Ratio of High-Fat Diet Intake of Pups Nursed by Dams Fed Combination Diet Was Lower Than That of Pups Nursed by Dams Fed High-Fat or Low-Fat Diet

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(Received June 19, 2006)

Summary To investigate the influence of fat-feeding dams on the food choice of their pups after weaning, each of three groups of dams was fed a low-fat diet (LHD), a high-fat diet (HFD) or a two-choice diet of LFD and HFD during pregnancy and lactation. Immediately after weaning, all pups were placed on a two-choice diet program for 5 wk. The fat energy ratio (F ratio) for dams fed the two-choice diet was 31%. Although no significant differences in body weight or calorie intake were observed between these three groups of dams, liver and perirenal fat tissue weights and plasma and liver triglyceride and total-cholesterol concentrations were lower in dams fed the two-choice diet than in dams fed LHD or HFD. Both groups of pups nursed by dams fed LFD or HFD continued to eat a large amount of HFD after weaning (F ratio was over 40%). Although within first week after weaning, no significant difference in the ratio of HFD intake was observed among the three groups of pups, the ratio for pups nursed by dams fed the two-choice diet decreased after the second week. The F ratio for pups nursed by dams fed the two-choice diet was 32%. These data lead us to conclude that if dams ate more than one diet in an adequate PFC ratio, their pups would have the ability to eat adequately after weaning.

Key Words prenatal nutrition, self-selection, fat-feeding, weaning pups

Several empirical studies have shown that weaning pups are strongly influenced in their initial choice of diet by dietary preferences learned from adult rats. It appears that the odor or the flavor associated with the mother’s diet is transmitted via her milk to nursing pups and nibbling during the weaning period might have affected the pups’ dietary preference, and that these cues influence pups’ initial choice of diet (1–3). Bellinger et al. suggested that exposure to maternal undernutrition in early life would program a specific preference for energy-dense foodstuffs and hyperphagia (4). Other workers have demonstrated that in rats, early life exposure to undernutrition programs obesity in response to hyperenergetic feeding (5, 6).

Recently, young people have come to prefer a Western-style diet to a Japanese-style diet, and now more than 25% of the calories consumed by young people in Japan are provided by fat (7). In particular, animal products such as meat, fish, milk, milk products, and eggs contribute about half of the total fat in Japanese diets. Although young people in Japan have increased the fat content of their diet, this is associated with higher caloric intake as well as increased body weight, body fat, and adipose tissues depositions. Indeed, physiological homeostasis is maintained through complex nutrient metabolic pathways regulated by hormones and the central nervous system (8, 9). Although the physiological factors contributing to fat-diet selection are unknown, dietary fat is a key environmental component influencing the development of obesity in animals and humans (10). It has also been recognized that the amount of fat, especially animal fat, ingested is related to high blood cholesterol levels, hyperlipidemia and risks of atherosclerotic vascular disease, coronary heart disease and obesity (11).

There have been many studies of macronutrients (carbohydrate, fat and protein) consumption by rats placed on a self-selection regimen from weaning to maturity (10, 12–17), and most have indicated that rats selected balanced diets and thrived well when given separate macronutrients sources. We were particularly interested in examining whether fat-feeding of dams during pregnancy and lactation has any effect on the food choice of their pups after weaning. In a previous paper, we reported that two groups of dams were fed either a low-fat diet (LFD) containing 3.5% soybean oil or a high-fat diet (HFD) containing 7% soybean oil and 14% lard during pregnancy and lactation. When their pups were placed on a self-selection regimen after weaning, pups of both groups preferred the HFD (18). The ratio of HFD intake to the total amount of dietary intake by pups of dams fed LFD and HFD was 81 and 88%, respectively. The reason for this finding is considered to be that plasma triglyceride concentration in dams fed LFD was high for the reason that the soybean oil in the LFD was substituted for cornstarch and the excess carbohydrate in the diet increased the rate of lipogenesis in the liver (19). The plasma triglyceride concentration in dams fed HFD was naturally high as they consumed HFD. If dams were fed the diet that does
MATERIALS AND METHODS

Animals and diets. Twelve sperm-positive pregnant rats of the Sprague-Dawley strain (10 wk old) were commercially obtained from Japan Clea (Tokyo, Japan) on day 5 of pregnancy. They were housed individually in plastic cages in a room kept at a constant temperature (23 ± 1°C) and illuminated with a 12-h light/12-h dark cycle. Dams and pups had free access to food and distilled water. They were weighed and food intake was measured every day to determine daily intake during the experimental period.

The composition of diets shown in Table 1 was based on the AIN-93G diet (20). Basically, the amount soybean oil in the LFD was reduced to 1/2 that in AIN-93G, and lard (14%) was added to the HFD and substituted for cornstarch. The fat-energy ratios of the LFD and the HFD were estimated to be 10.7 and 42.7%, respectively. The LFD and the HFD supplied 3.5 and 4.6 kcal/g of diet, respectively.

