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Profound differences in fat versus carbohydrate preferences in CAST/EiJ and C57BL/6J mice:

Role of fat taste

Anthony Sclafani, Austin S. Vural, and Karen Ackroff

Department of Psychology, Brooklyn College of the City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210 USA

Abstract

In a nutrient self-selection study, CAST/EiJ mice consumed more carbohydrate than fat while C57BL/6J (B6) mice showed the opposite preference. The present study revealed similar strain differences in preferences for isocaloric fat (Intralipid) and carbohydrate (sucrose, maltodextrin) solutions in chow-fed mice. In initial 2-day choice tests, percent fat intakes of CAST and B6 mice were 4–9% and 71–81% respectively. In subsequent nutrient vs. water tests, CAST mice consumed considerably less fat but not carbohydrate compared to B6 mice. Orosensory rather than postoral factors are implicated in the very low fat preference and intake of CAST mice. This is supported by results of a choice test with Intralipid mixed with non-nutritive sweeteners vs. non-sweet maltodextrin. The preference of CAST mice for sweetened fat exceeded that of B6 mice (94 vs. 74%) and absolute fat intakes were similar in the two strains. When given unsweetened Intralipid vs. water tests at ascending fat concentrations CAST mice displayed reduced fat preferences at 0.1 – 5% and reduced intakes at 0.5 – 5% concentrations, compared to B6 mice. The differential fat preferences of CAST and C57BL/6 mice may reflect differences in fat taste sensing or in central neural processes related to fat selection.

Keywords

Sucrose; Maltodextrin; Intralipid; Sucralose; Saccharin; Strain differences

1. Introduction

The intake of foods rich in fat and/or sugar are thought to contribute to the prevalence of human obesity [4]. It is well documented that such foods promote overeating and obesity in rodents, and high-fat and high-sugar diet-induced obese animal models have been the subject of considerable research [8,21]. The attractive sweet taste of sugar is one factor that stimulates the overconsumption of sugar-rich foods [21]. High-fat foods also have an

Correspondence to be sent to: Anthony Sclafani, Department of Psychology, Brooklyn College of CUNY, 2900 Bedford Avenue, Brooklyn, NY 11210. AnthonyS@brooklyn.cuny.edu.

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attractive flavor and recent studies indicate that, in rodents at least, fat taste receptors contribute to the palatability of fat-rich foods [5]. Rodent studies further indicate that sugar and fat have postoral actions that stimulate the intake and preference for foods rich in these nutrients through an appetition process, which is distinct from postoral satiation that suppresses food intake [25,26].

Studies of inbred mouse strains demonstrate the importance of genetic factors in nutrient preferences and diet-induced obesity [2,6,15]. In particular, some strains display weak preferences for sugars and non-nutritive sweeteners, particularly at low concentrations, while other strains show strong preferences [3,13,18,23]. These differences in sweet “subsensitive” and “sensitive” mouse strains are attributed in part to genetic variations in the T1R3 component of the T1R2 + T1R3 sweet taste receptor [20]. Significant inbred strain differences in fat preferences have also been reported in experiments investigating two-bottle choice tests with vegetable oil emulsions (soybean oil, corn oil) [2,14,24,35]. In principle, these differences may be related to genetic variations in fat taste receptors but this remains to be determined. Interestingly, some mouse strains that are subsensitive to sugar taste also show relatively weak preferences for fat (e.g., 129, A/J) while other sweet subsensitive strains display strong fat preferences (e.g., BALB/cJ, AKR/J) [13,14].

One inbred strain not included in these strain comparisons of sugar and fat flavor preferences is the CAST/EiJ strain. This strain is of interest because in a study of differences in macronutrient diet selection, CAST/EiJ mice showed the lowest preference for the fat source (vegetable shortening) and the highest preference for the carbohydrate source (starch + sucrose) (Smith et al. 2000). Among the strains showing the strongest fat preferences was the C57BL/6J strain which notably also shows strong preferences for sugar and fat in nutrient vs. water choice tests [13,14,24]. Genetic and behavioral tests indicate that CAST/EiJ and B6 are both sweet sensitive strains so sugar taste sensitivity does not readily account for their differential preferences for fat and carbohydrate foods [20]. A recent study [17] reported that CAST mice display weaker preferences for starch-derived maltodextrins than do B6 mice, which also does not explain why CAST mice consume more carbohydrate than B6 mice. Instead, strain differences in the preference for fat taste (or flavor) and/or in the postoral satiating or appetite stimulating effects of fat may be an important factor. The preference for fat emulsions has been extensively studied in B6 mice, but there are no published data on the CAST strain [2,7,14]. The present study, therefore, compared the preferences of CAST and B6 mice for fat vs. sugar, vs. maltodextrin, and vs. water in 2-day choice tests.

