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Intragastric fat self-administration is impaired in GPR40/120 double knockout mice

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Abstract

Mice acquire strong preferences for flavors paired with intragastric (IG) fat infusions. This IG fat conditioning is attenuated in double knockout (DoKO) mice missing GPR40 and GPR120 fatty acid receptors. Here we determined if GPR40/120 DoKO mice are also impaired in IG fat self-administration in an operant lick task. In daily 1-h sessions the mice were trained with a sipper spout that contained dry food pellets; licks on the spout triggered infusions of IG fat (Intralipid). The training sessions were followed by test sessions with an empty spout. GPR40/120 DoKO mice self-infused more 20% fat than wild type (WT) C57BL/6 mice in training with a food-baited spout (2.4 vs. 2.0 kcal/h) but self-infused less 20% fat than WT mice in empty spout tests (1.2 vs. 1.7 kcal/h). The DoKO mice also self-infused less 5% fat than WT mice (0.6 vs. 1.3 kcal/h) although both groups emitted more licks for 5% fat than 20% fat. The DoKO and WT mice did not differ, however, in their self-infusion of 12.5% glucose (1.5 vs. 1.6 kcal/h), which is isocaloric to 5% fat. A second 5% IL test showed that the DoKO mice reverted to a reduced self-infusion compared to WT mice. When the infusion was shifted to water, WT mice reduced licking in the first extinction session, whereas DoKO mice were less sensitive to the absence of infused fat. Our results indicate that post-oral GPR40/120 signaling is not required to process IG fat infusions in food-baited spout training sessions but contributes to post-oral fat reinforcement in empty spout tests and flavor conditioning tests.

Keywords

Post-oral nutrient sensing; Intragastric infusions; Operant licking; Glucose

Introduction

The attraction to dietary fat has been intensively studied in laboratory rodents. Various methods have been used to distinguish the contributions of orosensory and post-oral nutritive effects to fat appetite. Several studies demonstrate that rodents are attracted to the

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orosensory properties of fat by using procedures that minimize post-oral exposure. Concentration-dependent licking and/or intakes have been reported in mice given 5-sec tests with Intralipid (soybean oil emulsion) [15], in 10-min tests in mice consuming corn oil [50,51,58] and in rats sham feeding corn oil emulsion [34,47]. Brief tests also showed that fatty acids (oleic, linoleic) are attractive to rats and mice [14,23,58]. In addition, non-nutritive oil emulsions are attractive to rodents [1,28] although their effects are not as potent as those of nutritive oils [10,42].

An important post-oral component to fat appetite was revealed in conditioning studies in which rodents were trained to associate a novel flavored solution with intragastric (IG) or intestinal infusions of a fat emulsion [3]. We first reported in 1989 [26] that rats acquired a preference for a flavor (the CS+) paired with IG corn oil infusions over another flavor (the CS-) paired with IG water infusions; this has been replicated in many subsequent studies [2,27,29,36,55,56]. Mice are particularly responsive to the flavor conditioning actions of IG fat infusions [41,43]. We observed that C57BL/6 (B6) mice increased their licking response to a novel CS+ flavor paired with IG infusions of Intralipid within 15 min in the first session, displayed even greater increases in CS+ licking in subsequent sessions and preferred the CS+ over the CS- flavor in two-choice tests [59]. This post-oral stimulation of intake and flavor preference, a process referred to as *appetition* [45], is affected by fat concentration [4]. B6 mice showed little post-oral fat *appetition* when infused with 1.6% Intralipid, but self-infused increased amounts of fat and displayed stronger CS+ preferences when infused with 3.2%, 6.4%, and 12.8% Intralipid [4].

Oral attraction to fat was long thought to reflect non-gustatory aspects of flavor such as odor and texture cues, and post-oral enhancement of fat intake was presumed to reflect the provision of energy. Recent discoveries of multiple putative fatty acid receptors in the oral cavity and in the gut have led to a detailed analysis of the chemosensory basis for fat appetite. The CD36, GPR120 and GPR40 receptors have received the most attention. CD36 is important in the orosensory but not the post-oral determination of fat preference [24]. This is indicated by the findings that CD36 knockout mice show deficits in their preference for fatty acids or fat emulsions in two-bottle choice tests but a normal flavor conditioning response to IG infusions of Intralipid [24,43].