Experimental design. Twelve pregnant rats were divided into three equal groups. Each group received the LFD, the HFD or the two-choice diet during the experimental period (pregnancy and lactation) as shown in Table 1. The two-choice diet group was placed on the two-choice diet program in which they self-selected from two food cups, each containing either the LFD or the HFD. Within 24 h of birth, litters were weighed and culled to 10 pups each and nursed by their dams. On days 10, 20 and 34 after birth, two pups each from the litters of dams fed the LFD, the HFD or the two-choice diet were sacrificed by decapitation and their blood collected. After weaning (at 20 d of age), the remaining pups were housed individually and provided both the LFD and the HFD, placed in separate cups. Dietary ingestion of both the LFD and the HFD and body weight were measured. The remaining pups were sacrificed on day 55 after birth (on day 35 after weaning) and dams were sacrificed immediately after weaning their pups. They were anesthetized and killed by cardiac puncture. Blood was collected with a heparinized syringe. After centrifugation (3,000 rpm×15), plasma was removed. Livers, stomachs (10 d-old pups) and perirenal fat tissues were removed from dams and pups and weighed. Blood in livers was removed by perfusion with physiological saline. These collected samples were frozen immediately and stored at −80°C until analysis. All procedures accorded with the guidelines for Seitoku University.

Methods of analysis. Hepatic lipids were extracted by the method developed by Folch et al. (21). Triglycerides and total cholesterol concentrations in blood and liver were measured using test kits (Triglycerides: Triglyceride E-test Wako, Total cholesterol: Cholesterol test-E Wako) (Wako Pure Chemical Industries, Osaka, Japan).

Statistical Analysis. Values are presented as individual group means ±SD. Repeated-measures analysis of variance (ANOVA) was used to evaluate the effects of preference group and time on food intakes. Differences in mean values between groups were tested by Scheffe’s multiple-range test. Student’s t test was used for all pairwise comparisons. Differences were considered significant at p<0.05.

RESULTS

Food and calorie intake, body, liver and perirenal fat tissue weight and concentration of plasma and liver lipids in dams fed a LFD, HFD or two-choice diet

No significant difference in body weight was observed among these three groups during pregnancy or lactation (Table 3). Table 2 shows food and calorie intakes during pregnancy and lactation for the dams fed the LFD, the HFD and the two-choice diet. During pregnancy and lactation, no significant difference in calorie intake was observed among the pregnant rats fed these three diets. The average ratio of the HFD intake to total diet intake by dams during pregnancy and lactation in the two-choice program was 64.8 and 63.5%, respectively.

Male and female litter size in the group receiving the LFD was 7.0±1.4 and 7.0±1.4, respectively, that in group receiving the HFD was 7.5±0.6 and 6.8±1.5.
Liver lipids in dams fed the two-choice diet was significantly lower than the HFD. Plasma total-cholesterol concentration in dams fed the two-choice diet was significantly lower than that in dams fed the LFD and HFD. Liver total fat, triglycerides and total-cholesterol concentrations in dams fed the LFD were significantly higher than those in dams fed the HFD and the two-choice diet.

Perirenal fat tissue weights and concentrations of plasma and liver lipids in dams.

Table 2. Food and calorie intakes during pregnancy and lactation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Food intake (g)</th>
<th>Calorie intake (kcal)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnant period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-fat diet</td>
<td>338 ± 31ab</td>
<td>1,182 ± 129</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>255 ± 37ab</td>
<td>1,174 ± 146</td>
</tr>
<tr>
<td>Two-choice diet</td>
<td>287 ± 27ab</td>
<td>1,209 ± 108</td>
</tr>
<tr>
<td>Low-fat diet</td>
<td>101 ± 11ab</td>
<td>353 ± 36</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>186 ± 15ab</td>
<td>856 ± 77</td>
</tr>
<tr>
<td>HFDI/TDI (%)</td>
<td>64.8 ± 5.5</td>
<td>70.8 ± 6.2</td>
</tr>
<tr>
<td><strong>Nursing period</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-fat diet</td>
<td>963 ± 92ab</td>
<td>3,371 ± 318</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>721 ± 64b</td>
<td>3,136 ± 291</td>
</tr>
<tr>
<td>Two-choice diet</td>
<td>802 ± 62b</td>
<td>3,368 ± 352</td>
</tr>
<tr>
<td>Low-fat diet</td>
<td>293 ± 29ab</td>
<td>1,026 ± 110</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>509 ± 47ab</td>
<td>2,341 ± 179</td>
</tr>
<tr>
<td>HFDI/TDI (%)</td>
<td>63.5 ± 4.8</td>
<td>69.5 ± 5.2</td>
</tr>
</tbody>
</table>

Values represent means ± SD, n=4. Within a column, values not sharing a common superscript letter are significantly different at p<0.05. Pregnant period: from day 5 of pregnancy to delivery (on day 21 of pregnancy). Nursing period: from delivery to weaning (pups were weaned at 20 d of age). HFDI/TDI: High-fat diet intake/Total diet intake.

respectively, and that in group receiving the two-choice diet was 7.8 ± 1.9 and 6.8 ± 2.1, respectively. Therefore, these three maternal diets did not affect litter size or ratio of female vs. male pups.

