compared the Intralipid preferences of *Trpm5* KO and *Calhm1* KO mice. This was accomplished by giving the KO mice 24-h, one-bottle exposure to 1%, 2.5%, and 5% Intralipid prior to two-bottle Intralipid vs. water preference tests. The one-bottle experience allows animals to unambiguously associate the orosensory properties of nutritive solutions with their postoral appetition effects. In two-bottle tests, postoral flavor-nutrient conditioning can be complicated by the tendency of animals to consume more than one fluid during individual drinking bouts [10]. We previously used this one-bottle training procedure to investigate monosodium glutamate and sucrose preferences, respectively, in *Trpm5* KO and *Calhm1* KO mice [1,37].

3.1. Materials and methods

3.1.1. Animals—Naïve *Trpm5* KO mice (5 males, 4 females) bred in the lab from stock described in the first experiment were used. Naïve *Calhm1* KO (7 males, 3 females) and WT mice (4 males, 5 females) on a C57BL/6 background were bred using stock obtained from Philippe Marambaud (Feinstein Institute for Medical Research, Manhasset, NY) [32,44]. The *Trpm5* KO, *Calhm1* KO, and B6 WT mice were 10, 11, and 11 weeks old, respectively,

at the start of testing and did not significantly differ in mean body weights (20.6, 19.0, and 21.4 g, respectively). The mice were housed as in Experiment 1.

3.1.2. Procedure—The mice were adapted to drink from two water bottles in their home cages for 5 days. They were then given one-bottle access to 1% Intralipid and water on four alternating days. This was followed by a 2-day, two-bottle test with 1% Intralipid vs. water. The mice were then given identical series of one- and two-bottle tests with 2.5% Intralipid and water and 5% Intralipid and water. Throughout one-bottle training and two-bottle testing, the left-right positions of the fat and water bottles were counterbalanced over days.

3.1.3. Data analysis—Intralipid and water intakes during the one- and two-bottle tests were averaged over the 2 days at each concentration. Fat preferences in the two-bottle tests were expressed as percent intakes (Intralipid intake/total intake × 100). Group differences in Intralipid intakes during the one- and two-bottle tests and two-bottle preference tests were evaluated using separate mixed model analyses of variance (ANOVAs) with group (genotype) and concentration as between-group and within-group factors, respectively. Significant interaction effects were evaluated using simple main effects tests. Within group differences in Intralipid and water intakes were evaluated using ANOVAs with fluid and concentration as within-group factors.

3.2. Results

In the one-bottle training sessions, *Trpm5* KO and *Calhm1* KO mice consumed less Intralipid than WT mice at all three concentrations although the differences were greatest with 1% Intralipid (Group × Concentration interaction, $F(4,50) = 10.9$, $P < 0.001$) (Fig. 2). In the two-bottle tests, the KO mice consumed less Intralipid than WT mice at 1% and 2.5% concentrations, but the group differences were not significant at the 5% concentration (Group × Concentration interaction, $F(4,50) = 6.8$, $P < 0.001$). The one- and two-bottle Intralipid intakes of the *Trpm5* KO and *Calhm1* KO mice did not significantly differ. With respect to two-bottle fat preference, the *Trpm5* KO and *Calhm1* KO mice displayed lower preferences for 1% Intralipid than did WT mice, but the groups did not differ at the 2.5% and 5% concentrations (Fig. 2). The 1% Intralipid preference of the *Calhm1* KO mice (78%) was slightly but not significantly greater than that of the *Trpm5* KO mice (66%) and the groups showed very similar preferences for 2.5% and 5% fat (94 – 99%) (Fig. 2).

