Mice bearing \textit{Acads} mutation display altered postingestive but not 5 s orosensory response to dietary fat

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RUNNING TITLE: Altered postingestive response to fat in \textit{Acads} -/- mice

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ABSTRACT

A previous survey of mouse inbred strains revealed a wide range in self-selected fat intake, from 26 to 83% of energy. The BALB/cByJ strain selected a lower percentage of fat intake (36%) than all other strains tested except for the CAST/Ei. BALB/cByJ mice are deficient in the short chain acyl-CoA dehydrogenase (SCAD) enzyme due to a spontaneous mutation in Acads. We hypothesized that this deficiency would alter fat appetite and used three behavioral test paradigms to compare the response of BALB/cByKz.Acads -/- and BALB/cByKz.Acads +/- mice to fat stimuli. First, during 10 d exposure to a macronutrient self-selection diet, Acads -/- mice consumed proportionately less fat and more carbohydrate than Acads +/- mice, yet total energy intake was similar between strains. Next, in 48 h two-bottle preference tests, Acads +/- mice displayed a preference for 50% corn oil but Acads -/- mice did not. Finally, in brief-access taste tests employing successive, 5 s presentations of corn oil in an ascending concentration series ending with 50%, there were no effects of strain on total licks indicating that Acads does not alter acute orosensory response to this fat stimulus. With 15 s presentations however, the Acads +/- mice licked more of the 50% oil than Acads -/- suggesting orosensory effects related to the increased exposure time. In contrast to corn oil, there were no strain differences in licking response to sucrose solution in either the two-bottle or brief-access taste tests. The observation that SCAD-deficient mice display altered postingestive responses to dietary fat provides further evidence for the metabolic control of feeding.
INTRODUCTION

In a phenotypic survey of mouse inbred strains, self-selected fat consumption ranged from 26% to 83% of total energy across strains in a choice paradigm containing separate sources of carbohydrate, fat, and protein (43). The BALB/cByJ mice selected a lower percentage of fat intake (36%) than all other strains tested with the exception of the CAST/Ei (25%) (43). The BALB/cByJ mouse strain bears a spontaneous deletion in the SCAD structural gene (Acads) (38, 52) which results in missplicing of the mRNA and the absence of functional short-chain acyl-Coa dehydrogenase (SCAD) (18; for review, see 38 & 51). SCAD is a mitochondrial enzyme that catalyzes the first reaction in the β-oxidation of C4-C6 fatty acids. We hypothesized that this deficiency in beta-oxidation of fatty acids may underlie the observed dietary preference.

Fatty acid oxidation is thought to be a key factor in the metabolic control of food intake (10) because rodents are stimulated to eat after administration of inhibitors that interfere with β-oxidation of fatty acids (36-37). For example, administration of mercaptoacetate (MA) stimulates feeding in mice by inhibiting the acyl-CoA dehydrogenases that catalyze mitochondrial β-oxidation (8). A similar effect has also been shown by administering methyl palmoxirate and emeriamine (8) which suppress fatty acid oxidation by inhibiting carnitine palmitoyltransferase I (CPT I), a transporter of long-chain fatty acids into mitochondria. In studies that examined patterns of macronutrient intake in response to MA (33, 42), the metabolic inhibitor failed to enhance fat consumption in the 3-macronutrient self-selection diet, or in tests in which a single fat source was available. However MA did increase the intake of carbohydrate or protein depending on the diet paradigm. This evidence supports a link between dietary fat selection and metabolic cues related to fatty acid oxidation (32).
The aim of the present study was to investigate the effects of the *Acads* mutation on behavioral feeding responses to fat. Specifically, we sought first to replicate, with the appropriate genetic strain control, the low fat intake and preferred consumption of dietary carbohydrate in *Acads* -/- mice that we observed in a previous study (43). Self-selected fat intake was assessed in a choice between two diets: fat/protein (containing vegetable shortening as the fat source) and carbohydrate/protein, over a 10 d period. We hypothesized that SCAD deficiency in *Acads* -/- mice would suppress appetite for dietary fat compared to *Acads* +/+ mice with the same genetic background. Next we examined corn oil preference in the commonly used 4 d, two-bottle choice test (39). Last, based on the premise that preference or avoidance behavior for dietary fat in rodents is conditioned by the postingestive effects of the nutrient (17, 40), we investigated the effects of *Acads* on taste responsiveness to corn oil emulsions during brief-access (5 or 15 s) tests using an automated lick sensor. We aimed to capture responses of these mouse strains to the hedonic properties of the test solutions by employing the brief-access taste test (5, 12, 44). This method measures brief periods of tongue activity thereby dissociating the postingestive effects of ingested fat from orosensory factors (53). To rule out possible effects of the *Acads* mutation on taste responsiveness to palatable stimuli other than fat, we also examined responses to sucrose in both the two-bottle preference and the brief-access taste tests.