2. Experiment 1: Fat vs. Sugar Preferences

This experiment measured fat and sugar preferences using soybean oil emulsions (Intralipid) and sucrose solutions that have been extensively used in prior studies [13,14,24]. We first compared fat vs. sugar in a direct choice test followed by fat vs. water and sugar vs. water tests. Finally, a second fat vs. sugar test was conducted to determine how prior experience with the two nutrients affects their relative preference. Isocaloric concentrations of 3.2% Intralipid and 8% sucrose were used because these concentrations produce peak or near-peak volume intakes in 24-h tests [29].

2.1. Methods

2.1.1. Subjects—Male CAST/EiJ (n=10) and C57BL/6J mice (n=10) were purchased from Jackson Laboratories (Bar Harbor, ME). The mice were 9 weeks old at the start of the experiment and CAST mice weighed significantly less than B6 mice (15.1 vs. 23.5 g, $t(18) = 17.1$, $P < 0.001$), which is characteristic of these strains. The mice 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 chow (5001, PMI Nutrition International, Brentwood, MO) and water except where noted. 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—A 3.2% fat emulsion was prepared by diluting 20% Intralipid (Baxter, Deerfield, IL), a stable soybean oil emulsion, with deionized water. An isocaloric 8% sucrose solution was prepared using food-grade sugar (Domino Foods, Yonkers, NY) and deionized water. The test solutions or water were presented in two 50-ml plastic tubes with stainless steel sipper spouts with a 1.5-mm opening designed for mice (Ancare, Bellmore, NY). The tubes were placed on the tub cage tops to the right of the 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. Intakes were corrected for fluid spillage as estimated by recording the daily weight changes of two drinking tubes placed on an empty cage.

2.1.3. Procedure—The mice were adapted to two bottles of water for 6 days in their home cages. They then were given a series of 2-day, two-bottle choice tests as follows: Test 1 (days 1–2) Intralipid vs. sucrose; Test 2 (days 3–4) Intralipid vs. water, Test 3 (days 5–6) sucrose vs. water, and Test 4 (days 7–8) Intralipid vs. sucrose. The left-right position of the tastants and/or water bottles were switched from the first to second day of each test.

Daily fluid intakes were averaged over the 2 days of each test and nutrient preferences were expressed as percent solution intakes (e.g., Intralipid intake/total intake $\times 100$). Intakes were analyzed using a mixed model analysis of variance (ANOVA) with test and solution as repeated factors. One ANOVA included results from Tests 1 and 4 and determined if the relative intakes of the two sugars changed across the two tests. A second ANOVA included results from Tests 2 and 3 and compared the preference for each sugar over water. Additional ANOVAs are described below. Percent nutrient preferences were analyzed with *t*-tests.

2.2. Results

The CAST and B6 mice differed substantially in their preference for fat vs. sugar. In Test 1, CAST mice consumed sucrose almost exclusively while B6 mice consumed significantly more Intralipid than sucrose [Group $\times$ Nutrient interaction, $F(1,18) = 117.3$, $P < 0.001$] (Fig. 1). The percent fat intakes of CAST and B6 groups were 4% and 71%, respectively ($t(18) = 13.8$, $P < 0.001$); CAST mice displayed low fat preferences on both days 1 and 2 of the test (5%, 3%). In Test 4, CAST mice continued to consume very little fat while B6 mice now consumed less fat than sucrose [$F(1,18) = 37.3$, $P < 0.001$]. Nevertheless, the percent fat

intake of CAST mice remained below that of B6 mice (6% vs. 36%, $t(18) = P < 0.001$). Overall, B6 mice consumed more total nutrient than CAST mice in Test 1 [$F(1,18) = 40.1$, $P < 0.001$] but total nutrient intakes did not differ in Test 4. Within group comparisons indicated that CAST mice increased their sucrose intake from Test 1 to 4 while their fat intake did not change [$F(1,9) = 23.7$, $P < 0.01$]. The B6 mice increased their sucrose intake but decreased their fat intake from the first to last test [Test $\times$ Nutrient interaction, $F(1,9) = 50.6$, $P < 0.001$]. In Test 2, both groups consumed more fat than water but B6 mice consumed three times more ($P < 0.001$) fat than did CAST mice [$F(1,18) = 117.3$, $P < 0.001$]. The percent fat intake of CAST mice was also less than that of B6 mice (84% vs. 99%), but this difference was not significant. (One CAST mouse consumed more water than fat in Test 2; the remaining CAST mice had a mean fat preference of 93%.) In Test 3, the groups consumed more sucrose than water and CAST mice consumed more ($P < 0.001$) sugar than did B6 mice [$F(1,18) = 13.1$, $P < 0.01$]. Within group comparisons revealed that CAST mice consumed more ($P < 0.001$) sucrose than fat in Tests 2 and 3 while B6 mice consumed similar amounts of the two nutrients.

2.3. Discussion

Consistent with the early report [34] that CAST and B6 mice self-selected low and high-fat diets, respectively, CAST and B6 mice displayed low and high preferences for fat over sugar in Test 1. In addition, CAST mice consumed less fat but more sugar than did B6 mice in Tests 2 and 3, respectively.

