Bottle-choice Tests in Sprague-Dawley Rats Using Liquid Diets That Differ in Oil and Sucrose Contents

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Bottle choice tests using liquid diets were done with Sprague-Dawley (SD) rats. SD rats ingested more oil-and-sucrose-enriched milk (hi-fat) and less oil-enriched milk (hi-fat-no-carb) than sucrose-enriched (hi-carb) milk by two-bottle choice tests after they were habituated to liquid diets for 4 days. Chronic food restriction didn’t increase hi-fat ingestion but hi-fat-no-carb. Rats ingested less without habituation, and overnight food deprivation increased intake. This increment was maintained after rats were free-fed. The difference in fat content of the maintenance diet had little effect on fat preference. These results showed SD rats prefer a sweet and fatty liquid diet than a sweet and lean liquid diet. Habituation and food restriction were more important than the composition of the maintenance diet to demonstrate a clear preference for the fatty liquid diet.

Key words: food preference; optional food; bottle choice test; rats; high fat diet

Fat is believed to be one of the most important factors of sensory appeal in foods, although triglyceride, the main constituent of dietary fat, has a bland taste. Texture, sensory properties, the flavor of fat-soluble volatile compounds, and postabsorptive metabolic effects might be reasons for the appeal of fat.¹–³ On the other hand, the palatability, high energy density, and postabsorptive processing of fat are thought to be causes of hyperphagia and obesity.²–⁷

Human studies have indicated that obese people prefer fatty foods and ingest more fat.⁸–¹⁰ There is a hypothesis that fat becomes more attractive with repeated ingestion of fatty foods. Studies about intravital or behavioral reactions induced by a high fat diet, like changes of biochemical pathways,¹¹–¹⁴ central opioid activity,¹⁵–¹⁷ and learned flavor-calorie associations¹⁸,¹⁹ would explain the theory. However, there is little direct evidence to prove fatty foods increase the appetite for fat. Reed et al. indicated that rats raised with high fat food ingested more fat than controls.¹⁰ However, as a considerably high caloric percentage (more than 60 kcal %) of fat was used as a component of the maintenance diet, it is not clear whether to trigger an appetite for fat, a high fat composition or lack of carbohydrates is more important. Warwick and Synowski reported that Long-Evans (LE) rats maintained on fat-enriched laboratory chow (48% kcal from fat) ingested more high-fat liquid diets than controls in a 30-min two bottle choice test.²¹ This is the sole report indicating an increased preference for fat caused by a realistic high fat food, as far as we know.

The bottle-choice test that measures voluntary fluid intake of animals is one of the most common methods for studying ingestive behavior. A 30-min bottle-choice test has frequently been used to study fat appetite. Rats given a test solution would cease ingesting in 30 min. So the results of a 30-min bottle choice test are considered to indicate the consequence of appetite for the test solution and negative-feedback effects by ingested solution (post-ingestive factors). The liquid diet used by Warwick and Synowski was equicaloric sucrose-enriched milk and oil-and-sucrose-enriched milk.²¹ Both solutions contained sucrose that rats innately prefer. So they would be innately preferred by rats and the main difference between them was fat contents.

The SD strain of albino rat is the most frequently used animal for the study of feeding behavior tests. However, there is little information about a bottle-choice test using nutrient-enriched milk by SD rats. So we checked their behavior toward isocaloric oil-and-sucrose-enriched milk (hi-fat) and sucrose-enriched (hi-carb) liquid diets using the same procedure as Warwick and Synowidki.²¹ Then we investigated the effects of the maintenance diet’s composition on fat preference, using a modified method.