METHODS

*Animals*

It has been determined that the *Acads* mutation occurred spontaneously sometime between 1981 and 1982 (34) in the BALB/cByJ production line. The BALB/cByJ mice are descendents of the BALB/cBy strain maintained originally by Donald Bailey at the Jackson
Laboratory. The best SCAD-normal control line for the BALB/cByJ strain is thought to be the BALB/cBy because the two lines are considered to be coisogenic (51). The BALB/cByKz.Acads -/- and BALB/cByKz.Acads +/- mice were obtained from a colony maintained by Dr. Leslie P. Kozak at Pennington Biomedical Research Center in Baton Rouge. The BALB/cByKz.Acads +/- substrain was separated from the research colonies at the Jackson Laboratory in 1996. Due to the length of time that these substrains have been separated, we cannot rule out undetected spontaneous mutations in genes other than Acads that could affect behavioral responses to dietary fat.

Animals were individually housed in plastic shoebox cages and fed a low-fat (12% by energy) rodent chow (no. 5001; LabDiet®, Richmond, IN) and water ad libitum. In experiment 1, the bedding was removed and stainless steel wire floor inserts were placed in the cages when experimental diets were initiated at 7-9 wks of age. Polyvinylchloride nesting tubes (1½ in. diameter) were provided to reduce time spent on the wire bottom. Mice were maintained on a photoperiod of 12:12 with lights on at 0600 and at an ambient temperature of 24-26 deg. C. All mice appeared clinically normal during the experiments. Separate cohorts of experimentally naïve male mice were used in experiments 1 (n = 15-16 per strain at 7-10 wks of age) and 2 (n = 7 per strain at 6.5-17 wks); two naïve cohorts were used in experiment 3 (n = 8-9 per strain at 9-14 wks, and n = 14 per strain at 12-16 wks).

Procedures

Macronutrient diets. Dietary intake was assessed in a selection paradigm providing for 10 d a choice between the Carbohydrate/Protein (C/P) diet containing 78% and 22% of energy from carbohydrate (corn starch and powdered sugar) and protein (casein), respectively, or the
Fat/Protein (F/P) diet containing 78% and 22% of energy from fat (vegetable shortening) and protein, respectively (see Table 1). Providing a source of protein in both diet choices, rather than separately, prevents the long-known problems associated with the aversive taste of casein in rodent diet studies (28, 41). The F/P and C/P diets, when presented simultaneously for 10 d, comprise a nutritionally complete diet for short-term studies according to National Research Council guidelines (26) but may not be optimal for long-term use, i.e., >3 mos, or for early growth phase or reproduction. Each of the diets was presented in custom 2 oz. glass jars (Unifab, Kalamazoo, MI) and covered with a stainless steel lid with an opening that measured 7/8 inch in diameter. Under the lid on top of the diet, a stainless steel disc with six circular openings (each 7/16 in. diameter) was placed to allow food access while minimizing spillage. To ensure freshness, the fat/protein (F/P) diet jar was replaced every other day; on alternate days, the lid was removed and fresh diet was added. The jar containing carbohydrate/protein (C/P) diet was topped with fresh diet every 24 h. Jars and spillage were weighed daily to the nearest 0.1 g midway through the light period. Notably, F/P spillage was nonexistent with this method. Any spilled C/P diet (minus feces) was caught on cardboard pads placed beneath the wire floor insert and the powder was collected into a container with a brush.
Table 1. Composition of experimental diets.

<table>
<thead>
<tr>
<th>2-CHOICE DIET PROTOCOL</th>
<th>Carbohydrate/Protein†</th>
<th>Carbohydrate/Protein‡</th>
<th>Fat/Protein†</th>
<th>Fat/Protein‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn starch</td>
<td>49.62</td>
<td>13.70</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Powdered sugar</td>
<td>21.24</td>
<td>5.86</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Casein</td>
<td>19.88</td>
<td>5.49</td>
<td>32.77</td>
<td>5.50</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.29</td>
<td>0.08</td>
<td>0.49</td>
<td>0.08</td>
</tr>
<tr>
<td>Vegetable shortening</td>
<td>0.00</td>
<td>0.00</td>
<td>51.93</td>
<td>8.71</td>
</tr>
<tr>
<td>AIN-76A vitamin mix*</td>
<td>1.00</td>
<td>0.28</td>
<td>1.53</td>
<td>0.26</td>
</tr>
<tr>
<td>AIN-76A minerals mix*</td>
<td>3.20</td>
<td>0.88</td>
<td>5.33</td>
<td>0.89</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.18</td>
<td>0.05</td>
<td>0.31</td>
<td>0.05</td>
</tr>
<tr>
<td>Cellulose (alphacel)</td>
<td>4.92</td>
<td>1.36</td>
<td>7.62</td>
<td>1.28</td>
</tr>
</tbody>
</table>

Energy density (kcal/g) | 3.61 | 5.96 |
Macronutrient content (% energy) | 78/22 | 78/22 |

† Ingredients expressed as percent by weight.
‡ Ingredients expressed as g/100 kcal.
* The vitamin and mineral mixes contain 97% and 12% sucrose respectively.