Postoral factors do not readily account for the very low fat preference of CAST mice in Test 1 because the mice consumed so little fat (< 0.03 g/day of nutrient) that postoral effects were minimal. Nevertheless, CAST mice showed a strong fat preference when given the choice between Intralipid vs. water in Test 2. However, their Intralipid intake was low and did not significantly exceed their pre-test water baseline intake (5.9 vs. 4.9 g/day) whereas B6 mice consumed substantially more Intralipid in Test 2 than their baseline water intake (20.1 vs. 5.0, $P < 0.001$). The low Intralipid intake of CAST mice in Test 2 may reflect their weak attraction to the flavor of the Intralipid. However, it may also reflect a strong postoral satiating action of the fat and/or a weak postoral appetite-stimulating action of the nutrient. Note that the low Intralipid emulsion intake of CAST mice in Test 2 cannot be attributed to their low body weights relative to B6 mice. In Test 3, CAST mice consumed more sugar solution than did B6 mice (24.4 vs. 19.9 g/day) despite their reduced body size. When intakes are expressed on a body weight basis, CAST mice consumed twice as much sugar as did B6 mice (32.4 vs. 16.9 g/20 g bw/day).

The CAST mice maintained their low preference for fat relative to sucrose in Test 4 whereas B6 mice significantly altered their nutrient preference from the first to last test. That is, while they consumed more fat than sugar in Test 1, they consumed more sugar than fat in Test 4. This preference change was likely related to their separate experience with fat and sugar in Tests 2 and 3 that allowed them to associate the flavor of each nutrient with its postoral nutritive consequences. In an early study we observed that intragastric (IG) infusions of isocaloric Intralipid and sucrose conditioned equally strong preferences (96–98%) for an arbitrary cue flavor (conditioned stimulus) in B6 mice, indicating that the two

nutrients have similar postoral reinforcing actions. However, we did not directly compare the fat-and sugar-conditioned preferences in a direct test. The finding that CAST mice did not alter their nutrient preference after separate experience with fat and sugar in Tests 2 and 3 suggests that the postoral actions of fat did not enhance fat preference in this strain. Alternatively, CAST mice may not have consumed a sufficient amount of fat in Test 2 to experience its postoral appetite-stimulating effects.

The present findings are similar to results of a preliminary study in which naive CAST and B6 mice were given the choice between isocaloric 6.4% Intralipid vs. 16% sucrose (see Supplementary Fig. S1). Male and female mice were included in this study and there were no sex differences in nutrient preferences.

3. Experiment 1B 2: Fat vs. non-nutritive sweetener preferences

The first experiment suggests that CAST mice are more strongly attracted to the flavor of sucrose than that of Intralipid. Furthermore, their low preference for fat remained unchanged after separate experience with Intralipid and sucrose in Tests 2 and 3. This may have occurred because fat has little postoral appetite stimulating action in CAST mice or because they consumed insufficient amounts of the nutrient, particularly compared to their high sugar intakes. Experiment 1B compared the preferences of CAST and B6 mice for fat vs. a non-nutritive sweetener that has an attractive flavor but no postoral appetition actions. The sweetener was a mixture of sucralose and saccharin, which prior work indicates is highly attractive to both CAST and B6 mice [30].

3.1. Method

The mice from the first experiment were given 2 days of water only following Test 4. They were then given a series of 2-day choice tests with 3.2% Intralipid and a mixture of 0.1% sucralose (Tate & Lyle) and 0.1% saccharin (Sigma-Aldrich, St. Louis, MO) referred to as SS. In Tests 1 and 4 they had the choice of Intralipid vs. SS, while in Tests 2 and 3 the choices were between Intralipid vs. water and SS vs. water, respectively.

3.2. Results and discussion

In Test 1, CAST mice consumed considerably more SS than Intralipid while B6 mice consumed significantly more fat than SS [Group $\times$ Nutrient interaction, $F(1,18) = 79.5$, $P < 0.001$]; the percent fat intakes of CAST and B6 groups were 9% and 63%, respectively ($t(18) = 10.4$, $P < 0.001$) (Fig. 2). Both groups then increased their fat intake and decreased their SS intake in Test 4 and now CAST mice consumed similar amounts of the two tastants while B6 mice consumed substantially ($P < 0.001$) more Intralipid than SS [Group $\times$ Tasant interaction, $F(1,18) = 54.0$, $P < 0.001$]; the percent fat intakes of CAST and B6 groups were 44% and 85%, respectively ($t(18) = 6.8$, $P < 0.001$). In Tests 2 and 3, both groups consumed much more ($P < 0.001$) Intralipid and SS than water, respectively, but CAST mice consumed less fat and more sweetener than did B6 mice [Group $\times$ Tasant interaction, $F(1,18) = 79.6$, $P < 0.001$]. In addition, CAST mice consumed more ($P < 0.05$) SS than Intralipid whereas B6 mice consumed more ($P < 0.001$) Intralipid than SS in these tests.

