Regulation of Essential Fatty Acid Intake in the Rat: Self-Selection of Corn Oil

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Summary The present investigation was conducted to determine if rats can recognize the essential fatty acid (EFA) in the diet. Weanling rats were pre-fed a 30% hydrogenated coconut oil (30HCO) diet containing 1% cholesterol for 2 or 4 weeks to produce EFA deficiency, then allowed to choose between the fat-free (FF) diet and the 0.5, 1.0, or 2.0% corn oil diet, and between the 0.5% HCO diet and 0.5% corn oil diet. Rats pre-fed the 30HCO diet for 2 weeks selected the corn oil-containing diets for a few days at the beginning of the experiment, but the rats pre-fed for 4 weeks could not recognize the EFA-containing diet. The non pre-fed animals continued to prefer the EFA-containing diet for about 2 weeks. From these results we could demonstrate that rats have a mechanism to distinguish the EFA in their diet, but this EFA selection ability was only in the early period of the experiment, and diminished soon after.

Key Words essential fatty acid intake, self-selection, intake regulation, corn oil intake

Previously the existence of the regulation for energy and specific nutrients in rats has been well investigated. Animals ate food to meet their energy requirement (1–3). Rats deficient in some B vitamins (4–6) or calcium (7) preferred a diet containing these elements. Normal rats took phosphorus (8) under the feedback regulation. Ashley and Anderson (9) demonstrated that the protein intakes are regulated by a different mechanism from the energy intake. Our previous reports (10–16) made clear that rats have an ability to regulate the intake of each essential amino acid to meet requirement for the amino acid.

To our knowledge, no study to date has been made to test the possible regulation of the EFA intake. We conducted the present investigation to clarify if rats can recognize the EFA in their diet. Self-selection feeding technique, which has
been shown to be useful for the determination of the essential amino acid requirement (10-16), was used to investigate the regulation of EFA intake in weanling rats.

MATERIALS AND METHODS

Male weanling rats of Wistar strain (Shizuoka-ken Jikken Dobutsu Nougyokyo-dokumiai, Hamamatsu) (35-40 g) were offered a 25% casein (25C) diet for 3 to 4 days before the experiments. Rats were individually housed in suspended screen-bottom cages (25 × 18 × 18 cm) in a room maintained at 24 ± 1 °C and lighted for 12 h daily (06:00-18:00). The composition of the 25C diet was as follows (%): corn oil, 5.0; casein, 25.0; salt mixture (17), 5.0; vitamin mixture (17), 1.0; choline chloride, 0.1; and α-starch to make 100. The composition of experimental diets is

Table 1. Composition of experimental diets.

| Ingredient                  | 0.5CO | 1.0CO | 2.0CO | 0.5HCO | 3.0HCO | FF |
|-----------------------------|-------|-------|-------|--------|--------|----|
| Corn oil                    | 0.5   | 1.0   | 2.0   | —      | —      | —  |
| Hydrogenated coconut oil    | —     | —     | —     | 0.5    | 30.0   | —  |
| Cholesterol                 | —     | —     | —     | 1.0    | —      | —  |
| Casein                      | 25.0  | 25.0  | 25.0  | 25.0   | 25.0   | 25.0|
| Salt mixture¹               | 5.0   | 5.0   | 5.0   | 5.0    | 5.0    | 5.0|
| Vitamin mixture⁴            | 1.0   | 1.0   | 1.0   | 1.0    | 1.0    | 1.0|
| Choline chloride            | 0.1   | 0.1   | 0.1   | 0.1    | 0.1    | 0.1|
| α-Starch                    | 58.4  | 57.9  | 56.9  | 58.4   | 27.9   | 58.9|
| Sucrose                     | 10.0  | 10.0  | 10.0  | 10.0   | 10.0   | 10.0|

¹ Muramatsu, K. et al. (1976): J. Nutr. Sci. Vitaminol., 22, 397-403 (25).