The situation with GPR40 and GPR120 is the reverse: they appear to be more involved in post-oral rather than oral determination of fat preference [6,46], although early data suggested a role in fatty acid preference [7]. In particular, we reported that knockout (KO) mice with genetic deletion of GPR40, GPR120, or both fatty acid receptors displayed normal preferences for Intralipid over water in choice tests with a wide range of concentrations (0.001 - 20%). However, the double knockout (DoKO) mice missing both receptors were deficient in their *appetition* response to IG Intralipid infusions. That is, the DoKO mice showed much weaker stimulation of intake and preference for a CS+ flavor paired with IG Intralipid infusions than wildtype (WT) control mice [46]. Single KO mice missing only GPR40 or GPR120 had smaller impairments in the post-oral stimulation of intake and preference, suggesting that both GPRs play a role in post-oral detection of fat reward.

Other studies of the oral and post-oral effects on fat appetite have used the conditioned place preference (CPP) procedure, in which fat is paired with a distinctive location [21,22,49,51]. The CPP reward effect has been shown to depend on the post-oral action of nutritive oil in mice. That is, mice learned a preference for a place that was paired with the oral consumption of a nutritive fat (corn oil) but not for a place paired with the consumption of a palatable but non-nutritive fat substitute [32,48]. A CPP was produced, however, by oral intake of the fat substitute accompanied by an IG corn oil infusion [32,48], yet IG corn oil alone, without oral intake, did not support a CPP [48]. Taken together, these findings suggest that both the flavor and post-oral actions of fat are essential for place preference conditioning.

Recent studies by de Araujo and colleagues have used an operant IG self-infusion method that bypasses oral ingestion to investigate the post-oral control of fat appetite [13,53]. In this operant licking method, “dry licks” on an empty sipper spout drive IG infusions of fat (Intralipid). The de Araujo lab reported that following initial training with a “food-baited” sipper spout, mice showed robust licking of an empty spout that was reinforced with IG Intralipid infusions. Furthermore, the dry licking response was modulated by the caloric density of the fat infusion: as Intralipid concentration decreased (from 30 to 15 to 5%) the mice licked more so as to maintain a constant caloric intake. These findings were taken as evidence that orosensory stimulation is not required for the reinforcing actions of IG fat infusions, which contrasts with the results obtained with the place preference conditioning procedure (but see Discussion). de Araujo and colleagues did not investigate the post-oral fat sensing processes that mediated operant licking for IG Intralipid. Rather, they focused on the role of striatal dopamine systems in regulating fat energy intake in the absence of flavor cues [13,53].

In view of our findings implicating GPR40 and GPR120 fatty receptors in post-oral fat appetite, in the present study we investigated the role of these receptors in the dry licking response for IG fat infusions. Toward this end, we compared operant licking for IG Intralipid in GPR40/120 DoKO mice and wild-type B6 mice. Intralipid concentrations of 20% and 5% were used to determine if DoKO mice, like WT mice, show concentration-dependent shifts in their dry licking behavior. In addition, operant licking for IG infusions of 12.5% glucose, which is isocaloric to 5% Intralipid, was measured to determine if DoKO mice show nutrient-specific deficits in dry licking.

Materials and Methods

Animals

Adult male GPR40/120 double knockout (DoKO) mice (n=12) and C57BL/6J wild-type (WT) mice (n=12) were used. The DoKO mice were generated by cross breeding GPR40 and GPR120 mice that had been generated on a C57BL/6J background, as previously described [7]. The genotype status of all DoKO mice was confirmed by real-time PCR of ear biopsies (Transnetyx, Cordova, TN). The mice were 15 wk old, and the DoKO mice weighed more than the WT mice (28.3, 26.2 g, $p < 0.001$). They were singly housed in plastic tub cages kept in a room maintained at 22°C with a 12:12-h light-dark cycle and ad libitum access to chow (LabDiet 5001; PMI Nutrition International, Brentwood, MO) and

water. During testing, they were maintained at 90% of ad libitum body weight by feeding them fixed-size chow pellets (0.5 or 1 g, Bio-Serv, Frenchtown, NJ), which allowed for precise adjustment of daily food rations. 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.

Surgery

Mice were fitted with IG catheters (0.84 mm OD $\times$ 0.36 mm ID, Micro-Renathane tubing, MRE-033; Braintree Scientific, Braintree, MA) while anesthetized with 2% isoflurane inhalation, as previously described [44]. About 10 days after surgery, the mice were briefly (5 min) anesthetized with isoflurane, and tubing was attached to the gastric catheter and then passed through an infusion harness with a spring tether (CIH62; Instech Laboratories, Plymouth Meeting, PA). The tubing and spring were then attached to an infusion swivel mounted on a counterbalanced lever (Instech Laboratories). The body weight of each mouse was measured before and after it was fitted with the infusion tether/swivel system; daily body weights were monitored by weighing the mouse with the attached infusion tether/swivel system. Each animal was then returned to a tub cage, and the swivel's counterbalanced lever was attached above the cage.