Methods

Subjects. Male SD rats (Japan SLC, Hamamatsu, Japan) were used for all experiments. Ages of rats were depended the age at which the choice test begun

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Table 1. Composition of High Fat and Low Fat Diets

<table>
<thead>
<tr>
<th></th>
<th>High fat</th>
<th>Low fat</th>
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<tr>
<td>Crude protein</td>
<td>21.6</td>
<td>21.6</td>
</tr>
<tr>
<td>Crude fat</td>
<td>25.4</td>
<td>4.0</td>
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<tr>
<td>Fiber</td>
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<td>3.8</td>
</tr>
<tr>
<td>Ash</td>
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<tr>
<td>Carbohydrate</td>
<td>43.6</td>
<td>65.0</td>
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<tr>
<td>kcal/100 g diet</td>
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<tr>
<td>Total dietary energy</td>
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<td>37.0</td>
</tr>
<tr>
<td>kcal/100 kcal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>17.0</td>
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</tr>
<tr>
<td>Carbohydrate</td>
<td>36.0</td>
<td>68.8</td>
</tr>
<tr>
<td>Fat</td>
<td>47.0</td>
<td>9.4</td>
</tr>
</tbody>
</table>

Diet composition was made by adding fat (high fat) or starch (low fat) to the CLEA Rodent Diet CE-2 in the proportion of one part to four. The fat was a mixture of soy oil (The Nishin OillO Group, Ltd., Tokyo, Japan) and lard (Ashall Denka Co., Ltd., Tokyo, Japan). The fatty acids composition of each diet was HF: 19.3% (C16:0), 7.5% (C18:0), 32.5% (C18:1), 32.97% (C18:2), 4.43% (C18:3); LF: 19.8% (C16:0), 3.4% (C18:0), 22.4% (C18:1), 42.7% (C18:2), 4.94% (C18:3).

Maintenance diets. A commercial diet (F-2, Funabashi Farm, Funabashi, Japan) was used in the acclimatization period and Experiments 1. The macronutrient composition of the diet was 5.2% crude fat, 63% carbohydrate (as nitrogen-free extract), and 23% crude protein (expressed by percent weight as dry matter).

A chow-starch diet for the low-fat group (LF) and a chow-oil diet for the high-fat group (HF) were used as maintenance diets in Experiments 2. The compositions of these diets were slightly modified from Warwick and Synowski. Namely, starch was added to CLEA Rodent Diet CE-2 (CLEA Japan, Inc., Japan) to reduce the difference in protein concentration between the two diets, and corn oil was replaced with a mixture of soy oil and lard (1:1) as a source of fat for the chow-oil diet. The ingredients and macronutrient composition are listed in Table 1.

Test solutions. The test solutions were prepared as described by Warwick and Synowski. In brief, extra carbohydrate and/or fat emulsion were added to the evaporated milk. They contained similar amounts of protein and energy (2.3 kcal/ml). There were several variations in composition and constituent; i.e. soybean lecithin was used as an emulsifier instead of sodium stearoyl lactylate. The formulas of test solutions are listed in Table 2.

Bottle choice test. Food and water were removed one hour before the test to minimize the influence of accidental intake. The bottle choice test was started at 1200 h unless specified otherwise by the experimental procedure. Test fluids were presented in 20-ml plastic tubes equipped with a leakage-free stainless steel drinking spout. A spout was fixed to the front of each cage. The 30-min intake of each solution was measured by weight, converted to volume by gravity, and expressed as a result.

Statistical analysis. The test solution intake was expressed as the mean ± standard errors. The preference was defined as a percentage that is total fluid intake as one of a pair of values. They were expressed as the median with an interquartile range, because the preference would not follow the Gaussian distribution and tended to be distributed randomly when rats failed to differentiate between two samples. A nonparametric test (The Wilcoxon Signed Rank Test) was done to find whether the median of the percentage is different from the hypothetical median (50%). Differences between means in intakes of two test solutions were assessed with Student’s paired t tests. General Linear Model Repeated Measures (GLM-RM) using SPSS Advanced Models 11.0J for Windows (SPSS Inc., Chicago, IL) was used for comparison of test solution intake between two groups. GLM is a general procedure for analysis of variance in SPSS. The MANOVA program was used.
for the analysis of a simple effect following significant interaction.

Experimental

Experiment 1.

Bottle-choice test under the varied conditions.

Experiment 1a. In experiment 1a, we studied a bottle-choice test with the same procedure as that of Warwick and Synowski.21) Six 6-week-old rats were acclimated for 5 days. In the following 4 successive days, they were given 15 ml of the two test solutions (hi-fat and hi-carb) for 24 hr for habituation in addition to food and water. Two days after this acclimation period, two-bottle choice tests between hi-fat and hi-carb were done for 3 successive days. Then, one-bottle choice tests were done for 4 successive days. The test solution was hi-fat or hi-carb every two days. The order in which the test solution was given was counterbalanced like AABB or BBAA.