Table 2. Fatty acid composition of dietary fats.¹

| Fatty acid²   | Corn oil | Hydrogenated coconut oil |
|---------------|----------|--------------------------|
| 10:0          | —        | 17.4                     |
| 12:0          | —        | 46.8                     |
| 14:0          | —        | 16.6                     |
| 16:0          | 11.4     | 8.3                      |
| 18:0          | 2.2      | 10.8                     |
| 18:1          | 37.0     | —                        |
| 18:2          | 47.4     | —                        |
| 18:3          | 1.5      | —                        |
| 20:0          | 0.6      | —                        |

¹ Expressed as percentage of total peak areas. ² Number carbons: number double bonds.
given in Table 1, and the fatty acid composition of fats used for the experimental diet is shown in Table 2. The fixed feeding experiment was conducted to determine the condition readily producing EFA-deficient rats, which was used in self-selection feeding experiment. Fifteen rats were separated into three groups of 5 rats and had access to only a single diet which was the 25C diet, the fat free (FF) diet, or the 30% hydrogenated coconut oil (30HCO) diet containing 1% cholesterol for 6 weeks. The other 64 rats were used for the self-selection feeding experiment: Twenty rats were pre-fed the 30HCO diet ad libitum for 2 weeks and separated into four groups of 5 rats; another 20 rats were pre-fed equally for 4 weeks and separated into four groups of 5 rats; the remaining 24 rats were not treated by any prefeeding and separated into four groups of 6 rats. Each of these four self-selection feeding groups in the three different situations of prefeeding and age were offered a choice between these two diets: the FF diet and the 0.5% corn oil (0.5CO) diet, the 1.0% corn oil (1.0CO) diet, or the 2.0% corn oil (2.0CO) diet; the 0.5% hydrogenated coconut oil (0.5HCO) diet and the 0.5CO diet.

Test diets and water were supplied ad libitum for 2 or 4 weeks in the pretreated and non-treated groups, respectively. For the self-selection feeding period, two diets were kept in opposite corners of the cage, and the position of the cups was alternated daily to avoid any positional effects.

Analysis of fatty acid profiles of dietary fats was performed by gas-liquid chromatography (Hitachi Model 163, Hitachi Ltd., Tokyo) with a stainless column packed with DEGS. Temperature at the injection port and column was 250 and 240°C, respectively.

The data of the body weight gain and food intake were analyzed by Duncan’s multiple range test, and the data of food preference were analyzed by Student’s t-test.

RESULTS

The growth of rats offered the 25C, FF, or 30HCO diet by the fixed feeding method is shown in Fig. 1. When rats were fed the FF diet, growth retardation was observed after more than 2 weeks, and mild skin lesions were observed externally in all rats at 4 weeks. The addition of hydrogenated coconut oil (HCO) to an EFA-deficient diet accentuated the development of EFA deficiency, i.e. the growth retardation and the external skin lesions, in comparison with the FF diet.

From the results of this fixed feeding experiment, a following self-selection feeding experiment of EFA was undertaken using rats deficient in EFA by prefeeding the 30HCO diet for 2 or 4 weeks, and the results are shown in Table 3. When rats, pre-fed the 30HCO diet for 2 weeks, were given a choice of two diets which contained corn oil or not, the body weight gain of the 0.5CO vs. FF group was significantly less than the 0.5CO vs. 0.5HCO group in the first week, and both 0.5CO vs. FF and 0.5CO vs. 0.5HCO groups weighed significantly less than those of the 1.0CO vs. FF and 2.0CO vs. FF groups in the second week. There was no
Fig. 1. The body weight of rats fed the 25C, FF, or 30HCO diet for 6 weeks. ●, 25C; ○, FF; □, 30HCO; each symbol is a mean of 5 rats and vertical bars show the standard deviation.

Fig. 2. Food preference in rats offered a choice of two diets containing different levels of corn oil or 0.5% HCO after being pre-fed the 30HCO diet for 2 weeks. Hatched bars = FF; open bars. (a) = 0.5CO, (b) = 1.0CO, (c) = 2.0CO, (d) = 0.5CO; and dotted bar = 0.5HCO.

significant difference in total food consumption among all the groups throughout the experimental period. In the first week, rats of all groups ate a much greater amount of the corn oil diet than of the diets deficient in EFA. However, this preference to the EFA diminished in the second week and the rats ate more of the EFA-deficient diet.