Apparatus

IG infusion sessions were conducted in plastic infusion cages (15 $\times$ 15 $\times$ 32 cm) [40]. The mice licked sipper spouts through vertical slots (5 $\times$ 20 mm, 32 mm apart) in a stainless steel plate fixed on the wall of the cage. Licking was monitored by an electronic lickometer (ENV-250B, Med Electronics, St. Albans, VT) connected to a computer, which operated syringe pumps (A-99; Razel Scientific, Stamford, CT). The pump infused liquid at a nominal rate of 0.5 ml/min into the gastric catheters as the animals licked the sipper spout, but the animal controlled the overall infusion rate and volume by its licking pattern. The pump was activated for 3 s (delivering 0.025 ml) when a criterion lick was recorded, as described in the Procedure.

Solutions

The IG infusates were 20% Intralipid (soybean oil emulsion, 2 kcal/ml; Baxter, Deerfield, IL), 5% Intralipid (prepared by diluting the 20% stock with water), and 12.5% glucose (Honeyville Food Products, Rancho Cucamonga, CA). 20% IL delivered 0.051 kcal/3 sec infusion, and 5% IL and 12.5% glucose provided 0.013 kcal/3 sec infusion.

Procedure

For 2 days the mice were given six 45-mg pellets (grain-based rodent tablet 5TUM, TestDiet, St. Louis, MO) in their home cages to accustom them to the food used to bait the sipper tube. On the second of these days the mice were adapted to the infusion cages in a 1-h session with no food or spout present. The following day chow was removed from the home cages and the mice were given restricted food rations each day to maintain them at 90% of their ad libitum body weight. Daily 1-h sessions were conducted with IG infusions

contingent on licking the spout. The first lick of the session was reinforced with a 3-s nutrient infusion; during infusions additional licks were recorded but had no consequence. The next lick after termination of an infusion activated a new 3-s infusion. The mice were trained to lick the dry sipper spout to obtain IG nutrient infusions by placing two 45-mg pellets in the spout. They could contact the first pellet, which protruded slightly from the 1.5 mm hole in the spout tip; the second pellet served as a “weight” to limit movement of the first pellet. Measurements revealed that the animals removed less than 1 mg of food from the pellet during a 1-h session. Food rations were provided in the home cage 1 h after the daily sessions.

The mice were first given 4 Training sessions with a food-baited spout and were reinforced with IG infusions of 20% Intralipid. This was followed by 8 Test sessions with a clean empty spout to determine their operant licking for IG 20% Intralipid infusions in the absence of food stimuli. The same mice were then Trained with a food-baited spout (4 sessions) and Tested (4 sessions) with an empty spout with reinforced licks delivering IG infusions of 5% IL. The mice were then given the same Training and Test schedule but with licks reinforced with an IG infusion of 12.5% glucose. Following the last glucose Test session, the mice were given 6 additional Test sessions with licks reinforced with 5% IL infusions. During this period one DoKO mouse was removed from the experiment because its catheter became unusable. Finally, the infusion was switched to water for 6 sessions to evaluate the extinction of dry licking in the absence of nutrient.

Data analysis

Total licks and IG infusions (equivalent to reinforced licks) were averaged in 3- or 4-session blocks for each test period. The longer 8-day period of 20% IL test sessions thus was divided into two 4-session means, and the final 6-day period of 5% IL testing was divided into two 3-day means. To evaluate these test measures relative to the training period data collected with food-baited spouts, the same measures were averaged for the 4 days of the training periods. The data were evaluated with a mixed model analysis of variance (ANOVA) with a group factor (DoKO, WT) and a repeated measures factor (training vs. test period). To compare across infusates, 4-day mean kcal/h were calculated for training and test periods for the first exposure to each infusate, with the exception of an 8-day mean for the longer 20% IL test period, and analyzed with mixed model ANOVA. To evaluate the behavior and delivery of infusions within sessions, mean cumulative infusion curves were generated for each infusate (last 4 test days) and infusion rates were also expressed as infusions per 10-min bin for each infusate. The infusion bin data for the two groups were analyzed for each infusate with repeated measures ANOVA. Extinction of dry licking was evaluated by repeated measures ANOVA comparing the means of total licks and infusion numbers of the last 3 sessions of 5% IL infusion with the 6 sessions of water infusions. Significant main effects were evaluated with Newman-Keuls tests, and significant interactions with simple main effects tests.