Two-bottle preference tests. Solutions were presented in 50 ml conical tubes fitted with rubber stoppers and stainless steel spouts of uniform size. Mice were first adapted to drinking from two bottles containing deionized water for 3-4 d. During the experiment, one bottle containing vehicle (deionized water + emulsifier) and the other containing the oil emulsion were mounted on the wire feeder top with a distance of 7.5 cm between the two spouts. Food pellets were placed in between, and the right-left position of the bottles was alternated every 24 h to control for side preferences. Preference scores were calculated as the ratio of the 48 h (right + left bottle position) taste solution intake (g) to total fluid (taste solution + vehicle) intake (g). The use of preference ratios eliminates the effect of body weight on solution intake. The concentrations of tastant chosen were those that have been shown to be near the peak of the behavioral preference function in other studies (13, 23).
Solutions. Reagent grade sucrose (ICN Biomedicals; Aurora, OH) was dissolved in deionized water. The corn oil emulsions (Mazola, Best Foods, Englewood Cliffs, NJ) were prepared as oil in deionized water mixtures (% wt./wt.) and stabilized with a combination of sodium stearoyl lactylate (Emplex, Patco, Kansas City, MO) and xanthan gum (Sigma, St. Louis, MO) using 0.2% total emulsifier per 10% corn oil. The oil emulsions were prepared fresh daily using a bench top laboratory homogenizer (PRO Scientific, Monroe, CT) at high speed (15,000 rpm x 10 min) and then cooled to room temperature before presentation. The stability of corn oil emulsions over 24 h has been demonstrated previously in our laboratory (44).

Choice of nutrient stimuli. Because the macronutrient diet protocol described above was already established in our laboratory, we chose initially to test the effect of SCAD deficiency on nutrient selection using this paradigm. After observing the marked reduction in fat intake by the BALB/cByKz.Acads -/- strain with a fat source high in long-chain fatty acids, the use of short-chain fatty acids seemed unnecessary. The decision to use corn oil for the two-bottle preference and lickometer tests was based mainly on its physical form at room temperature, e.g., vegetable shortening cannot be used in solution tests. All together, the results show that the avoidance of dietary fat by Acads -/- mice generalizes to different fat sources, and to both solid and liquid forms.

Lickometer apparatus. In experiment 3, licking activity during brief-access to stimuli was determined using a MS-160 Davis Rig behavioral data acquisition and analysis system (DiLog Instruments, Inc. Tallahassee, FL). This system was designed to record the latency to lick and all interlick intervals with a resolution of 1 ms. The system consists of a test cage with clear plastic sides and back and a stainless steel front with an opening for access to the sipper tube, a movable bottle carriage and a sipper tube access shutter. The bottle carriage and tube
access shutter are moved by computer-controlled stepper motors, allowing smooth acceleration as well as a warning move of the shutter prior to closure. Tongue contact with the sipper tube is detected via a radio frequency contact circuit and licks are stored in a data file. The sipper tube is connected to the circuit through a capacitor to prevent direct current. To minimize possible olfactory cues (31), the system has been modified to include an air pump which provides a continuous air flow across the end of the sipper tube.

Lickometer training. A period of training is necessary for the mice to approach the spout and begin licking during test sessions. For this purpose, mice were water deprived approximately 23 h per day in their home cage. On the first day, each mouse was placed in the test cage with the access shutter open and allowed to drink for ten minutes from the sipper tube. Once the mice learned to find water in the apparatus, they received several more days of training with gradually shorter presentations to acclimate to the shutter action. In our experience, 5-10 d of training are necessary depending on mouse strain. Mice were weighed before and after each training session; to help maintain body weight, they were supplemented with 15 min access to a water bottle in their home cage approximately 4 h after each training session. On weekends, mice were allowed to recover with ad libitum access to water. The Acads -/- mice weighed 22.8 ± 0.6 g at the beginning of training and 29.3 ± 0.5 g at the end of these experiments. Acads +/- mice weighed 25.8 ± 0.9 g and 29.9 ± 0.5 g, respectively. All mice maintained their body weight at ≥85% of their baseline values during training.

Lickometer (brief-access) tests. We developed a procedure for ensuring that the mice would approach the spout to lick palatable taste stimuli in the test apparatus when not motivated by overnight water deprivation (44). Each week, the animals received 2 d of training with overnight water deprivation, followed by 2 d of testing with a single tastant in varying
concentrations (see Table 2 for test sequence). Motivation to approach the spout on test days was induced by mild water deprivation (6 h) during the light period, i.e., water was removed at 0800 h and testing began at 1400 h. Concentrations of corn oil (0.5%, 1.5%, 5%, 15%, and 50%) or sucrose (0.05, 0.1, 0.2, 0.5, and 1.0 M) were tested in ascending series with one exception: at the completion of testing with lickometer cohort 1, an additional group of naïve mice were trained and tested for the purpose of conducting two corn oil tests with a descending series. On test days, animals received only one tastant and a single presentation of each concentration; all concentrations of a tastant were tested in a single session. The presentation length was either 5 or 15 s and the inter-presentation interval was 30 s. Thus the minimum length of a session ranged from 2.4 to 3.25 min. The same mice were used in all the lickometer tests listed in Table 2; these animals were naïve to corn oil in week 2 and to sucrose in week 3. The data collected during weeks 6 and 11 recapitulated the results obtained for corn oil (weeks 2 & 4) and sucrose (weeks 3 & 9), and therefore are not presented. After testing with this initial group of mice was completed, an additional cohort of naïve mice was trained and tested for taste responsiveness to corn oil in a descending concentration series (see Fig. 9).