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Table 3. Body weight gain, food, intake, and food preference of rats selected corn oil diet and FF, or 0.5HCO diet after the treatment of prefeeding the 30HCO diet for 2 or 4 weeks.

| Groups | Body weight gain | Food intake |
|--------|-----------------|-------------|
|        | (g/week)        | Total       | Food preference<sup>1</sup> |
|        |                 | (1)         | (2) | P |

1st week

- 0.5CO vs. (2) FF: 35.9 ± 2.1<sup>a</sup> vs. 93.6 ± 1.6<sup>a</sup>, 59.3 ± 4.9 vs. 34.3 ± 6.2, *p<0.05
- 1.0CO vs. (2) FF: 40.2 ± 1.4<sup>ab</sup> vs. 94.6 ± 2.7<sup>a</sup>, 56.6 ± 4.2 vs. 38.0 ± 3.2, **p<0.01
- 2.0CO vs. (2) FF: 40.0 ± 1.3<sup>ab</sup> vs. 93.2 ± 3.9<sup>a</sup>, 66.3 ± 7.6 vs. 26.9 ± 8.0, **p<0.01
- 0.5CO vs. (2) 0.5HCO: 41.4 ± 1.4<sup>b</sup> vs. 97.4 ± 3.4<sup>a</sup>, 61.6 ± 6.5 vs. 35.8 ± 5.0, *p<0.05

2nd week

- 0.5CO vs. (2) FF: 35.8 ± 2.5<sup>a</sup> vs. 105.8 ± 2.7<sup>a</sup>, 46.9 ± 3.3 vs. 58.9 ± 4.2, NS
- 1.0CO vs. (2) FF: 39.6 ± 1.2<sup>b</sup> vs. 111.2 ± 2.4<sup>a</sup>, 44.0 ± 3.4 vs. 67.2 ± 2.6, ***p<0.001
- 2.0CO vs. (2) FF: 39.2 ± 1.3<sup>b</sup> vs. 108.4 ± 3.2<sup>a</sup>, 40.6 ± 5.0 vs. 67.8 ± 5.2, **p<0.01
- 0.5CO vs. (2) 0.5HCO: 34.7 ± 1.4<sup>b</sup> vs. 111.0 ± 2.8<sup>a</sup>, 50.5 ± 4.0 vs. 60.5 ± 3.7, NS

Treated prefeeding the 30HCO diet for 4 weeks

1st week

- 0.5CO vs. (2) FF: 36.8 ± 0.8<sup>ab</sup> vs. 118.3 ± 1.6<sup>a</sup>, 56.4 ± 7.8 vs. 61.9 ± 8.3, NS
- 1.0CO vs. (2) FF: 39.9 ± 1.3<sup>a</sup> vs. 117.7 ± 3.8<sup>a</sup>, 59.6 ± 12.2 vs. 58.6 ± 9.9, NS
- 2.0CO vs. (2) FF: 37.8 ± 2.3<sup>a</sup> vs. 119.7 ± 3.2<sup>a</sup>, 77.8 ± 7.4 vs. 41.9 ± 5.8, **p<0.01
- 0.5CO vs. (2) 0.5HCO: 33.4 ± 0.8<sup>b</sup> vs. 116.8 ± 2.5<sup>a</sup>, 69.1 ± 7.4 vs. 47.7 ± 6.8, NS

2nd week

- 0.5CO vs. (2) FF: 22.7 ± 1.2<sup>ab</sup> vs. 121.0 ± 5.0<sup>a</sup>, 45.3 ± 5.0 vs. 75.8 ± 6.5, **p<0.01
- 1.0CO vs. (2) FF: 25.7 ± 1.8<sup>a</sup> vs. 123.1 ± 3.7<sup>a</sup>, 58.0 ± 10.0 vs. 65.0 ± 11.4, NS
- 2.0CO vs. (2) FF: 25.9 ± 2.8<sup>a</sup> vs. 122.5 ± 6.1<sup>a</sup>, 60.5 ± 16.0 vs. 61.9 ± 13.7, NS
- 0.5CO vs. (2) 0.5HCO: 19.5 ± 1.1<sup>b</sup> vs. 123.9 ± 3.1<sup>a</sup>, 65.8 ± 8.2 vs. 58.1 ± 5.7, NS

<sup>1</sup>Food preference of each group represents the amount of two diets ((1) or (2)) selected. T-test analysis was done within each pair of two diets ((1) and (2)). *p<0.05, **p<0.01, ***p<0.001; NS, not significant. <sup>2</sup>Mean ± SEM. Values in the same column without common superscripts are significantly different (p<0.05).

After prefeeding the 30HCO diet for 4 weeks, the rats offered a choice of the 0.5CO vs. the 0.5HCO diet grew less than the rats fed the 1.0CO, or 2.0CO vs. the FF diet. Total food intake did not differ in both the first and the second week. Rats fed the 2.0CO vs. the FF diet ate significantly greater amounts of the EFA-containing diets in the first week, and the rats fed the 0.5CO vs. FF diet selected more of the FF diet in the second week, and other groups did not recognize the diet containing EFA.