Results

During the 20% IL infusion periods, self-infusion changed differently across training and test periods for the DoKO and WT mice (Group $\times$ Period interaction $F(2,44) = 9.4$, $p < 0.01$) (Fig. 1A). Simple main effects tests showed that when trained to dry lick for 20% IL infusions with a food-baited sipper tube, the DoKO mice self-infused more ($p < 0.05$) lipid than the WT mice, but when tested without food in the spout, DoKO mice infused less lipid than did WT mice; this difference was significant ($p < 0.01$) during the second 4-day test period. Within-group analyses indicated that the number of IG infusions declined (Period $F(2,22) = 24.3$, $p < 0.0001$) from training to testing for the DoKO mice while the WT mice showed a smaller and non-significant decline in self-infusions. The session by session infusion numbers for this and subsequent infusates are plotted in Supplementary Figure 1. The total licks emitted by the mice during the 20% IL Training and Test periods exceeded their reinforced licks (equivalent to the number of IG infusions); the DoKO mice licked more during training and less during testing than did the WT mice (Group $\times$ Period interaction $F(2,44) = 4.66$, $p < 0.05$) (Table 1). The groups differed in their pattern of self-infusion in 20% IL sessions, and the data for Test period 2 are shown in Fig. 1B. The DoKO mice obtained fewer infusions during testing than WT mice (Group $F(1,22) = 7.1$, $p < 0.05$), due to their lower number of infusions during the first 10 min of the sessions (Group $\times$ Bin $F(5,110) = 3.8$, $p < 0.0001$). Infusion rates declined following the first 10 min in both groups ($F(5,110) = 22.3$, $p < 0.0001$) and their infusions did not differ in 10-min bins 2 to 6.

The mice were next trained to dry lick a food-baited spout for 5% IL infusions, followed by a 4-day test period without food in the spout. Overall, the DoKO mice self-infused less 5% IL than did the WT mice (Group $F(1,22) = 24.5$, $p < 0.01$) and their number of infusions declined ($p < 0.05$) from training to testing, while infusion number did not change in the WT mice (Group $\times$ Period interaction $F(1,22) = 4.45$, $p < 0.05$) (Fig. 2A). Total licks during training and testing were also less for the DoKO mice than the WT mice (Group $F(1,22) = 9.3$, $p < 0.01$) (Table 1). The groups differed in their pattern of self-infusions of 5% IL during testing (Fig. 2B). DoKO mice self-infused less 5% IL than did WT mice during 10-min bins 1-4, with the difference being most pronounced in the first bin (Group $\times$ Bin $F(5,110) = 6.1$, $p < 0.0001$). For both groups, there were more infusions in the first 10 min than in the remainder of the hour and more in the second 10-min bin than in bins 3-6 (Bin $F(5,110) = 35.8$, $p < 0.0001$).

The mice were next trained to dry lick for IG infusions of 12.5% glucose. With this nutrient, the DoKO and WT mice did not differ in their IG infusions (Fig. 3A) or total licks (Table 1). For both groups, IG infusions did not change from Training to Testing, but total licks increased significantly (Period $F(1,22) = 47.5$, $p < 0.001$). The DoKO and WT mice also did not differ in their pattern of infusions during testing (Fig. 3B), and self-infused substantially more glucose during the first 10-min bin than in the remainder of the hour (Bin $F(5,110) = 142.0$, $p < 0.0001$).

Further analyses compared the three nutrient infusates within each group, using means of the test periods; these data are plotted in Figure 4. In both DoKO and WT groups, there were more 12.5% glucose infusions than 5% IL infusions, which in turn exceeded the number of

20% IL infusions (Infusate $F(2,22) = 128.6, 111.3, p < 0.0001$). Because the 20% IL had four times the energy of the 5% IL and 12.5% glucose, the infusions were converted to kcal for a direct comparison of self-infused energy across nutrients during test periods. The DoKO mice self-infused more energy as 12.5% glucose than 20% IL, and more as 20% IL than 5% IL (Infusate $F(2,22) = 51.8, p < 0.0001$). The WT mice self-infused similar amounts of energy as 12.5% glucose and 20% IL, and less as 5% IL (Infusate $F(2,22) = 5.7, p < 0.05$).

