The Effect of Dietary Fatty Acid Composition on Food Choice Using the Bottle-Choice Test in Rats

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Abstract: Feeding behavior is thought to be one of the most important homeostatic mechanisms. Several studies have indicated that when animals are deprived of a certain amount of nutritional elements, they ingest more to compensate for the loss. In this study, we investigated whether unbalanced fatty acid composition of maintenance diet affects the choice of fatty acids. Rats were divided into two groups and fed a polyunsaturated fatty acid diet (P-rich diet) or a monounsaturated fatty acid diet (M-rich diet). To habituate the animals to the test procedure, a three-bottle-choice test using one bottle of a 2% sucrose solution and two bottles of water was conducted. Rats in the M-rich group tended to ingest more sucrose solution than rats in the P-rich group. In the test using emulsions of three kinds of triacylglycerol, rats of the M-rich group tended to ingest less. The difference in the fatty acid composition of the triacylglycerol used as the test solution didn’t affect the choice. A two-bottle-choice test between oleic acid (OA) and linoleic acid (LA) showed that rats in both groups ingested more LA and rats in the M-rich group preferred LA. A two-bottle-choice test between LA and linolenic acid (LN) did not produce a statistically significant outcome. The results indicate that dietary fat might affect feeding behavior in rats. However, the difference was very small, particularly when the oil used for the choice test was provided as a triacylglycerol.

Key words: bottle choice test, food selection, feeding behavior, ingestion value, dietary fatty acid

1 Introduction

Fatty acids that constitute dietary fat can be classified according to degree of unsaturation, i.e.; saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). Animals can synthesize SFA and MUFA but not n-6 and n-3 PUFA. So dietary PUFA is the sole source with which to regulate internal PUFA levels and referred to as essential fatty acid (EFA) (1,2).

Several studies have shown that when animals are deprived of a certain amount of nutritional elements like essential amino acids, sodium and calcium, they ingest more to compensate for the loss (3-9). Thus, feeding behavior is one of the most important homeostatic mechanisms. Yamamoto and Marumatsu studied whether rats can recognize the EFA in a diet using EFA-deficient animals (10). However, the results did not suggest an ability to select the EFA-containing diet to meet the EFA requirement.

There seem to be several difficulties in showing the
relation, if there is one, between EFA deficiency and the selection of EFA-rich foods by choice test. Triacylglycerols are generally considered to have a bland taste because the stimulation by fat is weaker than that by other taste stimuli, although there is some evidence that fats are perceptible orally (11,12). It might therefore be difficult to distinguish differences in the fatty acid composition of food orally. Besides, the term “EFA deficient” is ambiguous because there is a wide gap between a reduction in the reserves of EFA and the development of an EFA-deficient model (13). Severe EFA deficiency might cause some disadvantages in fat absorption (14) and could affect the results of choice tests. In addition, SFA which is frequently used to cause EFA deficiency is indicated to affect feeding behavior (15).

In this experiment, we reconsidered the relation between the fatty acid composition of daily diets and food choice. Rats were divided into two groups and given a daily diet rich in either polyunsaturated fatty acid (P-rich diet) or monounsaturated fatty acid (M-rich diet). The M-rich diet was not a severe EFA-deficient diet so that the malabsorption of oil caused by EFA-deficiency could be avoided. For the food choice tests, we used the bottle-choice method which measures voluntary optional fluid intake and is one of the most common methods of studying ingestive behavior. This approach is useful for estimating the intake of fat because the solutions have a simple composition and are free from the effect of other nutritive components that affect palatability and satiation. A 0.5% oil emulsion was used as the selectional solution because at this concentration, the emulsion was dense enough to be distinguished from the vehicle and dilute enough not to disturb the daily fatty acid concentration unless rats ingest it unusually much (16). Free fatty acid is used as solute because rats prefer long-chain fatty acid to its derivatives in short-term (5 min) two-bottle choice tests (17). This result indicated fatty acids are more detectable by rats than triacylglycerols. Each test lasted for 3 hr in this study, so rats had enough time to ingest as much emulsion as necessary. In this study, we investigated whether the fatty acid composition of daily diets affects the selection of optional foods.