Then a two-bottle choice test between hi-fat-no-carb and hi-carb was done for two days. Test solution intakes were compared by a paired t-test (hi-fat and hi-carb, hi-fat-no-carb and hi-carb).

Experiment 1b. In this experiment, we studied the effects of chronic food deprivation on the choice. Eight 15-week-old rats were given two-bottle choice tests. Before the experiment, they were fed 17 g (about 75% of free-fed rats) of the maintenance diet a day for 6 weeks. This food restriction continued during the experiment. The experimental schedule is outlined in Table 3. Test solution intakes were compared by paired t-tests.

Experiment 1c. In this experiment, we studied the effects of an overnight food deprivation on the choice. Six 6-week-old rats were acclimated for 1 week and then two-bottle choice tests were done. The experimental schedule is outlined in Table 4. The two-bottle choice test in the dark period began at 20:00.

Test solution intakes were compared by GLM-RM using within-subject factors of test solutions (hi-fat and hi-carb), experience of food deprivation (before and after), and period (light period and dark period).

Experiment 2.

The effects of fat content of maintenance diet on bottle-choice test.

Experiment 2a. In this experiment, we studied the effects on preference with rats with a short habituation before the test (innate preference). Sixteen 3-week-old rats were acclimated to the laboratory for 5 days. On the sixth day, they were given the two test solutions (hi-fat and hi-carb) for 6 hr for habituation in addition to food and water. All rats ingested the test solutions. Thirty minute two-bottle choice tests between hi-fat and hi-carb were done on the seventh day for grouping. Then, rats were divided into two groups with equal values of weight, maintenance food intake, test solution intake, and preference. The high fat group (HF) was fed a chow-oil diet, while the low fat group (LF) was fed a chow-starch diet.

Sixteen days later, a two-bottle choice test between hi-fat and hi-carb was done for 5 successive days. Test solution intakes were compared by GLM-RM using between-subject factors of dietary conditions (HF vs. LF) and within-subject factors of days and test solutions (hi-fat and hi-carb).

Experiment 2b. In this experiment, we studied the effects on preference with rats reinforced in their ingestion volume by an overnight food deprivation. Sixteen 3-week-old rats were acclimated to the laboratory for 4 days. From the fifth day, rats were given diluted evaporated milk for 3 days. Then, they were divided into two groups with equal values of weight, maintenance food intake, and evaporated milk intake. The HF was fed the chow-oil diet, while the LF was fed the chow-starch diet.

Four weeks later, rats were given a two-bottle choice test 3 times. The first and second trials were done after the rats were deprived of food overnight at two-day intervals. The third trial was under free-fed conditions after a two-day interval.

Subsequently, two-bottle choice tests using a less palatable fatty solution were done, as SD rats tended

| Day 1 Day 2 | One-bottle | hi-fat or hi-carb |
| Day 3 Day 4 | Two-bottle | hi-fat vs. hi-carb |
| Day 5 Day 6 | Two-bottle | 14% sucrose vs. 39% sucrose |
| Day 7 Day 8 | Two-bottle | hi-fat-no-carb vs. hi-carb |

| Day 1 | Dark | Free fed |
| Day 2 | Light | Free fed |
| Day 3 | Light | Deprived |
| Day 4 | Light | Free fed |
| Day 5 | Dark | Free fed |
| Day 6 Day 7 | Light | Free fed |
| Day 8 Day 9 | Light | Free fed |