<table>
<thead>
<tr>
<th>Week</th>
<th>Tastant</th>
<th>Training</th>
<th>Presentation length</th>
<th>Water deprivation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>3 d</td>
<td>10 min → 15 s</td>
<td>Overnight</td>
</tr>
<tr>
<td>2</td>
<td>Corn oil</td>
<td>3 d</td>
<td>15 s, 15 s</td>
<td>6 h</td>
</tr>
<tr>
<td>3</td>
<td>Sucrose</td>
<td>2 d</td>
<td>15 s, 15 s</td>
<td>6 h</td>
</tr>
<tr>
<td>4</td>
<td>Corn oil</td>
<td>2 d</td>
<td>5 s, 5 s</td>
<td>6 h</td>
</tr>
<tr>
<td>5</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Corn oil</td>
<td>2 d</td>
<td>15 s, 5 s</td>
<td>6 h</td>
</tr>
<tr>
<td>7</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sucrose</td>
<td>2 d</td>
<td>5 s, 5 s</td>
<td>6 h</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Sucrose</td>
<td>2 d</td>
<td>15 s, 5 s</td>
<td>6 h</td>
</tr>
</tbody>
</table>
Data Analysis. For experiment 1, diet intakes were examined as kilocalories and proportion of total calories. Data were analyzed by ANOVA with repeated measures using a mixed model, with strain as the between-group factor and day as the within-group factor. For experiment 2, data were analyzed both as solution intakes and preference ratios using a one-factor analysis of variance (ANOVA). Results for analyses of solution intakes were essentially no different than those for preferences and therefore are not presented.

For experiment 3, mean interlick interval (ILI) was calculated from data obtained during training day 1; the reciprocal of the ILI represents the lick rate within bursts of licking. ILIs <10 ms and >200 ms were excluded for functional assessment of oromotor response between strains, i.e., frequency distribution of ILI, as well as mean ILI and total licks. ILIs >200 ms are thought to represent either short (e.g., missed licks) or longer (e.g., deprivation state) pauses between bursts (48). For each corn oil or sucrose trial, the number of licks was examined by three-way, mixed ANOVA, with strain as a between-subjects factor, and concentration and test (trial 1 or trial 2) as within-subject, doubly repeated factors. This experiment was designed originally with the goal of averaging across two identical trials for each mouse, performed on consecutive days each week. However when the results were analyzed, a trial effect was discovered, thus the results of each trial were presented separately. In general, when a main effect was observed, individual comparisons were assessed using Tukey’s protected t-test.

The use of standardized lick ratios, based on each mouse’s licking response during training, has been advocated to control for individual differences in interlick interval (also known as within-burst lick rate) (12). However the use of ratios to remove the confounding effects of a denominator variable can be problematic in the context of hypothesis testing, by violating
statistical assumptions and creating difficulties in interpretation (1). When our data (Expt. 3) were transformed into standardized scores, the results and interpretations were found to be essentially the same. This was not surprising due to the small amount of intra- and inter-strain variation in ILI. Thus we have chosen to evaluate and present the unadjusted data for total licks in response to palatable stimuli.

RESULTS

Experiment 1: Macronutrient diet (2-choice) test

Macronutrient diet preference (kcal). The effect of the Acads mutation on self-selected macronutrient diet intake across days is illustrated in Fig. 1. Relative to the carbohydrate/protein diet, the Acads +/- strain consumed more calories overall from the fat/protein source, while the Acads +/- mice consumed significantly less fat/protein kcal, [strain x diet; $F(1, 29) = 30.33, P > 0.0001$]. Furthermore an analysis of diet selection over time revealed contrasting patterns in the two strains [strain x diet x day; $F(9, 29) = 5.89, P = 0.0001$]. Neither strain displayed a diet preference on day 1 (Acads -/-, $P = 0.15$; Acads +/-, $P = .74$). However Acads +/- mice displayed a preference for the F/P diet on days 7-10 (see Fig. 1A). Notably, the Acads -/- mice decreased their fat/protein intake in a stepwise manner on days 2-4, suggesting a conditioning effect, and reached a plateau that continued to the end of the study (Fig. 1B). This reduction in energy intake from fat was compensated by an increased consumption of the C/P diet.

Total kilocalories and percent fat intake. There were no effects of strain or day on total daily calorie intake [$F(1, 29) = 0.01, P = 0.913; F(9, 29) = 1.89, P = 0.093$, respectively] as shown in Fig. 2A. When expressed relative to total calories, F/P intake was similar between strains on day 1 of the 2-choice diet presentation ($P = 0.50$), representing approximately 40% of
total energy. Over the next 3 days however Acads -/- mice decreased their proportion of fat intake incrementally [strain x day: \( F(9, 29) = 6.72, P < 0.0001 \)] (Fig. 2B).