Figure 2 shows that rats pre-fed the 30HCO diet for 2 weeks are able to...
Table 4. Body weight gain, food intake and food preference of rats selected corn oil diet and FF, or 0.5HCO diet.

| Groups             | Body weight gain | Food intake |                        |                        | P       |
|--------------------|-----------------|-------------|-------------------------|-------------------------|---------|
|                    |                 | Total       |                         |                         |         |
|                    |                 | (g/week)    | (1)                     | (2)                     |         |
| 1st week           |                 |             |                         |                         |         |
| (1) 0.5CO vs. (2) FF | 25.4 ± 1.3 ± 2 | 57.0 ± 2.8a | 48.6 ± 4.8              | 8.4 ± 3.0               | ***     |
| (1) 1.0CO vs. (2) FF | 27.3 ± 2.3a    | 57.2 ± 2.0a | 52.9 ± 2.8              | 4.3 ± 1.2               | ***     |
| (1) 2.0CO vs. (2) FF | 26.5 ± 1.0a    | 56.5 ± 1.9a | 53.1 ± 3.0              | 3.4 ± 0.5               | ***     |
| (1) 0.5CO vs. (2) 0.5HCO | 28.1 ± 0.5a | 60.4 ± 3.0a | 38.8 ± 8.6              | 21.6 ± 0.6               | NS      |
| 2nd week           |                 |             |                         |                         |         |
| (1) 0.5CO vs. (2) FF | 28.2 ± 1.8a    | 75.8 ± 3.1a | 51.9 ± 4.1              | 23.9 ± 5.3               | **      |
| (1) 1.0CO vs. (2) FF | 32.3 ± 1.5a    | 80.0 ± 2.3a | 67.1 ± 3.2              | 12.9 ± 2.2               | ***     |
| (1) 2.0CO vs. (2) FF | 28.8 ± 2.4a    | 77.9 ± 3.3a | 58.0 ± 4.5              | 19.9 ± 6.1               | ***     |
| (1) 0.5CO vs. (2) 0.5HCO | 28.0 ± 1.7a | 75.2 ± 2.1a | 52.8 ± 3.2              | 22.4 ± 1.2               | ***     |
| 3rd week           |                 |             |                         |                         |         |
| (1) 0.5CO vs. (2) FF | 28.6 ± 2.1a    | 87.3 ± 3.1a | 42.3 ± 4.3              | 45.0 ± 5.5               | NS      |
| (1) 1.0CO vs. (2) FF | 27.3 ± 2.7a    | 91.6 ± 3.4a | 52.3 ± 6.9              | 39.3 ± 4.5               | NS      |
| (1) 2.0CO vs. (2) FF | 30.9 ± 2.8a    | 82.5 ± 2.5a | 43.4 ± 3.1              | 39.1 ± 3.0               | NS      |
| (1) 0.5CO vs. (2) 0.5HCO | 31.5 ± 2.2a | 82.2 ± 3.3a | 58.5 ± 6.1              | 23.7 ± 4.6               | **      |
| 4th week           |                 |             |                         |                         |         |
| (1) 0.5CO vs. (2) FF | 33.5 ± 2.3a    | 108.0 ± 3.5a | 60.9 ± 4.2              | 47.1 ± 8.2               | NS      |
| (1) 1.0CO vs. (2) FF | 35.5 ± 3.5a    | 110.2 ± 3.3a | 63.7 ± 6.0              | 46.5 ± 5.2               | NS      |
| (1) 2.0CO vs. (2) FF | 37.9 ± 3.0a    | 105.0 ± 2.6a | 50.9 ± 3.2              | 54.1 ± 3.7               | NS      |
| (1) 0.5CO vs. (2) 0.5HCO | 33.7 ± 2.8a | 98.8 ± 3.1a | 64.1 ± 3.7              | 34.7 ± 5.1               | ***     |

1 Food preference of each group represents the amount of two diets (1) or (2) selected. T-test analysis was done within each pair of two diets (1) and (2). *p < 0.05, **p < 0.01, ***p < 0.001; NS, not significant. 2 Mean ± SEM. Values in the same column without common superscripts are significantly different (p < 0.05).

recognize diets containing EFA from the beginning of the experiment and continued to prefer the diets containing EFA for a few days in the first week, but this food intake pattern diminished after that period.