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<tr>
<th>Period</th>
<th>Condition</th>
<th>Choice test</th>
<th>Test solution</th>
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<tbody>
<tr>
<td>Day 1</td>
<td>Dark</td>
<td>Two-bottle</td>
<td>hi-fat vs. hi-carb</td>
</tr>
<tr>
<td>Day 2</td>
<td>Light</td>
<td>Two-bottle</td>
<td>hi-fat vs. hi-carb</td>
</tr>
<tr>
<td>Day 3</td>
<td>Light</td>
<td>One-bottle</td>
<td>hi-fat or hi-carb</td>
</tr>
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<td>Day 4</td>
<td>Light</td>
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<tr>
<td>Day 5</td>
<td>Dark</td>
<td>Two-bottle</td>
<td>hi-fat vs. hi-carb</td>
</tr>
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<td>Day 6 Day 7</td>
<td>Light</td>
<td>Two-bottle</td>
<td>hi-fat vs. mid-fat</td>
</tr>
<tr>
<td>Day 8 Day 9</td>
<td>Light</td>
<td>Two-bottle</td>
<td>hi-fat-no-carb vs. hi-carb</td>
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to ingest hi-fat more than hi-carb. Namely, rats were given a choice between hi-fat-no-carb and hi-carb for three days.

Results and Discussion

Experiment 1.

Bottle-choice test under the varied conditions.

At first, SD rats were put through a bottle-choice test using several liquid diets under the well-habituated conditions (Experiment 1a), food restricted conditions (Experiment 1b), and the habitation with overnight food deprived and subsequent liquid diet provided (Experiment 1c).

Experiment 1a. Rats were habituated to two test solutions (hi-fat and hi-carb) for 4 days. The results of the following bottle choice tests are shown in Fig. 1. The results for the first day of the test comprised significantly low values, probably because the rats had yet to adapt to the change in the duration of the test (24 hr to 30 min), and so were omitted. The one-bottle choice test shows the rats ingested hi-carb too but significantly less than hi-fat. The two-bottle choice test shows SD rats ingested almost entirely the hi-fat test solution. The result of the two-bottle choice test contradicted a previous finding in LE rats. In that report, intake values of both solutions were similar in the chow group in the two-bottle choice test. As an extremely slanted solution intake for hi-fat was inappropriate for the following test, we arranged the composition of the test solution (hi-fat-no-carb). Hi-fat-no-carb didn't content sucrose that was strongly preferred by rats and would be a trigger of test solution ingestion. In this case, rats significantly ingested more hi-carb.

This proved that a long habituation period poses a problem. Namely, the average solution intake for the 4 days was 13.6 ± 0.73 mL/24 hr (hi-fat) or 12.2 ± 1.51 mL/24 hr (hi-carb). Food intake during this period was decreased to 62.9% compared to just before the habituation period. This diminution (28.6 ± 2.9 kcal) was less than the total caloric intake of test solutions (59.8 ± 3.9 kcal); rats over ate in this period. The ratio of ingested fat to total caloric intake also changed (F=2; 11.6 cal%, average of habituation period; 27.4 ± 0.7 cal%).

Experiment 1b. As several reports indicated food restriction increases the preference for fats in rats under the food-restricted condition, rats were restricted in food to 75% of free-fed rats in this experiment (Fig. 2). Results of the one-bottle choice test showed a tendency for the hi-fat solution to be ingested more than the hi-carb solution although there wasn’t statistical significance (P = 0.368, df = 7, paired t test). The two-bottle choice test showed a significantly high hi-fat intake. However, hi-carb was also ingested. The sucrose concentration of the hi-carb solution was very high (39%), which may be one of the reasons for the greater intake of hi-fat solution. So a two-bottle choice test with sucrose solutions (14% vs. 39%) was done. These concentrations corresponded to the sucrose concentration of each test solution. Rats ingested the more concentrated (39%) sucrose solution in this case. This result showed rats prefer the highly concentrated sucrose solution. The result of the one-bottle choice test showed that acceptance of the hi-carb solution was almost the same as for the hi-fat solution. So avoidance or saturation for sucrose would not account for the lower intake of hi-carb solution. That is, these results confirmed that SD rats prefer the hi-fat solution to hi-carb.

Rats significantly ingested more hi-carb than hi-fat-no-carb in experiment 1a. In this experiment, both solutions were ingested (P = 0.76, df = 7, paired t test). So, the food restriction condition didn’t increase the preference for hi-fat but influenced the intake of hi-fat-no-carb, which is the less palatable liquid diet.