The CAST and B6 mice displayed similar preference profiles in Test 1 as they did in the first experiment. The CAST mice strongly preferred the non-nutritive sweetener to fat while B6 mice preferred fat to sweetener. However, after separate experience with Intralipid and SS in Tests 2 and 3, CAST mice no longer preferred SS to fat while B6 mice strongly preferred fat to SS. The increased in fat preference from Test 1 to 4 displayed by both strains can be attributed to the mice learning in Test 3 that the sweet taste of SS, unlike that of sucrose, is not associated with postoral reinforcement. In the case of CAST mice, the findings also imply that Intralipid had a postoral reinforcement effect; otherwise, there would be no reason for them to modify their preference from Test 1 to 4. Nevertheless, postoral fat appetite was not sufficient to induce CAST mice to prefer Intralipid to SS in Test 4.

4. Experiment 2A: Fat vs. maltodextrin preferences

In the first experiment CAST mice, unlike B6 mice, displayed overwhelming preferences for sugar over fat. However, this may not be the case for all carbohydrates. As noted in the Introduction, CAST mice show weaker preferences for starch-derived maltodextrin than do B6 mice [17]. Maltodextrin has a palatable, but non-sweet taste [16] mediated by taste receptors distinct from sweet receptors [36,37]. Given their weak preference for maltodextrin, CAST mice may prefer fat to this carbohydrate.

4.1. Method

Naïve male CAST/EiJ ($n=9$) and C57BL/6J mice ($n=10$) from Jackson Laboratories were housed as in experiment 1. The CAST mice weighed significantly less than B6 mice (14.5 vs. 24.5g, $p < 0.001$). Two-bottle preferences tests were conducted like those of the first experiment except with 3.2% Intralipid vs. 8% maltodextrin. The maltodextrin used was SolCarb (Solace Nutrition, Pawcatuck, CT) which is very similar in glucose polymer composition to Polycose, a maltodextrin extensively used in prior studies with B6 mice [1,2,23,37].

4.2. Results and discussion

In Test 1, CAST mice consumed substantially more maltodextrin than fat whereas B6 mice consumed significantly more fat than maltodextrin (Group $\times$ Nutrient interaction, $F(1,17) = 205.4$, $P < 0.001$) (Fig. 3). The CAST and B6 mice had percent fat intakes of 10% and 83%, respectively ($t(17) = 16.7$, $P < 0.001$); fat preferences of CAST mice were low on both days of the test (11%, 10%). In Test 4, CAST mice continued to consume relatively little fat, whereas B6 mice consumed slightly less fat than maltodextrin, although the Group $\times$ Nutrient interaction was not significant. Nevertheless, the percent fat intake of CAST mice was less than that of B6 mice in Test 4 (24% vs. 47%, $t(17) = 2.3$, $P < 0.05$). Overall, CAST mice consumed less total nutrients in Test 1 and 4 than did B6 mice ($F(1,17) = 62.4$, $P < 0.001$). Within group comparisons indicated that CAST mice consumed less fat than maltodextrin in both tests [$F(1,8) = 99.3$, $P < 0.001$] although they increased their fat intake slightly from Test 1 to 4. The B6 mice, in contrast, decreased their fat intake and increased their maltodextrin intake from the first to last test [Nutrient $\times$ Test interaction, $F(1,9) = 24.1$, $P < 0.001$]. In Test 2, both groups consumed more fat than water [$F(1,17) = 673.8$, $P < 0.001$] and had similar percent fat intakes, but B6 mice consumed three times more ($P <$

0.001) fat than did CAST mice [Group $\times$ Nutrient interaction, $F(1,17) = 175.0$, $P < 0.001$]. In Test 3, the groups consumed more maltodextrin than water [$F(1,17) = 238.8$, $P < 0.001$] and did not significantly differ in their maltodextrin intakes. Within group comparisons revealed that CAST mice consumed more ($P < 0.001$) maltodextrin than fat in Tests 2 and 3 while B6 mice consumed more ($P < 0.001$) fat than maltodextrin in these tests.

Despite prior findings that CAST mice are less attracted to maltodextrin than are B6 mice [17], the fat vs. carbohydrate preference profile obtained in this experiment was similar to that of the first experiment. The CAST mice strongly preferred maltodextrin to fat in both Tests 1 and 4 although the percent fat intakes (10% and 24%) were higher than those obtained with fat vs. sucrose in the first experiment (4% and 6%). The B6 mice, in contrast strongly preferred fat to maltodextrin in Test 1 but consumed slightly more maltodextrin than fat in Test 4. As in the first experiment, CAST mice consumed substantially less fat in Test 2 than did B6 mice. Unlike experiment 1 in which CAST mice consumed more sucrose than B6 mice in Test 3, the two groups did not differ in their maltodextrin intakes in Test 3. The present findings indicate that while CAST mice have a reduced attraction to maltodextrin than do B6 mice, this deficit is not nearly as great as their reduced attraction to fat.