Within group analyses revealed that WT mice consumed more Intralipid than water at all concentrations during the one and two-bottle tests ($F(1,8) = 295.1, 508.6$, $P < 0.001$) and they did not differ in their two-bottle percent preferences for the three Intralipid concentrations (Fig. 2). In contrast, the *Trpm5* KO mice consumed more Intralipid than water only at the 2.5% and 5% concentrations in the one and two-bottle tests (Solution × Concentration interactions, $F(2,16) = 12.4, 20.1$, $P < 0.001$). Their percent preferences for 2.5 and 5% Intralipid exceeded that for 1% Intralipid ($F(2,16) = 17.8$, $P < 0.001$). The *Calhm1* KO mice also consumed more 2.5% and 5% Intralipid than water in the one-bottle tests (Solution × Concentration interaction, $F(2,18) = 58.2$, $P < 0.001$), but in the two-bottle tests they consumed more Intralipid than water at all three concentrations ($F(1,9) = 111.8$, P

< 0.001). However, percent preferences of the *Calhm1* KO mice for 2.5% and 5% Intralipid exceeded that for 1% Intralipid (2,18) = 17.1, P < 0.001). In the 1% Intralipid two-bottle test, 8 of 10 *Calhm1* KO mice consumed more (≥ 60%) fat than water whereas only 5 of 9 *Trpm5* KO did so. This preference difference was not related to differences in one-bottle 1% Intralipid intakes; the *Trpm5* KO mice consumed slightly more 1% fat than did *Calhm1* KO mice during one-bottle training (6.3 vs. 5.9 g/day).

Fig. 3 presents the two-bottle Intralipid test results obtained with the "experienced" mice of the present experiment along with the two-bottle results obtained with mice which did not have prior one-bottle exposure to 1 – 5% Intralipid solutions; these animals are referred to as one-bottle "inexperienced" mice. The inexperienced *Trpm5* KO data are from Test 1 of the first experiment; the data for the inexperienced *Calhm1* KO and WT mice were obtained from Test 1 in our prior study which used a procedure identical to that of Experiment 1 [32]. For the WT mice, there were no differences between the two-bottle Intralipid preferences of the one-bottle fat experienced and inexperienced mice. The experienced mice, however, consumed more 1% and 2.5% Intralipid than did the inexperienced mice (Group × Concentration interaction F(2,34) = 3.8, P < 0.05) (Fig. 3). In contrast to WT mice, one-bottle fat exposure increased the two-bottle preferences of the experienced *Trpm5* KO mice relative to the inexperienced mice at all three concentrations (F(1,17) = 25.8, P < 0.001) and increased their Intralipid intake at the 2.5% and 5% concentrations (Group × Concentration interaction, F(2,34) = 10.6, P < 0.001) (Fig. 3). Similarly, the one-bottle experienced *Calhm1* KO mice displayed higher preferences than did the inexperienced *Calhm1* KO mice for all fat concentrations (F(1,17) =16.5, P < 0.001), and consumed more 2.5% and 5% Intralipid than did the inexperienced mice (Group × Concentration interaction, F(2,34) = 6.8, P < 0.01) (Fig. 3).

3.3. Discussion

The 24-h one-bottle access to Intralipid significantly enhanced the two-bottle fat preferences and intakes of the *Trpm5* KO and *Calhm1* KO mice. With one-bottle exposure, the *Calhm1* KO and *Trpm5* KO mice significantly preferred 1% and 2.5% Intralipid to water, respectively, whereas KO mice without such exposure preferred 2.5% and 5% Intralipid, respectively. Furthermore, one-bottle exposure increased Intralipid acceptance in both KO lines. In marked contrast, one-bottle exposure did not enhance the already high preferences WT mice displayed for the 1 – 5% Intralipid solutions, which documents the inherent avidity of naive WT mice for the fat solutions. The 24-h access to 2.5% and 5% fat induced preferences in the KO mice as great as those displayed by WT mice. In contrast, *Calhm1* KO mice show little or no attraction to 1 – 5% soybean oil in 3-min choice tests, which minimize postoral appetition [40].