**Experiment 2: Two-bottle preference tests (taste solution vs. vehicle)**

The mean 48 h preference scores by strain for corn oil are illustrated in Fig. 3A. Overall, preference scores were lower in Acads -/- mice compared to Acads +/+ \([F(1,13) = 12.19, P < 0.001]\) on both days 1-2 and 3-4, although scores were lower in both strains during the second 48 h test \([F(1,12) = 22.78, P < 0.001]\). Acads +/+ mice displayed a reliable preference for corn oil (scores different than .50) on both days 1-2 and 3-4 \((P < 0.001\) and \(P < 0.005\), respectively) while Acads -/- mice did not \((P = 0.11\) and \(P = 0.25\)). In contrast to corn oil, both strains showed a similar, strong preference for 4% sucrose solution \([F(1, 13) = 0.85, P = 0.37]\) and the results were similar for both of the two 48 h tests \([F(1, 13) = 1.80, P = 0.20]\) (Fig. 3B). Thus, Acads -/- mice were indifferent to corn oil while Acads +/+ mice showed a clear preference for this tastant.

Both strains displayed strong preference scores for 4% sucrose solution indicating its high palatability, and that taste responsiveness to this stimulus is unaltered by SCAD deficiency.

**Experiment 3: Brief-access tests (oromotor function, and concentration-dependent licking to corn oil, sucrose)**

*Licking response to water.* The licking response to water during the first day of sipper tube training was used to compare oromotor function between water-deprived Acads -/- and Acads +/+ mice. The training session on day 1 lasted 10 min, beginning when each mouse took its first lick. An interlick interval (ILI) distribution was calculated for each mouse and averaged by strain as shown in Fig. 4. The Acads -/- mice showed a mean ILI of 110 ± 2 ms compared
with 109 ± 2 ms in \( Acads^{+/+} \) mice (data not shown). The reciprocal of the ILI represents the rate of licking which was similar in the two strains, i.e., 9 licks/s. The total number of licks for this training session was inversely related to body weight \( (r = -0.55, P < 0.05) \). There was no effect of strain on the mean ILI or total number of licks \( [F(1, 13) = 0.18, P = 0.67 \text{ and } F(1, 13) = 0.44, P = 0.52] \), respectively, when body weight was used as a covariate.

**Concentration-dependent licking response to corn oil: ascending order.** Overall, the licking response to corn oil in the 15 s tests increased with increasing concentrations \( [F(4, 55.6) = 11.78, P < 0.0001] \) but \( Acads^{+/+} \) mice licked more of this nutrient than \( Acads^{-/-} \) [strain x concentration: \( F(4, 55.6) = 3.35, P < 0.05 \); Fig. 5]. Specifically, \( Acads^{+/+} \) mice licked more of the 50% corn oil emulsion than \( Acads^{-/-} \) mice in both trials 1 \( [F(1, 44.4) = 6.21, P < 0.05] \) and 2 \( [F(1, 38.5) = 4.99, P < 0.05] \) (Fig. 5). Additionally, both strains licked more in the second trial than in trial 1, suggesting a learning effect [concentration x trial: \( F(4, 66.1) = 12.01, P < 0.0001 \)]. By contrast during the 5 s presentations, the licking response was similar in the two strains [strain x concentration: \( F(4, 57.6) = 0.85, P = 0.51 \)] (see Fig. 6). There was a strong concentration effect, and again, both strains licked more corn oil in the second trial compared to trial 1 [concentration x trial: \( F(4, 65.7) = 11.97, P < 0.0001 \)]. The lack of a strain effect in the 5 s tests indicates that \( Acads \) does not alter the orosensory response to corn oil. However, the higher lick total for 50% corn oil by \( Acads^{+/+} \) mice compared to \( Acads^{-/-} \) mice in the 15 s tests suggests possible orosensory effects related to the increased exposure time.

**Concentration-dependent licking response to sucrose: ascending order.** To rule out the possibility that this \( Acads \) mutation affects taste responsiveness to palatable nutrients in general and not specifically to fat stimuli, we also examined responses to sucrose in both the two-bottle preference and the brief-access lick tests. In tests employing either 15 s or 5 s presentations of
sucrose solution, both the Acads -/- and Acads +/- mice licked more with increasing concentrations but there was no difference between strains [15 s, strain x concentration: $F(4, 54.8) = 0.13, P = 0.97$; 5 s, $F(4, 55.9) = 0.78, P = 0.54$] (Figs. 7, 8). Also, both strains licked progressively more sucrose as a function of concentration in trial 2 but not in trial 1 [15 s, concentration x trial: $F(4, 67.7) = 3.19, P < 0.05$; 5 s, concentration x trial: $F(4, 67.1) = 5.45, P < 0.001$].

**Concentration-dependent licking response to corn oil: descending order.** Because the Acads -/- mice licked less of 50% corn oil than Acads +/- in the ascending series, we questioned whether this response depended on prior experience with the lower concentrations in the same test. Thus we investigated whether these strains would respond in the same manner in a descending concentration series, i.e., if 50% corn oil was presented as the first stimulus (Fig. 5). For this purpose, an additional cohort of naïve mice were trained and tested. In a descending series of 15 s presentations, both strains licked less corn oil with decreasing concentration [$F(4, 112) = 6.09, P < 0.0005$] and in similar amounts [strain x concentration: $F(4, 112) = 0.50, P = 0.74$] (Fig. 9). Additionally, licking responses were somewhat erratic in the first trial compared to trial 2 (conducted on consecutive days), suggesting a learning effect [concentration x trial: $F(4, 117) = 3.66, P < 0.001$]. Overall, the Acads +/- mice responded to the descending concentrations by licking less of 50% corn oil (Fig. 9) compared with their response in the ascending series (Fig. 5), i.e., approximately 40 licks versus 80 licks, respectively. Thus for Acads +/- mice, prior experience with four lower concentrations of corn oil (within-test) resulted in an enhanced licking response to the 50% emulsion (Fig. 5).
DISCUSSION