2 Experimental

2.1 Animals and Diets

Twenty 3-week-old male SD rats (Japan SLC, Hamamatsu, Japan) were divided into two groups (P-rich and M-rich, n = 10) on the day of their arrival. The animals were maintained with free access to the experimental diet and water throughout the experiment. The experimental diet was a modified AIN 93G diet (18), i.e.; 50 g/kg fat, 549.5 g/kg α-potato-starch, 200 g/kg casein, 100 g/kg sucrose, 50 g/kg cellulose, 35 g/kg mineral mix (AIN-93G), 10 g/kg vitamin mix (AIN-93), 3 g/kg L-cystine and 2.5 g/kg choline bitartrate. The fat in the P-rich diet was a mixture of plant oils (soy oil, perilla oil and hydrogenated palm kernel oil) including 16.9% SFA, 22.7% MUFA and 58.1% PUFA (n-6/n-3 = 5.0). The fat in the M-rich diet was high oleic canola oil including 41.6% SFA, 48.0% MUFA and 10.0% PUFA (n-6/n-3 = 3.0). The rats were housed individually in a standard wire mesh cage under a 12:12 h light: dark cycle (lights off at 2000 h) throughout the experiments. All procedures were done according to the Guideline for Animal Experiments of Tohoku University (Decision of the university’s president, March 24, 1988, revised September 13, 1994).

2.2 Test Solutions and Bottle Choice Test

Triolein (99%), OA (99%), LA (98%) and LN (60%) were from Wako Pure Chem. Ind., Ltd. (Osaka, Japan) while trilinolein (95%) was from Tokyo Kasei Kogyo Co., Ltd. (Tokyo Japan). 0.5% (w/w) oil was emulsified in a 0.03% gum arabic (Nacalai Tesque Inc., Kyoto, Japan) solution by sonication. The emulsion was freshly prepared every day before the test. Food and water were removed 6 hours before and returned immediately after the experiment. Test solutions were presented in 20mL measuring cylinders equipped with a leakage-free stainless steel drinking spout (SE nozzle, Musashi Co., Saitama, Japan). Spouts were fixed to the front of each cage with springs. Amounts ingested were measured by reading the measuring cylinder once every hour. The test was conducted over two or three consecutive days. The data from the first trial was omitted because rats often refuse foods when provided them for the first time (neophobia) or ingest abnormally large amounts after several days of rest.


2.3 Habituation to the Test Process
To habituate the animals to the test procedure, a three-bottle choice test was performed for two consecutive days when the rats reached 6 weeks of age. Briefly, rats were provided with three bottles from 1500 to 2300 h. One bottle contained 2% sucrose solution (w/w) and the others water. After 5 days, three-bottle choice tests using triolein, trilinolein and perilla oil (53% LN) were conducted for two days from 2100 to 2400 h.

2.4 Two-Bottle Choice Test
When the rats reached 8 weeks of age, two-bottle choice tests between OA and LA were conducted for three consecutive days. After a 5-day interval, two-bottle choice tests between LA and LN were performed for three days. The test was carried out from 2100 to 2400 h.

2.5 Statistical Analysis
The intake of solution was expressed as the mean ± SE. The preference that is the percent intake of total solution as intake of emulsion of LA was expressed as the median with interquartile range because the preference would not follow the Gaussian distribution and tend to distribute randomly when rats failed to differentiate between two samples. A nonparametric test (The Wilcoxon Signed Rank Test) was used to determine whether the median of the percentage is different from the hypothetical median (50%). Oil emulsion intakes were analyzed by General Linear Model Repeated Measures (GLM-RM) utilizing SPSS Advanced Models 11.0J for Windows (SPSS Inc., Chicago, IL, USA). GLM is a general procedure for the analysis of variance in SPSS. The between-subject factor was group (P-rich vs. M-rich) and within-subject factor was solute (OA vs. LA or LA vs. LN).