While the fat-experienced *Trpm5* KO and *Calhm1* KO mice did not significantly differ in their fat preferences or intakes, the experienced *Calhm1* KO mice consumed more 1% Intralipid than water in the two-bottle test whereas the experienced *Trpm5* KO mice did not. Conceivably, the *Trpm5* KO mice were less sensitive than *Calhm1* KO to the postoral appetition actions of the fat at this low concentration since, as previously noted, TRPM5 is co-localized with GPR120 in gut cells [41]. Alternately, *Trpm5* KO mice may

be less sensitive than *Calhm1* KO mice to the non-taste orosensory stimuli (odor, texture) of Intralipid that serve as conditioned stimuli mediating postoral fat conditioning. This seems unlikely, however, because *Trpm5* KO and *Calhm1* KO mice did not differ in their preferences for 0.5 – 5% Intralipid when the ascending test series was repeated a second time (Test 2, Fig. S1). The Intralipid preferences of the naive *Trpm5* KO in Experiment 1 were very similar to those displayed by naive *P2x2/3* DKO mice, although the *Trpm5* KO mice consumed less 5% and 10% Intralipid than did the *P2x2/3* DKO mice [30]. These comparisons are complicated, however, by fact that the *Trpm5* KO and *P2x2/3* DKO background strains differed (B6 only vs. B6x129) and the mice were tested with different concentration ranges.

The source of the relatively small differences in the Intralipid preferences of the *Trpm5* KO and *Calhm1* KO mice observed in the present experiment could be investigated further using other test procedures. For example, conditioned taste aversion tests could evaluate the sensitivity of the two KO lines to the residual orosensory features of the fat solutions [20,45]. In addition, distinctive olfactory cues could be added to the Intralipid solutions to serve as CS+ flavors to evaluate postoral fat conditioning [31,34]. Alternatively, postoral fat appetition could be evaluated in the absence of distinctive orosensory stimuli by using an operant licking paradigm in which mice lick a dry sipper spout to obtain IG fat infusions [12]. We previously reported that mice missing GPR40 and GPR120 fatty acid receptors, which in part mediate postoral fat appetition [39], show reduced operant licking for IG Intralipid infusions but not for IG glucose infusions [38].

Consistent with the present findings that, compared to 1% Intralipid, one-bottle exposure to 2.5% Intralipid induced greater increases in fat acceptance and preference in KO mice are results obtained with B6 WT mice in an IG fat conditioning experiment. That is, IG infusions of 6.4% and 3.2% Intralipid conditioned significant CS+ flavor preferences whereas 1.6% Intralipid infusions had marginal effects; the 6.4%, 3.2% and 1.6% infusions were diluted in the stomach to 3.2%, 1.6% and 0.8% fat, respectively, by the consumed CS+ solution [2].

4. General Discussion

The present findings provide a more detailed description of the reduced fat preferences displayed by taste-deficient *Trpm5* KO mice [40]. Experiment 1 revealed that naive *Trpm5* KO mice display fat preference and intake deficits, relative to WT mice, as great or greater as those of *Calhm1* KO and *P2x2/3* DKO mice [30,32]. The somewhat greater fat deficits displayed by naive *Trpm5* KO mice compared to *Calhm1* KO and *P2x2/3* DKO mice may result because TRPM5, acting in gut cells, contributes to the postoral appetition actions of dietary fat, although this requires further investigation.

After their experience with Intralipid in the first test, *Trpm5* KO mice preferred all fat concentrations in Test 2, although their preferences were somewhat lower than WT mice at some concentrations. In contrast, *Trpm5* KO mice in our earlier study that provided more extensive fat experience, did not differ from WT mice in their preferences over a range of low Intralipid concentrations (0.039 – 2.5%) in a final test series [40]. Nevertheless,

the *Trpm5* KO mice in both the present and past studies consumed substantially less fat at intermediate concentrations compared to WT mice. Other KO mice with fat-specific (*Cd36* KO) or global taste deficits (*Calhm1* KO, *P2x2/3* DKO) also displayed near-normal preferences but reduced fat acceptance in their second test series [30,32,35]. These findings demonstrate that postoral fat appetition is sufficient to induce robust fat preferences in taste-impaired mice, but normal oral fat taste signaling is required to stimulate the high intakes observed in WT mice. Sugar-experienced, sweet-taste deficient *T1r3* KO mice also display normal preferences for glucose and sucrose solutions but reduced sugar intakes compared to WT mice [48,49]. Interestingly, *T1r3* KO mice given a 34.2% (1 M) sucrose solution containing 1% Intralipid, which they strongly prefer, consumed as much sugar and gained as much excess weight and adiposity, as did the WT mice [15]. Thus, the inherently attractive taste of fat stimulates not only fat intake and preference in normal mice but also sugar intake in sweet-taste deficient *T1r3* KO mice.