In this study, three behavioral test paradigms were used to compare the ingestive responses of Acads −/− and Acads +/+ mice to dietary fat stimuli. The results show that during long-term but not brief access (5 s) studies, Acads −/− mice consumed less fat. First, during 10 d exposure to a self-selection diet Acads −/− mice consumed proportionately less fat and more carbohydrate than Acads +/+ mice, while total calorie intake was similar between strains. Next, in 48 h preference tests, Acads +/+ mice displayed a preference for emulsified corn oil but Acads −/− mice did not, whereas both strains displayed equally strong preference scores for 4% sucrose solution. Third, data obtained during the first day of lickometer training demonstrate a lack of differences in oromotor function between strains. Subsequent brief-access taste tests employing 5 s presentations of corn oil showed no effects of strain on total licks, indicating the lack of an effect by Acads on the acute orosensory response to this fat stimulus. However in taste tests employing 15 s presentations, a strain difference was observed with 50% corn oil, suggesting orosensory effects related to the increased exposure time. In this case, naïve Acads +/+ mice licked more of 50% corn oil than Acads −/− in an ascending series but not when the concentrations were presented in descending order. No strain effects were found in the animals’ concentration-dependent licking responses to sucrose solution in either the 5 s or 15 s taste tests.

Mice with the Acads mutation are totally deficient for the SCAD enzyme (2, 49) resulting from a 278 bp deletion in the structural gene for SCAD (Acads) (18, 20). Surprisingly, Acads −/− mice have no apparent clinical disease (3). More subtle phenotypes include organic aciduria (38, 52); fatty liver and kidney (3); cold intolerance (16); fasting-induced hypoglycemia (52); and slowing of theta oscillations during sleep (49). SCAD deficiency also occurs in humans and in its most severe form is characterized by metabolic acidosis, nonketotic hypoglycemia, and short-
chain dicarboxylic aciduria (52). The lesser clinical severity observed in mice (2) has been attributed to an increased capacity for eliminating acyl-CoA derivatives, especially butyryl-CoA, by glycine conjugation (38, 52).

Notably in the two-choice diet paradigm SCAD-deficient mice did not display hyperphagia, but ate essentially the same amount of calories as Acads +/+ controls. The lack of effect by Acads on total energy intake stands in contrast to the stimulation of feeding in rodents induced by pharmacologic manipulations of fatty acid oxidation using metabolic inhibitors (10, 36-37, 42). For example, Del Prete et al. (8) showed that mercaptoacetate or emeriamine injections in mice, performed in the middle of the light phase, significantly increased food intake. Mercaptoacetate administration blocks the oxidation of long-chain fatty acids in the liver by inhibiting mitochondrial palmitoyl-CoA dehydrogenase activity (4) while emeriamine is a specific inhibitor of carnitine palmitoyltransferase I (8). How information about fat oxidation is sensed and integrated is not yet well defined (36) but recent evidence supports a decrease in hepatic energy status as a key signal for eating behavior induced by compounds such as these (21). In the current study a lack of short-chain fatty acid oxidation in Acads -/- mice did not stimulate increased food intake, indicating that adequate supplies of acetyl-CoA were available to the citric acid cycle through the β-oxidation of long chain fatty acids because long-chain acyl-CoA dehydrogenase function is normal. The fat sources used in the present study, i.e., vegetable shortening and corn oil, would have provided plenty of fuel for this pathway because their triacylglycerols contain >95% long-chain fatty acids (C16-C18).

In the diet choice paradigm, self-selected intake of the fat/protein mixture was similar between strains on day 1, representing approximately 40% of total energy. However on days 2-4, Acads -/- mice decreased their daily intake of the fat/protein diet in a stepwise manner.
indicating a conditioning effect, and then maintained a >2 fold lower fat intake compared to
*Acads +/+*. We hypothesize that a change in metabolism at the substrate level generated a signal
that resulted ultimately in a behavioral response to decrease fat intake. Based on the concept of
Pavlovian conditioning, conditioned food avoidance manifests when an animal chooses to refrain
from eating a particular form of food because it is able to detect an orosensory cue (conditioned
stimulus, CS) that has been previously associated with an aversive postingestive consequence
(unconditioned stimulus, US) of that food (6). Of interest, a similar, gradual reduction in fat
intake over time has been observed in the carbohydrate-preferring CAST/Ei strain (43, 46), thus
pointing to the possibility that CAST/Ei mice may be SCAD deficient. However in a previous
study of self-selected macronutrient diet intake, we found no linkage for the *Acads* locus on
chromosome 5 in an F2 mapping population generated from a C57BL/6J x CAST/Ei intercross
(46). Moreover, the C57BL/6J strain is SCAD enzyme replete (51).