5. Experiment 2B: Preference for sweetened-fat vs. maltodextrin

In the preceding experiments CAST mice displayed minimal preferences for Intralipid when offered an alternative tastant (Test 1) and also consumed considerably less fat than B6 mice when offered water as the alternative choice (Test 2). It appears likely that a reduced attraction to the flavor of fat rather than postoral factors are primarily responsible for the reduced fat preference and acceptance of CAST mice. If this is correct, then enhancing the palatability of Intralipid should increase the fat preference and acceptance of CAST mice. This possibility was investigated in the present experiment by giving the mice the choice of Intralipid sweetened with SS vs. non-sweet maltodextrin.

5.1. Method

The mice from experiment 2B were given 5 days of water only following Test 4. They were then given a series of 2-day choice tests with 3.2% Intralipid containing 0.1% sucralose + 0.1% saccharin (IL+SS) vs. 8% maltodextrin. The two nutrients were presented in Tests 1 and 4, while in Tests 2 and 3 the choices were between IL+SS vs. water and maltodextrin vs. water, respectively.

5.2. Results and discussion

In Test 1, both groups consumed more IL+SS than maltodextrin ($F(1,17) = 75.8$, $P < 0.001$) (Fig. 4). Although the difference was greater in CAST than B6 mice, the Group $\times$ Nutrient interaction was not significant. However, the percent fat intake of CAST mice exceeded that of B6 mice (94% vs. 74%, $t(17) = 3.3$, $P < 0.01$). The IL+SS intakes of CAST and B6 mice did not differ. In Test 4, CAST mice again consumed substantially more IL+SS but now B6 mice consumed more maltodextrin than IL+SS (Group $\times$ Nutrient interaction, $F(1,17) = 54.4$, $P < 0.001$). The percent fat intake of CAST mice exceeded that of B6 mice (74% vs.

30%, $t(17) = 9.1$, $P < 0.001$). Within group analyses indicated that CAST mice did not alter their nutrient choices and intakes from Test 1 to 4, whereas B6 mice decreased their IL+SS and increased the maltodextrin intakes from the first to last test (Test $\times$ Nutrient interaction, $F(1,9) = 39.9$, $P < 0.001$). In Test 2, both groups consumed substantially more IL+SS than water ($F(1,17) = 484.0$, $P < 0.001$) and CAST mice consumed as much sweetened fat as did B6 mice (or significantly more ($P < 0.001$) on a body weight basis; 26.0 vs. 13.9 g/day/20 g bw). In Test 3, both groups consumed more maltodextrin than water ($F(1,17) = 272.5$, $P < 0.001$), but CAST mice consumed less than half as much maltodextrin as did B6 mice (Group $\times$ Nutrient interaction, $F(1,7) = 44.3$, $P < 0.001$). Within groups comparisons indicated that CAST mice consumed significantly more IL+SS in Test 2 than maltodextrin in Test 3 (Nutrient $\times$ Test interaction, $F(1,8) = 75.5$, $P < 0.001$), whereas the nutrient intakes of B6 mice in the two tests were similar.

These findings demonstrate that enhancing the flavor of Intralipid with the addition of nonnutritive sweeteners reversed the relative preference for fat and maltodextrin displayed by CAST in the previous experiment. Furthermore, it stimulated the absolute intake of fat to a level comparable to that of B6 mice. Clearly, postnatal factors do not limit the ability of CAST mice to consume as much fat as B6 mice. Rather, it appears that their reduced attraction to the flavor of fat is responsible for their reduced preference and intake of this nutrient. The present results also revealed a suppressed attraction in CAST mice to maltodextrin that was not apparent in Experiment 2A. That is, not only did CAST mice consume almost no maltodextrin in the choice tests with fat, they also consumed substantially less maltodextrin than did B6 mice in the choice test vs. water (Test 3). This contrasts with their elevated sucrose intake, relative to B6 mice, in Test 3 of the first experiment. Surprisingly, the addition of SS to Intralipid did not appear to enhance the preference of B6 mice for the fat; the percent IL+SS preferences in Tests 1 and 4 were lower than the percent Intralipid preferences in Experiment 2A. However, the experience of B6 mice in Experiment 2A may have influenced their nutrient choices in Experiment 2B. Intralipid and IL+SS preferences need to be evaluated in naïve B6 mice.

In early studies we presumed that maltodextrin taste facilitated the identification of starch-rich foods [21,22]. However, subsequent studies indicated that rodents have separate tastes for maltodextrins and starches [19,31]. In view of the reduced maltodextrin intake displayed by CAST mice in Test 2 and the Poole et al. study [17], we compared the preference and intake of starch in CAST and B6 mice. As shown in Supplementary Figure S2, CAST mice consumed substantially less 4% and 8% starch than did B6 mice in 2-day choice tests vs. water; CAST mice also displayed a reduced preference for 4% starch compared to B6 mice. However, when given the choice of 8% starch sweetened with SS vs. water, CAST and B6 mice consumed similar amounts of starch. Thus, CAST mice are capable of consuming as much starch as B6 mice, but they are less attracted to the taste (flavor) of unsweetened starch compared to B6 mice.