Experiment 1c. In this experiment, naive rats were put through the two-bottle choice test at first. In both the light and dark periods, intakes of the two solutions were low before the one-bottle-choice test with food deprivation (Fig. 3A). Then, rats were deprived of their food overnight and did a one-bottle choice test. The results were 6.17 ± 3.06 mL (hi-fat group) and 5.43 ± 0.91 mL (hi-carb group). Subsequently, two-bottle-choice tests were done again (Fig. 3B). GLM-RM indicated a significant interaction of the experience of food deprivation × period (F(1, 5) = 22.78, P < 0.005). Simple effects for the experience of food deprivation were significant in the light period (F(1, 5) = 20.00, P = 0.007) and dark period (F(1, 5) = 38.13, P = 0.002). So it could be concluded that the one-bottle choice test under the food-deprived conditions triggered active solution intake. Simple effects
for the period were significant after the experience of food deprivation (F(1, 5) = 32.12, P = 0.002). That is, the intake of solution was greater in the dark period than light period. Although the intake in the dark was greater, the difference was not extreme and there were several advantages with the light period (like little maintenance of food intake and identity with previous reports), so we used the light period for experiment 2.

Subsequently, two-bottle choice tests between hi-fat and mid-fat solutions were done and more hi-fat solution was found to be ingested. Then two-bottle choice tests between hi-fat and hi-fat-no-carb solutions were done and there was no significant difference in this case (P = 0.90, df(5), paired t test) (Fig. 3C). These results show SD rats preferred fatty solutions but some amount of sucrose was needed.

**Experiment 2.**

The effects of fat content of maintenance diet on bottle-choice test.

Experiment 1 indicated SD rats prefer hi-fat to hi-carb, and habituation and food restriction affect the ingestion value of liquid diet. In this experiment, we studied the effects of fat content of maintenance diet on the choice test.

**Experiment 2a.** Rats were habituated to the test solution for only 6 hr and all rats ingested test solution in this period. The results of a five-day two-bottle choice test were analyzed and GLM-RM indicated a significant effect of groups (F(1, 14) = 14.9, P < 0.002) and test solutions (F(1, 14) = 7.3, P < 0.017), respectively. As days didn’t have an effect, the results for the 5 days were averaged (Fig. 4). Hi-fat was ingested more than hi-carb in both groups. Total solution intake was greater for the HF group than LF group (HF: 4.1 ± 1.6 ml/30 min, LF: 2.5 ± 1.1 ml/30 min, P = 0.017, df = 14, unpaired t test).

This experiment shows the rats innately ingested the liquid diet and preferred hi-fat to hi-carb, HF rats ingested more liquid diet than LF rats. But the ingestion value was less than that of rats well habituated to the test solutions beforehand.

**Experiment 2b.** Experiment 2a indicated the HF rats ingested more liquid diet. However, the ingestion volume was very small. Experiment 1c shows that the experience of solution intake after an overnight food deprivation triggered intake of the following bottle choice. In this experiment, rats were triggered to ingestion of the liquid diet when an overnight food deprivation was used.

Overnight food deprivation increased solution intake and more hi-fat was ingested than hi-carb, significantly, in the second trial in the HF group (Fig. 5A). On the other hand, LF group rats also increased their intake after food deprivation but there was no difference between the two solutions. Then, more hi-fat was ingested under the free-fed

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**Fig. 2.** Bottle Choice Test Using Food-restricted Rats (experiment 1b).
Dark gray bars: hi-fat, light gray bars: hi-carb, striped bar: 14% sucrose solution, open bar: 39% sucrose solution, filled bar: hi-fat-no-carb. *Significant difference between test solutions, p < 0.05. Values are means and standard errors (n = 8).

**Fig. 3.** The Effect of Overnight Food Deprivation on Test Solution Intakes (experiment 1c).
A: before the overnight food deprivation, BC: after the one-bottle test under the overnight food-deprived conditions. Dark gray bars: hi-fat, light gray; hi-carb, netted bar; mid-fat, filled bar; hi-fat-no-carb. *Significant difference between test solutions, p < 0.05. Values are means and standard errors (n = 6).
condition in both groups (Fig. 5B).