Immediately after the glucose test, the infusion was switched to 5% IL without an intervening training period. The numbers of infusions during the 5% IL test were analyzed as two 3-day means (sessions 1-3 and 4-6); these were compared with the immediately preceding glucose test to assess group differences in response to the nutrient change (Fig. 5). The glucose self-infusions were similar in the two groups, but the DoKO mice self-infused less 5% IL ($p < 0.05$) than did the WT group during sessions 1-3 and 4-6 (Group $\times$ Period interaction ($F(2,42) = 13.6, p < 0.0001$). Within-group analyses indicated that DoKO mice self-infused less 5% IL than 12.5% glucose and their IL infusions remained unchanged in sessions 1-3 and 4-6 (Period $F(2,20) = 48.2, p < 0.001$). The WT mice reduced ($p < 0.05$) their infusions when switched from 12.5% glucose to 5% IL in sessions 1-3 and then increased ($p < 0.05$) their IL infusions in sessions 4-6, although these remained lower ($p < 0.05$) than their glucose infusion level (Period $F(2,22) = 27.8, p < 0.001$). The groups declined to the same number of infusions on the first day of the change and began to differ thereafter (Supplementary Fig. 1). Infusions in 5% IL sessions 4-6 were compared with those in the first 5% IL (pre-glucose) test. Both groups self-infused more for 5% IL in the post-glucose sessions than in the pre-glucose test (Test ($F(1,21) = 39.6, p < 0.0001$) and the DoKO mice self-infused less 5% IL than did the WT mice in both tests (Group ($F(1,21) = 21.1, p < 0.001$).

Following the second 5% IL test, the infusion was switched to water for an additional 6 sessions. Total licks and numbers of infusions in these sessions were compared to the mean of sessions 4-6 of 5% IL infusion to assess the extinction of dry licking behavior. Total licks (Fig. 6A) declined across sessions ($F(6,126) = 27.3, p < 0.0001$) and the decline differed in the groups (Group $\times$ Session interaction $F(6,126) = 2.97, p < 0.01$). WT licking was greater than that of the DoKO mice during 5% IL infusion and on the first day of water infusion. The WT mice licked less for water than for 5% IL in all 6 water sessions ($p < 0.001$), but the DoKO mice did not reduce their licking significantly on the first 2 water sessions, differing only in sessions 3-6 ($p < 0.05$). The WT infusion numbers exceeded those of the DoKO group ($F(1,21) = 6.75, p < 0.05$) (Fig. 6B) and declined across sessions ($F(6,126) = 18.9, p < 0.001$). WT water infusions were less than 5% IL infusions by the second water session and continued to decline: infusions in water sessions 3-6 were lower than those in sessions 1 and 2 ($p < 0.05$). DoKO water infusions, in contrast, were significantly lower than 5% IL infusions only in water session 5 ($P < 0.05$).

Discussion

The present findings confirm and extend recent reports [13,53] that B6 mice will self-infuse IG Intralipid and glucose by licking a dry sipper spout and will adjust their operant licking

rate as a function of the caloric density of the nutrient infusion. The new finding here is that dry licking for IG fat is reduced in DoKO mice missing the GPR40 and GPR120 fatty acid receptors, although their operant licking for glucose is normal. This is consistent with our recent report that GPR40/120 DoKO are deficient in their flavor conditioning response to IG IL infusions [46].

The present and prior results show that dry licking for IG nutrient infusions, which removes taste and odor cues, is a readily acquired operant in mice. This presumably occurs because the mice had previously learned to lick a sipper spout to obtain water in the home cage, and spout licking needs only to be transferred to a new use. During lick training, the mice are initially attracted to the food pellet in the spout and their licking behavior is presumably also stimulated by the resulting IG nutrient infusions. Although very few licks are required (one per infusion), many more are executed, presumably reflecting the tendency of mice to lick in bursts [8], so that licking is sustained beyond each triggered infusion. It is likely that the mice do not detect the single-lick requirement for the infusions. Greater licking may also reflect the intake-stimulating effects of the nutrient infusion, although satiation effects can counteract appetite to reduce licking as the session proceeds.

In the dry licking tests with IL the DoKO mice licked significantly less than the WT mice. As a result, when tested with 20% and 5% IL they self-infused 35 and 50% less fat, respectively, than the WT mice. Yet, the DoKO mice self-infused as much 12.5% glucose as did the WT mice, showing that their operant licking deficit was specific to fat. We previously reported [46] that during 1-h training sessions with CS+ and CS- flavors paired with IG infusions of 6.4% IL and water, respectively, DoKO self-infused less fat and in a subsequent choice test displayed only a marginal CS+ preference (58%) compared to WT mice (81%). When training and test sessions were extended to 24 h/day, the DoKO mice developed a more substantial CS+ preference (75%), although still considerably less than that of WT mice (96%). These results indicate that GPR40 and GPR120 receptors have a significant role in post-oral fat conditioning but are not the only fat sensors involved. The present operant licking results are consistent with this interpretation. Overall the DoKO mice licked less for IL infusions than WT mice but still continued to dry lick for IL when food was removed from the sipper spout. In addition, like WT mice, they licked more for 5% IL than for 20% IL, indicating a sensitivity to lipid concentration.