6. Experiment 3: Fat preference as a function of concentration

In the fat vs. water tests of the first three experiments CAST mice consumed significantly less of the unsweetened fat choice than did B6 mice but the groups did not differ in their

percent fat preferences. We recently observed a similar discrepancy between fat acceptance and preference in B6 mice missing the putative fatty acid taste receptor CD36 [27]. That is, the CD36 knockout (KO) mice displayed similar preferences for 2.5 and 5% Intralipid as did B6 wildtype (WT) mice but consumed significantly less Intralipid than WT mice. At lower Intralipid concentrations, however, the KO mice displayed both reduced fat preferences and intakes relative to WT mice. To more fully compare the fat preference and acceptance of CAST and B6 mice, the present experiment conducted Intralipid vs. water choice tests with the same Intralipid concentrations used in the CD36 KO study [27]. The test series was repeated a second time because prior findings indicate that fat preference deficits disappear in KO mice with repeated testing [27].

6.1. Methods

Male CAST/EiJ ($n=10$) and C57BL/6J mice ($n=10$) from Jackson Laboratories were housed as in the first experiment. The mice were 9 weeks old at the start of the experiment and CAST mice weighed less than B6 mice (14.2 vs. 22.6, $t(18) = 16.8$, $p < 0.001$). The mice were adapted to drinking water from two bottles for one week. They were then given a series (Test 1) of 2-day choice tests with Intralipid vs. water at the following ascending fat concentrations: 0.1%, 0.25%, 0.5%, 0.75%, 1%, 2.5%, and 5%. The mice were then given water only for four days and then a second ascending series (Test 2) of Intralipid vs. water tests was conducted.

6.1.1. Results and Discussion—In Test 1, CAST mice consumed less Intralipid than B6 mice at 0.5 to 5% concentrations (Group $\times$ Concentration interaction, $F(6,108) = 45.4$, $P < 0.001$) (Fig 5). Overall, Intralipid emulsion intake increased and then decreased with concentration ($F(6,108) = 262.8$, $P < 0.001$) but when expressed as energy, fat intake simply increased with concentration (data not presented). With respect to percent fat intake, CAST mice had lower Intralipid preferences than did B6 mice at 0.1 to 1% concentrations (Group $\times$ Concentration interaction ($F(6,114) = 9.3$, $P < 0.001$). Within group analyses indicated that CAST mice consumed more ($P < 0.05$) Intralipid than water at 0.5% and higher concentrations, while B6 mice consumed significantly more ($P < 0.05$) at 0.25% and higher concentrations.

In Test 2, CAST mice again consumed less Intralipid than B6 mice but this time at 0.25 to 2.5% concentrations (Group $\times$ Concentration interaction, $F(6,108) = 21.3$, $P < 0.001$) (Fig. 5). Both groups now displayed strong ($>80\%$) preferences although the percent fat intakes of CAST mice were below those of B6 mice at 0.1 – 1% concentrations (Group $\times$ Concentration interaction, $F(6,108) = 10.4$, $P < 0.001$). Within group comparisons indicated that CAST mice increased their percent Intralipid intakes from Tests 1 to 2 at concentrations 0.1 – 1% (Test $\times$ Concentration, $F(6,60) = 20.7$, $P < 0.001$) and B6 mice increased their percent intakes at 0.1 and 0.5% concentrations (Test $\times$ Concentration, $F(6,54) = 22.1$, $P < 0.001$).

The results of the first test series confirm and extend the findings obtained with the 3.2% Intralipid vs. water tests of the previous experiments. That is, CAST and B6 mice did not differ in their preferences for 2.5 – 5% Intralipid, although CAST mice consumed less of

these emulsions than did B6 mice. However, with more dilute concentrations, CAST mice displayed lower fat percent as well as absolute intakes compared to B6 mice. In the second test series, the fat preferences of CAST mice were significantly enhanced although their percent intakes remained below those of B6 mice at the 0.1 – 1% concentrations. The differences in absolute fat intake in Test 2 were even greater between CAST and B6 mice at intermediate concentrations of 0.25 – 1% although the two strains consumed equivalent amounts of 5% Intralipid.

7. General Discussion

The present study revealed profound differences in the fat vs. carbohydrate preferences of CAST and B6 mice. Naïve CAST mice selected only 4% and 10% fat, respectively, when given the choice of soybean oil vs. sucrose or maltodextrin. In contrast, naïve B6 mice selected 71% and 83% fat, respectively. These results are consistent with a prior report that CAST and B6 mice displayed low and high fat preferences, respectively, when fed separate sources of fat, carbohydrate and protein. In choice tests with each nutrient vs. water, the two strains did not differ in their percent fat or carbohydrate intakes although CAST mice consumed significantly less fat than did B6 mice in these tests. In addition, CAST mice consumed less fat than B6 mice in tests with ascending concentrations of Intralipid vs. water and also displayed reduced preferences for 0.1 – 1% Intralipid. As discussed below, the findings suggest that CAST mice are less attractive to the flavor of fat than are B6 mice.