The body weight gain, food preference, and total food intake in rats not treated with any prefeeding and offered a choice of two diets differing in fat source and content for 4 weeks are summarized in Table 4. Rats offered a choice of the FF diet vs. the 0.5CO, 1.0CO, or 2.0CO diet ate the EFA-containing diet which constituted about 85, 93, or 94% of total food intake, respectively, in the first week. In the second week, these three groups selected the EFA-containing diet as about 69, 84, or 75% of total food intake, but in the third or the fourth week, rats of these three groups consumed their two diets equally. When rats were fed the 0.5CO and the
Fig. 3. Food preference in rats offered a choice of two diets containing different levels of corn oil or 0.5% HCO. Symbols in the graph are the same as in the footnote to Fig. 2.

0.5HCO diet, they could not select the EFA-containing diet in the first week, but ate significantly more of the EFA-containing diet in the second week and thereafter. The body weight gain and total food intake of all groups did not differ from each other throughout the experimental period.

Figure 3 shows the changes in food preference in rats not treated with any prefeeding for 4 weeks. The preference for the EFA-containing diet is clearly shown in the early 2 weeks of the experiment in the rats offered a choice between the EFA-containing diet and the FF diet (0.5CO vs. FF, 1.0CO vs. FF, 2.0CO vs. FF). The rats fed the 0.5CO and the 0.5HCO diet seemed not to distinguish between the diets containing the EFA or not in the early few days of experiment, but after then they preferentially selected the EFA-containing diet throughout the experimental period.

DISCUSSION

The results of the present fixed feeding experiment showed that rats were more deprived of EFA by feeding the 30HCO diet than by feeding the FF diet, when the reduction in weight gain was used as a criterion of EFA deficiency. The addition of saturated fat, such as HCO to an EFA-deficient diet, or the inclusion of cholesterol...
in an EFA-deficient diet has been shown to enhance the development of dermal signs of EFA deficiency in rats (18–21). Then we used the treatment of prefeeding with the 30HCO diet to produce EFA deficiency in the subsequent self-selection experiment.

The self-selection feeding experiments showed that rats treated by the prefeeding with the 30HCO diet for 4 weeks could little recognize the EFA, and the rats treated equally for 2 weeks selected the EFA-containing diet for only a few days of the experiment. On the other hand, rats not treated by any prefeeding exclusively preferred the EFA-containing diet to the FF diet in the first and the second week. From these results we concluded that weanling rats offered a choice of two diets containing EFA or not have an ability to select an EFA-containing diet, and this ability seemed to diminish as the rats grew.

The result that the rats treated by the prefeeding with the 30HCO diet for 4 weeks did not select the EFA-containing diet, although they were in a state of EFA deficiency, seemed to show that rats do not have an ability to recognize the EFA. But the studies concerning the requirements of the EFA for normal brain development show that the development of the brain is susceptible to EFA deficiency at the period from birth to about 9 weeks (22, 23). Then the 4-week prefeeding period after weaning may be the time when rats are strikingly susceptible to EFA deficiency. Though the mechanisms by which the weanling rats select the EFA-containing diet is not known, the EFA requirement for the tissue, especially brain, development may control the intake of EFA.

It is conceivable that the preference for the EFA-containing diet to the FF diet in the weanling rat is indicative of the appetite rather to the fat than to the EFA. But the result in this study that the weanling rat preferred the EFA-containing diet to the diet which contained the same amount of HCO after the first week of self-selection experiment (Table 4) demonstrated that rats have an ability to recognize the EFA in the diet.

Holman (24) has established the linoleate requirement of the young male rat to be approximately 1% of total energy intake when the triene-tetraene ratio of the tissues was used as the principal parameter of adequacy, and gain in weight as a supporting criterion of adequacy. The EFA level of diets used in this experiment, i.e. 0.5CO, 1.0CO, 2.0CO, was about 0.6, 1.2, and 2.4 of energy percentage, respectively, which was calculated from the data that the EFA contents (linoleic acid + linolenic acid) of the corn oil used in this experiment is about 49.0%. The amount of the EFA-containing diet consumed by the 0.5CO vs. FE, 1.0CO vs. FF, and 2.0CO vs. FF groups in the self-selection experiment of weanling rat (Table 4) did not correlate to the EFA content in the diet. Then whether the ability of the rats to select the EFA-containing diet is to meet the EFA requirement or not, is not suggested in this experiment.

We could demonstrate from this study that rats have an ability to recognize EFA in diet. But this ability to select the EFA-containing diet was shown only in the early period after weaning, and diminished as the rats grew.
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