A new finding of the present experiment is that two-bottle exposure to high (5% or 10%) fat concentrations is not required to induce robust fat preferences in KO mice. In Experiment 2, one-bottle exposure to 2.5% Intralipid induced a 94% preference for this solution in *Trpm5* KO mice, whereas KO mice without such exposure did not prefer this fat concentration in a 24-h choice test. In addition, the experienced *Trpm5* KO mice consumed three times more 2.5% Intralipid than did the inexperienced mice. One-bottle fat experience also significantly increased the preference *Calhm1* KO mice displayed for 2.5% fat, compared to inexperienced KO mice (from 81% to 98%), and also induced a mild but significant 78% preference for 1% Intralipid as well.

The current results confirm and extend prior findings indicating critical roles for TRPM5 and CALHM1 signaling in triglyceride oil acceptance and preference [40]. The CALHM1 channel mediates the release of the ATP neurotransmitter from taste cells which act on postsynaptic P2X2/P2X3 receptors on gustatory nerves [13]. Involvement of these receptors in fat preference is documented by the Intralipid preference deficits displayed by *P2×2/3* DKO mice [30]. Liu et al. [19] also provided evidence for TRPM5 involvement in fatty acid taste signaling in mice. They observed that *Trpm5* KO mice, unlike WT mice, failed to prefer linoleic acid in a 24-h, two-bottle choice test. However, the linoleic acid concentration tested (30 uM, 0.0008%) was far lower than the linoleic acid concentrations (0.2 – 2%) reported to be preferred by WT mice in other studies [7,8,22,25,35,42,46]. Future experiments should compare the fatty acid preferences of *Trpm5* KO as well as *Calhm1* KO and *P2x2/3* DKO mice over a range of concentrations. It would also be of interest to investigate if one- and/or two-bottle exposure to fatty acid solutions enhances the fatty acid intakes and/or preferences of KO mice. We reported that naive *Cd36* KO mice that were indifferent to 0.025 – 2% linoleic acid in 24-h preference tests preferred these concentrations after two-bottle access to soybean oil solutions. However, it remains to be determined if fatty acid experience alone is sufficient to induce linoleic acid preferences in *Cd36* KO or global taste KO mice. This question is of particular interest because, while IG infusions of triglycerides condition flavor preferences in WT and KO mice [2,33,35,39], the effectiveness of IG fatty acid infusions to do so has not been established.

We previously reported that *Calhm1* KO mice display greater deficits in Intralipid preferences than do *Cd36* KO mice, and the current and prior findings indicate that this is the case for *Trpm5* KO and *P2x2/3* DKO mice as well [30,32]. The greater deficits observed with these three global taste KO models suggest that CD36 is not the only taste receptor influencing fat preference. Although some researchers suggested that the GPR120 fatty acid receptor functions along with CD36 to promote fat preference [27], the normal Intralipid preferences displayed by *Gpr120* KO and *Gpr40/120* DKO mice question this proposal [3,39]. As discussed elsewhere, in addition to fatty acid receptors, it is possible that triglyceride receptors contribute to fat preference in rodents and perhaps humans [28,32,33]. Nevertheless, our findings of experience-enhanced intakes and preferences for fat in KO mice with global taste deficits indicate that animals can also associate non-taste characteristics of fat (odor, texture) with its rewarding postoral actions [32]. This secondary path may operate in parallel with fat taste to provide multisensory detection of an important dietary resource. Further research is needed to fully explain the role of gustation and other orosensory systems in the appetite for fat.