In prior dry licking studies, WT mice showed precise matching of energy self-infusions across 1-h sessions with IL concentrations of 5% to 30% [13,53]. The WT mice in the present study obtained closely, but not perfectly matched energy infusions when tested with 5% and 20% IL. This contrasts with our finding that IL energy self-infusions increased monotonically with concentration in separate groups of B6 WT mice trained in 1-h sessions with CS+ flavors paired with IG infusions of 1.6%, 3.2%, 6.4% and 12.8% IL [4]; similar results were obtained with IG infusions of 2% - 32% glucose [60]. Ferreira et al. [13] also observed that WT mice self-infused more 30% IL than 5% IL when the infusions were paired with unique spout position cues. Thus, precise energy matching with self-infused nutrients may depend upon the absence of external cues (flavor, place) linked to different nutrient concentrations. This can explain why mice orally consume more Intralipid or sugar

when offered concentrated rather than dilute nutrient solutions that differ in their flavor intensity [42,61].

Although the DoKO mice self-infused less 20% IL than WT mice in the empty spout test, they self-infused more 20% IL than the WT mice in the training period with the food-baited sipper spout. It is possible that licking in the training sessions was limited by the satiating action of the IG 20% IL infusions and deletion of the GPR40/120 receptors reduced fat-induced satiation. Consistent with this view are findings implicating GPR40 and GPR120 receptors in fat-stimulated release of the satiety hormones CCK and GLP-1 [9,16,17,25,52]. To evaluate a possible satiation deficit in GPR40/120 DoKO mice, we used a test designed to create a high level of intake of an attractive noncaloric solution, so that satiating effects can be observed as reductions in intake. We have used this method to evaluate the satiating effects of other infusions [29,37-39]. Naive DoKO mice and WT mice were trained to lick a 0.1% sucralose + 0.1% saccharin solution paired with matched-volume IG water infusions. The mice were then tested with the same solution paired with IG infusion of 20% IL. Both groups reduced their licking when switched from IG water to IG IL, and the group difference was not significant (Supplementary Fig. 2). The DoKO mice suppressed licking by 27% from the IG water to IG IL tests, which did not differ significantly from the 34% suppression of the WT mice. Thus, this test did not reveal a deficit in GPR40/120 DoKO mice and further evidence would be needed to document a satiation deficit in these animals.

The IG nutrient infusions were all satiating, as shown by the cumulative and 10-min bin infusion curves. The greater satiating effect of the concentrated 20% IL was reflected in the lower total licks and infusions than with infusates of lower energy density. Satiation was also shown by the rapid reduction in infusions from the first 10 min of a session to the rest of the session. These within-session infusion patterns also reveal a more sustained difference between the groups during 5% IL sessions, with more persistent levels of infusions by the WT mice in the first 40 min.

Following the 12.5% glucose dry licking test, the mice were retested for their operant licking for 5% IL. The finding that they licked more for 5% IL in the post-glucose sessions than in the pre-glucose test indicates that experience with the IG glucose infusions (or more extensive training) up-shifted their response to the IL infusions. Nevertheless, the DoKO mice continued to self-infuse less 5% IL than the WT mice even though the groups had self-infused similar amounts of glucose. The weak reinforcing action of 5% IL in the DoKO mice is further indicated by their minimal change in operant licking when switched from 5% IL to water infusions. The DoKO mice self-infused less water than 5% IL in only one of the 6 extinction sessions whereas the WT mice self-infused less in 5 of the 6 extinction sessions. The apparent group difference in extinction responses, however, may be related to their different starting points, i.e., WT mice self-infused more 5% IL than DoKO mice during the reinforced sessions. Note that in the transition from glucose to fat, licking declined in the first session in both groups, which shows that the 5% IL was a weaker reinforcer than isocaloric glucose, adding to the evidence that the post-oral nutrient signal does not reflect caloric density per se. Other extinction work examined dry licking when the infusions were replaced by sham infusions, using pump activation without infusion, and found substantial increases in licking in the first sham session that gradually declined across sessions [53]. In

another experiment [53], mice learned to prefer an active spout over an inactive spout, with infusions accompanied by an auditory cue. During 3 extinction sessions with sham infusions, only the group that continued to hear the auditory cue maintained preference for the spout but both groups reduced licks across 3 sham-infusion sessions. The authors suggest that external cues are sufficient to maintain behavior, drawing parallels to the effects of drugs on operant responding. Our data suggest that the internal cues generated by fluid infusion may be part of the stimulus that maintains licking when the nutrient is no longer present. Nevertheless, it is surprising that WT and DoKO mice continued to lick at relatively high rates for water infusions over 6 extinction sessions.