In Experiment 1 CAST mice displayed a strong preference for sucrose over fat in Test 1 and, after separate experience with the two nutrients in Tests 2 and 3, maintained this sucrose preference in Test 4. The CAST mice also strongly preferred the non-nutritive SS sweetener over fat in Test 1 (Exp. 1A) although in this case they lost this preference in Test 4 after separate experience with fat and non-nutritive sweetener in the intervening tests. This preference change indicates that CAST mice were sensitive to the different postoral consequences of Intralipid and SS. Yet, this sensitivity did not induce CAST mice to strongly prefer the fat to the non-nutritive sweetener in Test 4 as it did with B6 mice. Consistent with these findings, we previously reported that experienced CAST mice strongly preferred non-nutritive SS to nutritive glucose and fructose solutions, even though IG infusion experiments indicated CAST mice were sensitive to the postoral reinforcing actions of the two sugars [30]. It appears that the strong sweet taste attraction of CAST mice overrides postoral nutritive effects. (CAST mice displayed the strongest preference for non-nutritive saccharin in a study of 30 inbred mouse strains [20].)

In contrast to their robust sweet preference, CAST mice have an attenuated preference for maltodextrin taste compared to B6 mice [17]. Yet, in Experiment 2 CAST mice, unlike B6 mice, strongly preferred fat to maltodextrin in both Test 1 and 4. Taken together with other findings [17], the relative preferences of naïve and experienced CAST mice are sucrose > maltodextrin > soybean oil. In contrast, the relative nutrient preferences of naïve B6 mice are soybean oil > sucrose > maltodextrin although this profile changes after experience with the nutrients [1]. The position of starch in these preference profiles remains to be established although our supplementary data show that CAST mice have a weaker attraction to starch than do B6 mice.

The very low Test 1 Intralipid intakes of CAST mice in Experiments 1A and 2A indicated that orosensory rather than postoral factors determined the strong carbohydrate preferences observed in these tests. Consistent with this interpretation, CAST mice strongly preferred Intralipid to maltodextrin in Experiment 2B when the fat emulsion was sweetened with SS. Furthermore, CAST mice consumed as much sweetened fat (or more on a body weight basis) as did B6 mice in Test 2 which indicates that postoral factors did not constrain the fat intake of CAST mice. A reduced attraction to the flavor of fat, therefore, could account for the low fat preferences observed in the first two experiments. The findings obtained with sweetened Intralipid emulsion suggest that the low fat preference displayed by CAST mice in a prior macronutrient self-selection experiment [34] might be reversed if the animals are given access to sweet fat (vegetable shortening + SS), non-sweet carbohydrate (starch, maltodextrin), and protein (casein) sources.

Experiment 3 provides additional evidence that CAST and B6 mice differ in their preference for fat flavor. Compared to B6 mice, CAST mice displayed low intakes and preferences for dilute Intralipid concentrations that have minimal postoral nutritive effects. Only concentrated (2.5 – 5%) fat emulsions stimulated intakes above water baselines and produced strong preferences in CAST mice. We recently reported similar fat intake and preference deficits in naive B6 mice missing the putative fatty acid taste receptor CD36 [27], which suggests that the weak fat preferences of CAST mice may be due to a subsensitive fatty acid taste receptor. After experience with the various Intralipid concentrations in Test 1, CAST mice, like B6 mice, displayed strong preferences for all concentrations in Test 2 although CAST mice consumed less Intralipid than did B6 mice at most concentrations. Interestingly, CD36 KO mice show a similar enhancement in Intralipid preference but not intake when given a second series of Intralipid vs. water tests [27]. KO mice with global taste deficits (TRPM5, CALHM1, P2X2/P2X3) also show significant experience-induced increases in Intralipid preferences, which can be attributed to conditioning by the postoral reinforcing actions of fat [27,31].

The similar fat preference deficits displayed by CAST mice and CD36 KO mice suggest that CAST mice have a subsensitive or insensitive CD36 taste receptor. This is not the only possible explanation of the present results, however. We recently reported that CALHM1 KO mice show greater deficits in fat preference than do CD36 KO mice, which suggests that CD36 is not the only taste receptor that mediates the attraction to fat [27]; see also [28]. Thus, CAST and B6 mice may differ in the sensitivity of taste receptors other than CD36. Alternatively, CAST and B6 mice may differ in central neural processes that influence fat preferences and intakes. Of particular relevance to the present findings, Laque et al. [12] recently reported that KO mice missing leptin receptors on hypothalamic galanin-expressing neurons strongly preferred sucrose to Intralipid in 24-h choice tests whereas wildtype mice equally preferred the two nutrients. The KO mice also consumed less Intralipid than WT mice in short-term acceptance tests. Since galanin and leptin receptors are found in some lingual taste receptors, the possibility that taste signaling is disrupted in KO mice needs to be considered [9,32]. The genetic basis for the differential fat and carbohydrate preferences of CAST and B6 has been investigated in congenic mice derived from interbreeding the two strains [10,11,33]. These studies implicated a segment (*Mnic1*) on chromosome 17 as

containing genes that influence macronutrient selection. In view of present findings, the role of these genes in fat flavor preference is worthy of investigation.