Supplementary Material

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Highlights

- Taste receptors mediate the preference for dietary fat via TRPM5 and CALHM1 ion channels
- *Trpm5* knockout (KO) mice had greatly reduced preferences for 0.5 – 5% Intralipid in 24-h tests
- One-bottle access to 2.5 – 5% Intralipid enhanced the preference of *Trpm5* KO mice for these solutions
- One-bottle exposure to 1 – 5% Intralipid enhanced the preference of *Calhm1* KO mice for these solutions
- Exposure effects are attributed to postorally-conditioned preferences for non-taste fat orosensations

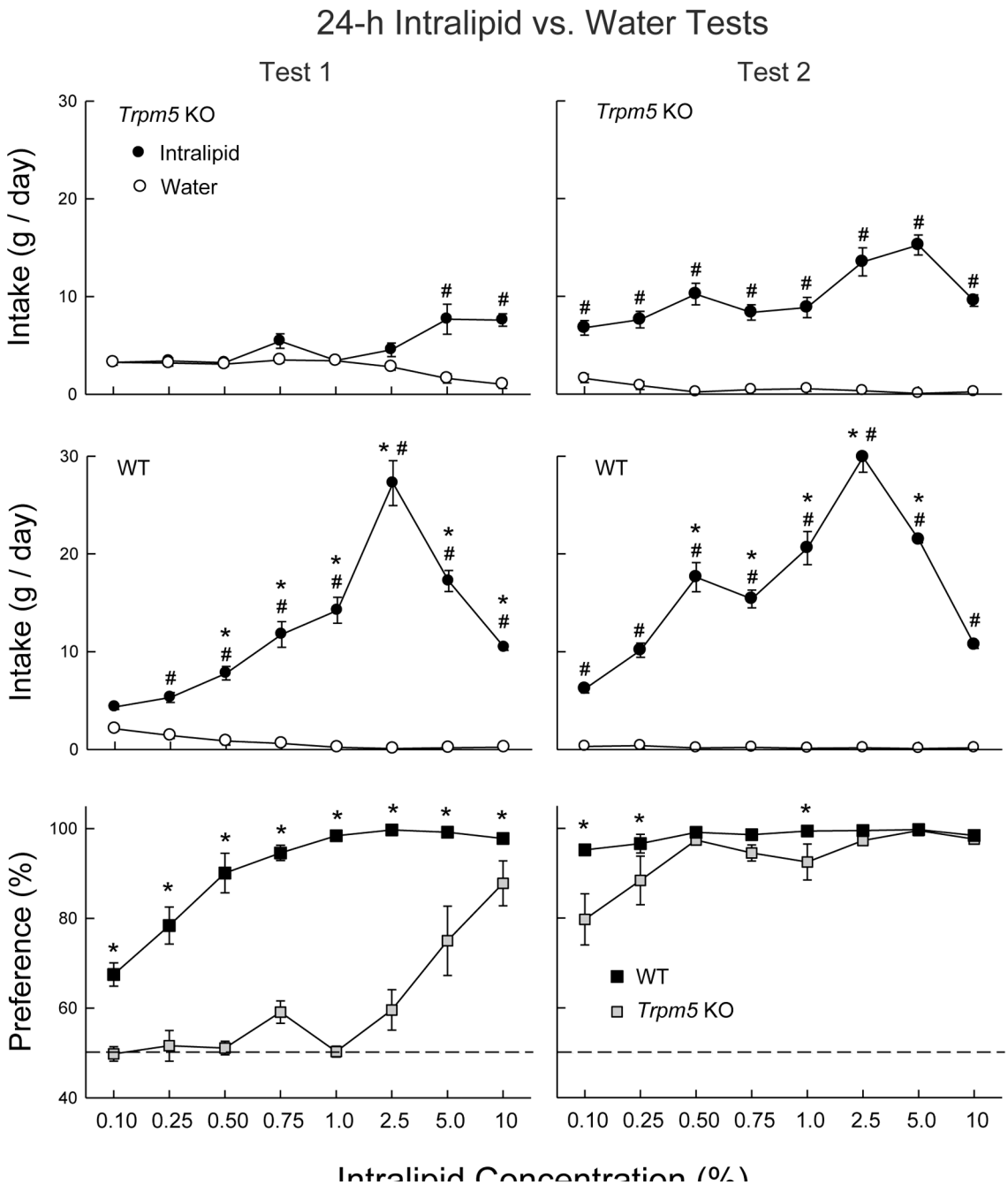

Fig. 1.