The US underlying the suppression of fat intake in the SCAD-deficient BALB/cByJ mice
is not known but a signal could originate from metabolic consequences of the mutation such as
changes in the availability of free fatty acids, acylcarnitines, or oxidative metabolites. For
example, it has been reported that SCAD-deficient mice have significantly lower concentrations
of acetyl-CoA in both liver and cerebrum (29). Also, mutants have slightly higher levels of \(\alpha\)-
ketoglutarate and lactate in cerebrum, and of liver pyruvate, compared to BALB controls when
maintained on rodent chow (29) but is not clear whether the mild accumulation of intermediate
metabolites in tissues of *Acads* -/- mice could account for the behavioral feeding effects observed
in the present study. Central nervous system neurons might sense changes in nutrient oxidation
in a manner similar to that proposed for brain glucose sensing (24), e.g., by the accumulation of
short-chain fatty acid (SCFA)-CoA molecules or changes in the rate of lipid oxidation in the
brain (27). Notably, the protein encoded by *Acads* and its mRNA are highly expressed in the hypothalamic paraventricular (PVN) and suprachiasmatic nuclei of wild-type but not mutant mice (49). More recent evidence indicating that the *Acads* mutation has tissue-specific effects on an enzyme involved in detoxification of ketones (49) point to the complexity of its metabolic effects thus making it difficult to hypothesize which pathways may be implicated in nutrient selection.

We also considered the possibility that mice bearing the *Acads* mutation may have developed a preference for carbohydrate, rather than a primary avoidance of fat. This seems highly unlikely for the following reasons: 1) the hypoglycemia associated with the *Acads* -/- genotype is fasting-induced (52), and mice were not fasted in the present experiments; and 2) there was no strain difference in sucrose responses as shown in both the solution preference tests and lickometer tests. Furthermore, we have measured non-fasting blood glucose levels in the BALB/cByKz. *Acads* -/- and BALB/cByKz. *Acads* +/- strains and found both to have values within normal range (Smith Richards, unpublished).

The equivalent response of *Acads* +/- and *Acads* -/- mice to corn oil in the 5s brief-access taste tests contrasts with the strain difference observed during long-term exposure to dietary fat, and leads us to conclude that short chain acyl-CoA dehydrogenase deficiency does not alter immediate orosensory response to fat. Unexpectedly in the 15 s tests, a strain difference in licking response to 50% corn oil was observed; in this case, *Acads* +/- mice licked significantly more of the 50% corn oil than *Acads* -/- . Mice were naïve to corn oil at the start of this test (see Table 2), thus their response to 50% corn oil was guided solely by cues received during the previous four, 15 s presentations in that test session. When a descending concentration series was performed in an additional cohort of naïve mice, no strain difference
was observed, i.e., both strains licked similar amounts of corn oil across all concentrations. Collectively, these results indicate that in brief-access tests of this duration, taste responsiveness to corn oil depends on both the concentration order and the length of the presentation. Either aspect of the experimental design could alter behavioral responses by invoking orosensory or postingestional, including pre-absorptive, effects.

That the difference in presentation length (5 s vs. 15 s) could alter the potential postingestive effects of stimuli seems unlikely because the minimum time elapsed from beginning to end of a concentration series would total 2.4 min compared to 3.25 min, respectively, taking into account the 30 s inter-presentation intervals and the average kcal intake from corn oil in ascending test series employing 15 s or 5 s presentations, i.e., 0.25 kcal versus 0.21 kcal, respectively. This indicates that only ~0.077 kcal/min would have cleared in the 3.25 min the mice were ingesting corn oil under the 15 s condition. This rate is comparable to data from Horn et al. (19) showing gastric emptying rates of ~0.08 kcal/min during the first 30 min after corn oil ingestion, an amount that did not affect subsequent food intake until 90 min later. The average energy intake from consumption of corn oil in the ascending series represents approximately 1.5% of total daily energy intake based on the results from experiment 1 (15 kcal/day). Although this amount is comparable to the caloric dose of linoleic acid found to inhibit sham feeding when infused into the small intestine (14), corn oil would have provided less linoleic acid than the same dose of pure fatty acid, and there is evidence that triglycerides are less satiating than pure fatty acids (13). Moreover, due to the very low level of gastric lipase activity in the mouse (9), it seems unlikely that free fatty acids would have been available to activate postingestive consequences, especially within such a short time period. These collective observations suggest that in the brief-access lick tests, a session length of 2.4 to 3.25 min may
not have been long enough for the ingested oil to empty from the stomach, undergo enzymatic digestion by gastrointestinal lipases, and reach sensitive intestinal sites. Thus postingestive effects may have been minimal.

It is possible that orosensory activation by corn oil may have contributed to results of the ascending concentration series. Oils have orosensory effects that can stimulate intake in the absence of postingestive metabolic effects, as demonstrated by studies of sham feeding in rats (25). This mechanism is sensitive (0.78% corn oil stimulated more intake than water), and the concentration curve shows an inverted U-shape over a range of 0.78 to 100% (25). The mechanisms underlying the oral response to oil may include chemosensory (11, 22) olfactory (30), and trigeminal (textural) (35) systems. Studies examining a basis for the chemosensory detection of fat have demonstrated gustatory cues for fats through transduction mechanisms for free fatty acids in taste receptor cells (11), and through the action of lingual lipase to release free fatty acids from dietary triacylglycerides within 1-5 s (22). These findings support the possible chemical detection of fat in our 5-15 s brief-access tests with corn oil. There is no evidence to date that a mutation in *Acads* could result in changes in oral lipase activity. Thus it remains unknown whether this defect could affect enzymatic degradation of fat in the oral cavity and thereby alter the orosensory perception of fat.