Numerous studies have investigated inbred strain differences in sugar and fat preferences [2,3,7,13,14,23,24,35]. One study that directly assessed fat vs. sugar preferences reported that SWR/J mice strongly preferred 8% sucrose to 20% corn oil while AKR/J mice preferred corn oil to sucrose in a 4-day, two-bottle test [35]. This resembles the fat vs. sugar differences observed with CAST and B6 mice in the present study. Note, though, that SWR and AKR displayed similar fat preferences in fat vs. water tests with ascending corn oil concentrations [35]. In contrast, SWR displayed stronger sucrose preferences than AKR mice in sucrose vs. water tests, which is consistent with other findings showing that SWR and AKR strains differ in sweet taste sensitivity [7,13,14,20]. Thus, the sensory basis for the fat vs. sugar differences in SWR and AKR strains appear to differ from that involved in CAST vs. B6 mice. It would be of interest in future studies to directly compare the taste and nutrient preferences in these four strains.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- CAST/EiJ mice strongly prefer fat to isocaloric carbohydrate (sucrose, maltodextrin).
- C57BL/6J mice show the opposite preference profile.
- CAST/EiJ mice show weaker fat preferences in fat vs. water tests compared to C57BL/6J.
- CAST/EiJ mice, like C57BL/6J mice strongly prefer sweetened fat to maltodextrin.
- Taste rather than postoral factors are implicated in the low fat preferences of CAST/EiJ mice.

Figure 1.

Experiment 1A. Mean intakes (+sem) of 3.2% Intralipid, 8% sucrose and water in 2-day choice tests with CAST (left panel) and B6 mice (right panel). (Order of testing is from left to right within each panel.) In Tests 1 and 4 the mice were given the choice of Intralipid vs. sucrose. In Test 2 the choice was Intralipid vs. water and in Test 3 it was sucrose vs. water. Numbers atop bars represent mean percent preference for that solution. Significant ($P < 0.05$) intake differences within the two-bottle tests are indicated by an asterisk (*).

Figure 2.

Experiment 1B. Mean intakes (+sem) of 3.2% Intralipid, 0.1% sucralose + 0.1% saccharin (SS), and water in 2-day choice tests with CAST (left panel) and B6 mice (right panel). (Order of testing is from left to right within each panel.) In Tests 1 and 4 the mice were given the choice of Intralipid vs. SS. In Test 2 the choice was Intralipid vs. water and in Test 3 it was SS vs. water. Numbers atop bars represent mean percent preference for that solution. Significant ($P < 0.05$) intake differences within the two-bottle tests are indicated by an asterisk (*).

Figure 3.

Experiment 2A. Mean intakes (+sem) of 3.2% Intralipid, 8% maltodextrin and water in 2-day choice tests with CAST (left panel) and B6 mice (right panel). (Order of testing is from left to right within each panel.) In Tests 1 and 4 the mice were given the choice of Intralipid vs. maltodextrin. In Test 2 the choice was Intralipid vs. water and in Test 3 it was maltodextrin vs. water. Numbers atop bars represent mean percent preference for that solution. Significant ($P < 0.05$) intake differences within the two-bottle tests are indicated by an asterisk (*).

Figure 4. Experiment 2B. Mean intakes (+sem) of 3.2% Intralipid + 0.1% sucralose + 0.1% saccharin (SS), 8% maltodextrin and water in 2-day choice tests with CAST (left panel) and B6 mice (right panel). (Order of testing is from left to right within each panel.) In Tests 1 and 4 the mice were given the choice of Intralipid + SS vs. maltodextrin. In Test 2 the choice was Intralipid + SS vs. water and in Test 3 it was maltodextrin vs. water. Numbers atop bars represent mean percent preference for that solution. Significant ($P < 0.05$) intake differences within the two-bottle tests are indicated by an asterisk (*).

Figure 5.

Experiment 3. Intake (mean $\pm$ sem) of Intralipid emulsions (top panels) and percent fat preference (bottom panels) of CAST and B6 mice during 2-day, 2-bottle Intralipid emulsion vs. water choice tests. In Test 1 (left), naïve mice were tested with 0.1–5% Intralipid and water. In Test 2 (right), the same mice were retested with Intralipid emulsions and water. An asterisk (*) indicates significantly ($P < 0.05$) higher values in B6 than CAST mice.