Experiment 1. Intake (means ± SE) of Intralipid and water for *Trpm5* KO mice (n = 10, top) and WT mice (n = 10, middle) and percent fat preference for both groups (bottom) during 24-h Intralipid vs. water choice tests. In Test 1 (left) naive mice were tested with 0.1 – 5% Intralipid vs. water. In Test 2 (right), the same mice were retested with Intralipid vs. water. # Concentrations at which Intralipid intake significantly ($p < 0.05$) exceeded water intake within each group. * Concentrations at which WT and *Trpm5* KO mice significantly ($p < 0.05$) differed in Intralipid intake or preference.

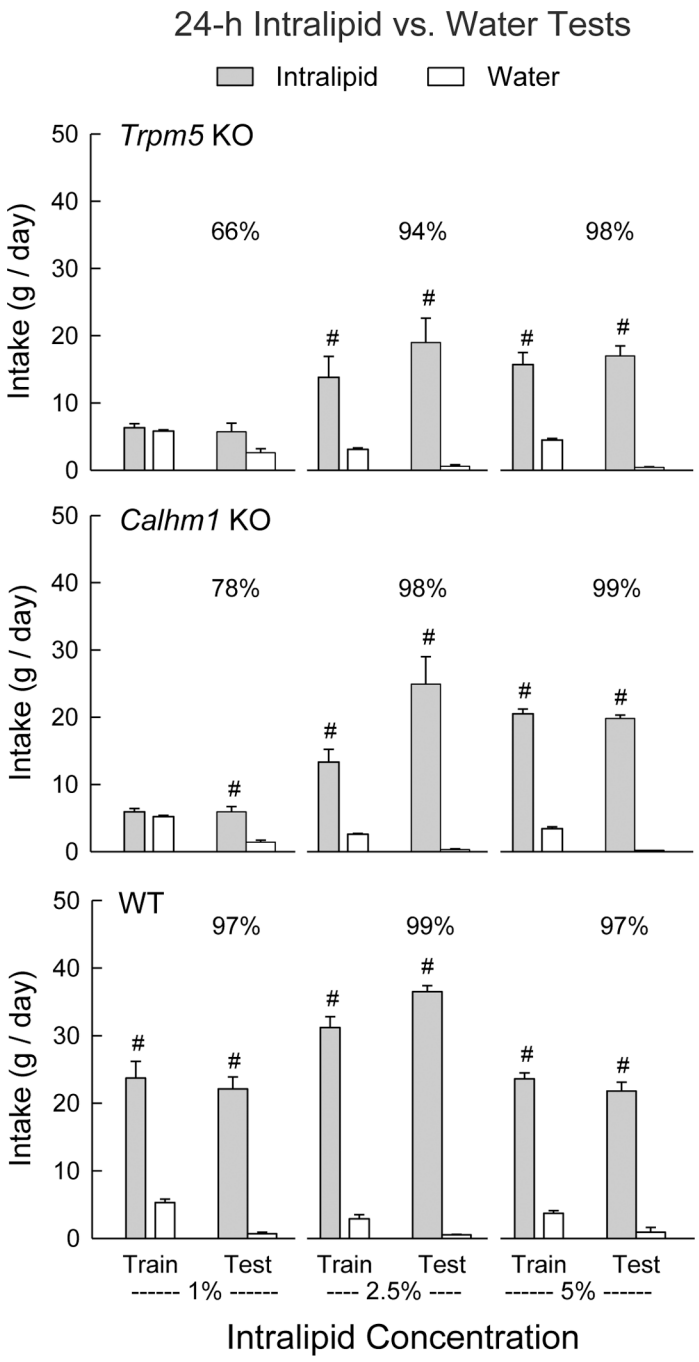

Fig. 2.