The ascending series order used in the present experiment was chosen originally based on evidence for lack of an order effect in brief access tests (45). However in the present experiment, 50% corn oil stimulated higher a lick count by the non-mutant *Acads* +/- strain in the ascending series than it did with descending concentrations in separate groups of naïve mice. This result may reflect a successive negative contrast effect (15). This finding differs from an earlier report that the number of licks per 30 s in rats increased as a monotonic function of
concentration regardless of the order of stimulus presentation (45). Possible explanations for these contrasting results include differences in the species and experimental methods used, e.g., the rats received previous exposure to at least one of the stimuli (carbohydrate solutions) during lick training, the same animals were used in all test series, and the design was not balanced to control for all possible carryover effects (45).

The brief-access lick test has several advantages for assessing taste responses in mice (see 12) including the ability to capture the behavioral response to hedonic characteristics of stimuli. Until recently, postingestive effects in 3-min (7) or 30 s (45) access tests were considered minimal or nonexistent for lack of adequate quantity of the stimulus solution in the gastrointestinal tract to induce a negative feedback signal. On the basis of current literature, tests of 30 s (44) or 5-15 s (5, 12) duration are thought to be sufficiently short intervals for discriminating between the orosensory and postingestive effects of the taste stimulus.

Our findings indicate that short-chain acyl-CoA dehydrogenase deficiency in Acads -/- mice gives rise to behavioral avoidance of dietary fat, but not carbohydrate, when the fat source contains >95% long chain fatty acids. This observation provides further evidence for the metabolic control of feeding. The mechanism controlling fat consumption in this model has not been determined but the results point to a signal arising from the postingestive consequences of fat intake and not from an acute effect of Acads on taste. Notably we have shown that this signal initiates a behavioral change in macronutrient diet selection to limit the consumption of dietary fat, but does not appear to signal decreased nutrient availability which would result in a positive feedback on total calorie intake. This genetic model offers a new tool for studying the role of fatty acid oxidation in regulating food choice.
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REFERENCES


FIGURE LEGENDS

Figure 1. Macronutrient diet intake in kilocalories (kcal) of Acads +/+ (top) and Acads -/- (bottom), mice self-selecting from a choice between carbohydrate/protein (○) and fat/protein (●). Data points represent mean ± SE for this and subsequent figures. n = 15-16 mice per strain. †P < 0.05, *P < 0.005.

Figure 2. Total daily kcal (top) and percent fat intake (bottom) of Acads +/+ and Acads -/- mice. Diet presentation began on day 0 and food intake was measured daily. †P < 0.05, *P < 0.005, Tukey-Kramer. ●, Acads -/-; ○, Acads +/+.

Figure 3. Mean 48 h preference ratios for 50% corn oil emulsion versus vehicle (A) and sucrose versus water (B) in two-bottle tests lasting 4 d. Analyzing the data as 48 h ratios eliminates the possible effects of tube position (right vs. left). Equal consumption of both solutions yields a preference ratio of 0.50 (see dotted line). n = 7 animals per strain. * P < 0.005, Acads +/+ vs. Acads -/-.

Figure 4. Rates of licking for water in response to 23-h water deprivation expressed as frequency distributions of the ILI. Data were collected on the 1st day of sipper tube training. The presentation length was 10 min. ●, Acads -/-; ○, Acads +/+; n = 8-9 animals per strain.

Figure 5. Number of licks per 15 s for ascending corn oil emulsions in trials 1 and 2, conducted on consecutive days. n = 8-9 mice per strain in this and subsequent figures. ●, Acads -/-; ○, Acads +/+. *P < 0.05.
Figure 6. Number of licks per 5 s for ascending corn oil emulsions in trials 1 and 2, conducted on consecutive days. The 5 s test was conducted one month after the 15 s corn oil test (see Table 2 for sequence of tests). ●, Acads -/-; ○, Acads +/+.

Figure 7. Number of licks per 15 s for ascending sucrose solutions in trials 1 and 2 conducted on consecutive days. ●, Acads -/-; ○, Acads +/+.

Figure 8. Number of licks per 5 s for ascending sucrose solutions in trials 1 and 2 conducted on consecutive days. ●, Acads -/-; ○, Acads +/+.

Figure 9. Number of licks per 15 s for corn oil emulsions in a descending concentration series, conducted in an additional cohort of naïve mice. Trials 1 and 2 were conducted on consecutive days. n = 14 mice per strain. ●, Acads -/-; ○, Acads +/+.
Figure 1

*Acads* +/+  
- **Fat/protein**  
- **Carbohydrate/protein**

*Acads* -/-

Food intake (kcal) over days 1 to 10.
Figure 2

Total Calorie Intake

Fat/Protein Diet Intake

\[ \text{Kcal} \]

\[ \% \text{ total kcal} \]

Day
Figure 3

**50% Corn Oil**

- **Acads +/+**
- **Acads −/−**

**4% Sucrose**

Days 1-2 and Days 3-4 preference ratio comparisons.
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9