The results of the two-bottle choice test between hi-fat-no-carb solution and hi-carb solution were not significant in either group (Fig. 6 upper). Although the intake values of the groups look similar, the distribution of preference for the hi-fat-no-carb solution was little different between the groups. Namely in the HF group, it was randomly distributed from 0% to 100% in the first trial but then showed partiality to 100%. On the other hand, the median of the preference of the LF group remained around 50% (Fig. 6 lower). However, there was no significant difference between the two groups, or each median of preference to the hypothetical median (50%) by the Wilcoxon Signed Rank Test in the groups. From these results, there was likelihood that HF rats prefer fatty solutions more than LF rats, the difference was too small to show statistical significance.

SD rats preferred a fatty solution (experiment 1a) when the liquid diet contained some sucrose. This result contradicted previous findings using LE rats. In that report, intake values of both solutions were similar in the chow group. The main point was that SD rats ingested less hi-carb. Fregly and Rowland reported that LE rats ingested more of a 5% glucose solution than SD rats in a 24-hr two-bottle choice test with water. LE rats probably prefer sucrose more than SD rats or have a higher tolerable limit for sucrose, so they preferred hi-carb as well as hi-fat. Meanwhile, a preference for fat hasn’t been reported as far as we know, although studies of strain differences in taste preferences or fluid ingestion behavior between SD rats and LE rats were published. So further study would be needed to confirm strain differences in fat preference.

The two-bottle choice test under food-restricted
conditions shows a relatively high hi-carb intake. This was unsurprising because rats ingested very much hi-carb in the one-bottle choice test of experiment 1a. As the hi-fat intake was similar to that in other experiments (experiment 1a, 1c, 2b), there must be some upper limit for hi-fat intake in a 30-min bottle-choice test. This assumption agreed with our previous report.20 In that, we did two-bottle choice tests between various concentrated oil emulsions and water under food-restricted conditions and suggested the ingested oil itself manipulated the negative feedback. So rats in experiment 1b probably were saturated with hi-fat and ingested hi-carb.

SD rats innately ingested test solutions (experiment 1c). However, the intake value was less than in former experiments. Rats often refuse optional foods when they are provided for the first time (neophobia). In this case, the average of total ingested solution was 2.50 ± 0.69 ml (dark phase) while the average 30-min water intakes of SD rats measured by the same technique was less than 2 ml.20 When rats were offered liquid diets for 24 hours, all rats ingesting liquid diets for the first time (experiment 1a; habituation period) decreased maintenance food intake to 62.9%. Although rats ingest less food during the light phase, these rats ingested 2.01 ± 0.56 ml of liquid diet and its energy corresponded to about 6 cal % of daily food intake. This data indicated that naive rats also prefer the liquid diet. So, less intake of liquid diet of rats without habituation might not be caused by neophobia but less interest.

On the other hand, some stimulation like long habituation or food deprivation would evoke a vigorous intake of liquid diet. The important thing in this case is that rats ingested highly calorific liquid diet during short test duration (30 min) in spite of it being light phase. So habituation or food deprivation might be related to acquisition of a stronger preference for the fatty liquid diet or to the upper limit of a 30-min ingestion.

As sucrose was the sole additive to the hi-carb solution while the hi-fat solution contained both sucrose and oil, it is possible that the preference for hi-fat can be accounted for by the mixture of energy sources rather than an attraction to the oil itself. The result of two-bottle choice tests between hi-fat and mid-fat (experiment 1c) ruled out this assumption. On the other hand, the two-bottle choice test between hi-fat-no-carb and hi-carb didn't show a one-sided preference. Therefore SD rats prefer a fatty solution but a certain amount of sucrose is needed to evoke a clear preference.

The naive HF group ingested more of both liquid diets than the LF group (experiment 2a). The HF group showed a slight tendency to prefer fatty solutions compared to the LF group (experiment 2b). So the effect of maintenance diet composition on fat preference, if it exists, is very small. Recently, Cooling and Blundell reported habitual food selection (high fat phenotype or low fat phenotype) is not related to taste preference in young adult males.20 They concluded that other factors such as habit and social desirability are equal or more important to food selection than taste sensation.

In our study, the fat content of the maintenance diet would not clearly affect on the fat preference. Rather, other factors like habituation seem to be more important.

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References