Experiment 2. Intake (+ SE) by *Trpm5* KO (top), *Calhm1* KO (middle) and WT (bottom) mice of 1%, 2.5% and 5% Intralipid and water during one-bottle training (Train, narrow bars) and two-bottle tests (wide bars). # Concentrations at which Intralipid intake significantly ($P < 0.05$) exceeded water intake within each group.

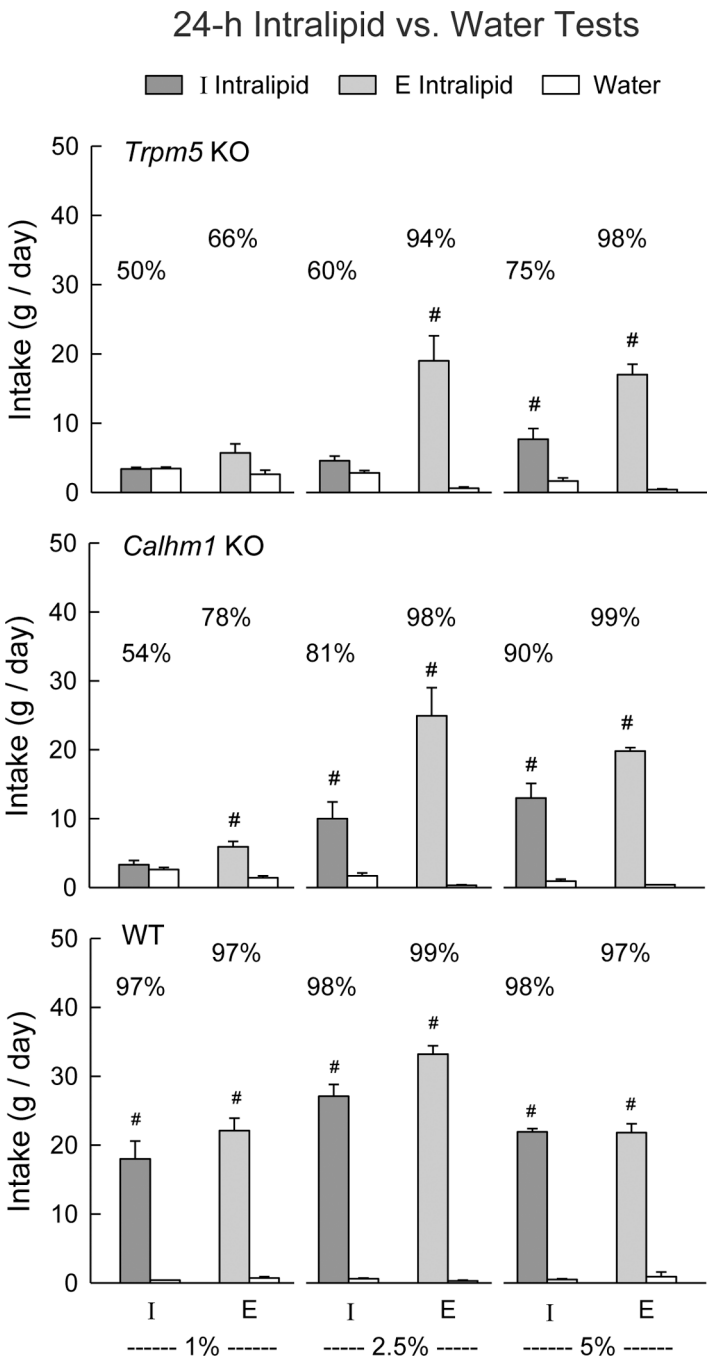

Fig. 3.

Experiment 2. Intake (+ SE) by *Trpm5* KO (top), *Calhm1* KO (middle) and WT (bottom) mice of 1%, 2.5% and 5% Intralipid and water during two-bottle tests of mice inexperienced (I) with one-bottle training and mice experienced (E) with one-bottle training. Data for inexperienced *Trpm5* KO mice are from Experiment 1. Data for inexperienced *Calhm1* KO mice are from [32] # Concentrations at which Intralipid intake significantly ($P < 0.05$) exceeded water intake within each group.