The reduced operant licking for IG fat in the GPR40/120 DoKO mice in the present experiment combined with their reduced preference for a flavor paired with IG fat in our prior studies [46,59] provides converging evidence that post-oral GPR40 and GPR120 fatty acid receptors have an important role in post-oral fat reinforcement. GPR40 receptors are located in intestinal enteroendocrine cells, pancreatic β -cells and perhaps brain neurons although this remains to be confirmed [19,30]. There is inconsistent evidence for GPR40 in lingual taste cells [7,31,35]. GPR120 receptors are located in intestinal enteroendocrine cells, lingual taste cells, pancreatic cells, adipocytes, and arcuate neurons [20]. Our findings that IG IL infusions stimulate licking of a flavored CS+ solution within 12-15 min in the first session, before little if any fat is absorbed, suggests an intestinal site of action for post-oral reinforcement, but post-absorptive sites may also contribute to this process [46,59].

The post-oral sensing process that maintains dry licking for fat and sensitivity to fat energy density in GPR40/120 KO mice remains to be determined. de Araujo and colleagues speculated that both pre- and post-absorptive fat sensing mechanisms contribute to the operant licking for IG fat, and specifically mentioned signals related to gastric emptying, cholecystokinin release, and lipid-sensing intestinal molecules (i.e., oleoylethanolamine) [13,53,54]. They further hypothesized that brain dopamine circuits mediate the caloric regulation displayed by mice dry licking for IG nutrient infusions of varying energy densities. Further studies of the operant licking for IG fat in GPR40/120 DoKO mice are needed to elucidate the peripheral and central mechanisms that control this behavior.

The present and prior IG fat operant licking and flavor conditioning studies in mice have used Intralipid as the fat source because it is a very stable emulsion of soybean oil. Soybean oil is similar in fatty acid composition to corn oil, which has been extensively used in flavor conditioning studies in rats and place preference conditioning studies of mice [51]. In unpublished work we observed that IG corn oil, like Intralipid was effective in stimulating the intake of a flavored saccharin solution in mice [59]. We also observed in rats that IG infusions of corn oil and safflower oil, which have similar fatty acid profiles, were equally effective in conditioning flavor preferences [2]. Flavor preferences are also conditioned in rats by other oils with different fatty acid profiles although they are less effective than corn oil [2]. These findings suggest that Intralipid would not be unique in its ability to reinforce dry licking for IG infusions although this needs to be confirmed in subsequent experiments.

The success of the dry licking procedure to maintain IG nutrient infusions when food cues are removed from the sipper tube is noteworthy in view of prior attempts to induce operant

IG nutrient self-infusion in the absence of oral ingestion. Early studies by Epstein and coworkers reported that IG nutrient infusions reinforced lever press responding in rats without “oropharyngeal sensations” [11,12,33]. However, a subsequent analysis by Holman [18] indicated that the use of a nasogastric catheter and cold liquid diet in these early studies provided oropharyngeal sensations that appear critical to maintaining lever responses for nutrient infusions [see also 57]. Holman reported that with a subcutaneous IG catheter (as used in mouse experiments) or warm diet, most rats did not lever press for IG nutrient infusions unless they “self-produced oral stimuli derived from licking and gnawing the response lever.” Holman's observation may explain the effectiveness of the dry licking response, which provides oral tactile stimulation, to maintain operant self-infusions of nutrients in mice. Note that this interpretation questions the claim by Ferreira et al. [13] that licking a dry sipper tube does not provide oropharyngeal sensations. Rather, their findings along with the present results demonstrate that specific taste or odor cues are not required to maintain a dry licking operant reinforced by IG nutrient infusions. In addition, the nutrient-reinforced dry licking by mice clearly demonstrate that changes in orosensory sensations are not always required for mice to respond to changes in the nutrient composition of IG infusions. This is further indicated by the recent findings that mice licking a flavored saccharin solution paired with IG water infusions showed stimulation of licking when switched to IG glucose infusions whether or not a new flavor was paired with the sugar infusion [5]. However, in the absence of specific flavor (taste, odor, texture, temperature) differences or other food cues (position, color, etc.), it would be difficult for animals to discriminate among potential food options based on their post-oral nutrient actions alone.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

GPR 40/120 double-knockout (DoKO) mice are impaired in post-oral fat sensing.

DoKO and wildtype (WT) mice learned to lick a dry spout for gastric nutrient infusions.

DoKO mice were impaired in intragastric self-infusion of 20% and 5% fat emulsions.

However, DoKO self-infusion of 12.5% glucose was similar to that of WT mice.

GPR40 and GPR120 sensors contribute to post-oral fat reinforcement signaling in mice.

Figure 1.