3 Results
3.1 Cumulate Intake of 2% Sucrose
Figure 1 shows the cumulate intake of the 2% sucrose solution with time. All rats ingested the sucrose solution and the preference for it was high (P-rich; 69 (54-86) %, M-rich; 97 (93-99) %) in the second trial. Most of the rats ingested the solution after dark. So the following test was conducted during the dark period. Rats of the M-rich group tended to ingest more of the sucrose solution.

3.2 Cumulate Intake of Oil Emulsion
Figure 2 shows the cumulate intake of oil emulsion with time. Rats were provided with three oils and showed no significant difference in preference. So the data was expressed as total emulsion intake. Rats ingested the 0.5% oil emulsions but in smaller amounts than the 2% sucrose solution. In contrast to the sucrose solution, rats of the P-rich group tended to ingest more of the oil emulsion.
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3.3 Two-Bottle Choice Test between LA and OA

GLM-RM indicated a significant effect of fatty acid emulsions (F (1, 18) = 13.42, P = 0.02). So rats ingested more of the emulsion of LA than OA. There was no significant effect between groups (Fig. 3A). In the M-rich group, the preference for LA differed significantly from the hypothetical median (50%) as determined using the Wilcoxon rank sum test. Indeed, almost all (nine) of the rats ingested more emulsion of LA than OA in the M-rich group. On the other hand, four rats ingested less emulsion of LA than OA in the P-rich group (Fig. 3B).

3.4 Two-Bottle Choice Test between LA and LN

Rats ingested both emulsions when given a choice of LA and LN (Fig. 4A) and preferences didn’t differ from the hypothetical median (50%) (Fig. 4B). The total intake of emulsions tended to be less in the M-rich group (4.63 ± 1.2 mL) than P-rich group (6.4 ± 1.1 mL), because four rats in the M-rich group didn’t ingest the test solution on the third day of the experiment.

4 Discussion

Although the effect of daily dietary fat on choice tests was ambiguous, several tendencies were observed in this study. Rats in the M-rich group tended to ingest less of the oil emulsions than those in the P-rich group. Notably, four rats in the M-rich group didn’t ingest the test solution on the third day of the two-bottle choice test between LA and LN. As they ingested more sucrose solution than water and showed a higher preference for it, negativity toward the choice test itself or inactivity would not be the reason. There might be several explanations for these tendencies like malabsorption of fat or behavioral change. However, more clear data will be need for consideration.

Rats ingested more LA than OA. This result agreed with the results of short-term (5 min) two-bottle tests using Wistar rats (17). So, rats seem to prefer LA to OA. However, this result was somewhat unexpected because the LA emulsion used here had a slight bitter taste. Wieser et al. reported emulsions of free unsaturated fatty acids have bitter taste (19). This bitter taste is perhaps due to a small quantity of contaminant generated by the oxidation of LA (20, 21). Indeed, even freshly opened LA had a detectable carbonyl value (4.88 meq/kg, by Kumasawa’s method (22)) and a different UV absorbance spectrum from pure LA (data not shown). However, whether it tastes good or bad, the LA used here was more stimulating than OA, which might explain the preference. The analysis of preference indicated that rats of the M-rich group more obviously preferred LA. As daily LA intake by these rats was less...
than the recommended requirement (13), the M-rich groups would be more sensitive to LA than the P-rich group.

The rats didn’t distinguish between LA and LN. This result contradicted that of short-term (5 min) two-bottle tests showing rats preferred LN to LA (17). However, the difference is less than between OA and LA even in the short-term. So, a relatively long test duration (3 hr) would eliminate the difference between LA and LN.

This study indicated that dietary fat affects fatty acid preference in rats. However, the effect was very small, and the rats didn’t differentiate when the oil was provided as triacylglycerol. So further study perhaps using a flavor cue would be needed to elucidate the effect of dietary fat on oil preference.

References