DoKO mice lick more for 20% Intralipid in training and less in tests than WT mice. A. Mean intragastric self-infusions of 20% Intralipid per 1-h session during Training and Test 1 (sessions 1 - 4) and Test 2 (sessions 5 - 8) for GPR40/120 double knockout (DoKO) and B6 wild-type (WT) mice. The mice licked a food-baited spout during Training sessions and an empty spout during Test sessions. Each reinforced lick activated the infusion pump for 3 sec; licks during infusions were without effect. Differences between the groups within periods are indicated by an asterisk. Differences from Training to Tests are indicated by the “less-than” sign. B. Mean 20% Intralipid infusions in 10-min bins of 1-h sessions during Test 2 in DoKO and WT mice. The inset shows the same data in cumulative form, with a solid line for the WT group and a dashed line for the DoKO group. Differences between the groups are indicated by an asterisk.

Figure 2.

DoKO mice lick less for 5% Intralipid in training and tests than WT mice. A. Mean intragastric self-infusions of 5% Intralipid per 1-h session during Training and Test periods for GPR40/120 double knockout (DoKO) and B6 wild-type (WT) mice. The mice licked a food-baited spout during Training sessions and an empty spout during Test sessions. Each reinforced lick activated the infusion pump for 3 sec; licks during infusions were without effect. Differences between the groups within periods are indicated by an asterisk. Differences from Training to Tests are indicated by the “less-than” sign. B. Mean 5% Intralipid infusions in 10-min bins of 1-h sessions during testing in DoKO and WT mice. The inset shows the same data in cumulative form, with a solid line for the WT group and a dashed line for the DoKO group. Differences between the groups are indicated by an asterisk.

Figure 3.

DoKO and WT mice do not differ in licking for 12.5% glucose. A. Mean intragastric self-infusions of 12.5% glucose per 1-h session during Training and Test periods for GPR40/120 double knockout (DoKO) and B6 wild-type (WT) mice. The mice licked a food-baited spout during Training sessions and an empty spout during Test sessions. Each reinforced lick activated the infusion pump for 3 sec; licks during infusions were without effect. The groups and periods did not differ statistically. B. Mean 12.5% glucose infusions in 10-min bins of 1-h sessions during testing in DoKO and WT mice. The inset shows the same data in cumulative form, with a solid line for the WT group and a dashed line for the DoKO group. The groups did not differ statistically.

Figure 4.

Infusates differed more in numbers of infusions than in energy infused. Mean intragastric self-infusions (A) and kcal (B) for 20% Intralipid (IL), 5% IL, and 12.5% glucose (Gluc) during the last 4 days of 1-h Test periods for GPR40/120 double knockout (DoKO) mice and B6 wild-type (WT) mice. Different numbers (for WT) or letters (for DoKO) above columns denote differences between infusates. Differences between the groups are indicated by asterisks.

Figure 5.

DoKO mice show sustained reduction in licking when shifted from glucose to 5%IL. Mean intragastric self-infusions of 12.5% Glucose and 5% Intralipid (IL) for GPR40/120 double knockout (DoKO) and B6 wild-type (WT) mice. The Glucose Test represents an average of 4 days and the two 5% IL periods are means of 3 days. Differences from Glucose to IL are indicated by the “less-than” sign. Differences between the groups within periods are indicated by an asterisk.

Figure 6.

Mice reduce operant licking and self-infusion under extinction conditions. Mean total licks (A) and intragastric self-infusions (B) of 5% Intralipid (IL, mean of last 3 sessions) and water (sessions 1-6) for GPR40/120 double knockout (DoKO) and B6 wild-type (WT) mice. Differences between the groups are indicated by an asterisk. Differences within groups between the IL mean and each water session are indicated by the “less-than” sign.

Table 1

Total licks (mean and (sem)) by GPR 40/120 double knockout (DoKO) and C57BL/6 wild-type (WT) groups during 1-h training and test sessions.

Infusate condition	DoKO		WT	
	Train	Test	Train	Test
20% Intralipid (1-8)	444.0 (47.5)	165.3 (28.5)	341.9 (26.9)	248.1 (40.0)
Test 1-4		181.9 (21.3)		233.0 (23.3)
Test 5-8		148.8 (44.5)		263.2 (63.2)
5% Intralipid (IL)	887.5 (116.2)	627.0 (168.8)	1394.1 (119.7)	1312.5 (190.4)
12.5% Glucose	1454.7 (150.6)	1821.9 (124.3)	1513.3 (164.6)	1946.8 (166.8)
DoKO n=11		1835.0 (135.4)		
5% IL 2 Test 1-3		1337.0 (132.6)		1670.9 (144.8)
5% IL 2 Test 4-6		1358.2 (140.3)		1882.8 (138.4)