At the end of lactation, the liver weight of dams fed the LFD was significantly higher than that of dams fed the HFD and the two-choice diet. Perirenal fat tissue weight of dams fed the HFD was significantly higher than for the other two groups (Table 3). Plasma triglyceride concentration in dams fed the two-choice diet was significantly lower than that in dams fed the LFD and the HFD. Plasma total-cholesterol concentration in dams fed the two-choice diet was significantly lower than that in dams fed the HFD. Liver total fat, triglyceride and total-cholesterol concentrations in dams fed the LFD were significantly higher than those in dams fed the HFD and the two-choice diet.

**Self-selection of LFD and HFD by weaning pups nursed by dams fed LFD, HFD and two-choice diet**

Within 24 h of birth, litters were weighed. The body weight of the male and female pups nursed by dams fed the LFD was 7.2 ± 0.4 and 7.3 ± 0.4 g, respectively, that of the male and female pups nursed by dams fed the HFD was 7.2 ± 0.2 and 6.7 ± 0.5 g, respectively; and that of the male and female pups nursed by dams fed the two-choice diet was 7.8 ± 0.5 and 7.3 ± 0.5 g, respectively. The male and female pups from these three groups were of similar weight at birth and no significant difference in body weight was observed among

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Ratio of HFD to total diet intake by weaning male and female pups nursed by dams fed the high-fat diet. The low-fat diet and the two-choice diet when they were placed on a self-selection regimen after weaning. Values are mean ± SD. Values not sharing a letter are significantly different (p<0.05). First and second week after weaning, n of male pups nursed by dams fed LFD, HFD and two-choice diet was 12, 11 and 12, respectively, and that of female pups nursed by dams fed LFD, HFD and two-choice diet was 11, 13 and 12, respectively. Third, fourth and fifth week after weaning, n of male pups nursed by dams fed LFD, HFD and two-choice diet was 8, 7 and 8, respectively, and that of female pups nursed by dams fed LFD, HFD and two-choice diet was 7, 9 and 8, respectively.
pups nursed by dams fed the LFD, the HFD and the two-choice diet during experimental period.

Immediately after weaning, all pups were placed on the two-choice diet program in which they self-selected from two food cups, each containing either the LFD or the HFD for 5 wk. The ratios of the HFD intake to total dietary intake by male and female pups in the two-choice program are illustrated in Fig. 1. In the first week after weaning all pups preferred the HFD and no significant difference in the ratio of HFD intake was observed among the pups nursed by dams fed the LFD, the HFD and the two-choice diet. However, after the second week, the ratio of male and female pups nursed by dams fed the two-choice diet decreased. In the fifth week after weaning, in both male and female pups, the amount of the HFD intake by pups nursed by dams fed the two-choice diet was significantly lower than that of pups nursed by dams fed the LFD and the HFD.

Liver and perirenal fat tissue weights and concentration of plasma and liver lipids in pups nursed by dams fed LFD, HFD and two-choice diet

On day 10 after birth, no significant difference in liver or perirenal fat tissue weights, plasma, liver or stomach content lipid concentration was observed among suckling pups nursed by dams fed the LFD, the HFD and the two-choice diet (data are not shown). However, the plasma triglyceride concentration of the 10-d old male suckling pups nursed by dams fed the LFD, the HFD and the two-choice diet was 283 ± 33, 303 ± 35 and 266 ± 38 mg/dL, respectively, and that of the female suckling pups nursed by dams fed the LFD, the HFD and the two-choice diet was 283 ± 33, 303 ± 35 and 257 ± 35 mg/dL, respectively. Therefore, in both the male and the female pups, plasma triglyceride concentration of the 10-d old suckling pups was higher than that of the 20- and 55-d old pups, and their dams

Values represent means ± SD. n = 4. Within a row, values not sharing a common superscript letter are significantly different at p < 0.05. T-Cholesterol: total cholesterol.
in our previous paper (18)]. We already reported that although no significant differences in body weights or calorie intake were observed when each of the three groups of male rats was fed a LFD (fat energy ratio 10.7%), a control diet (fat energy ratio 18.6%) or a HFD (fat energy ratio 42.7%), respectively for 9 wk, liver and fat tissue weights, and serum and liver lipid concentrations in male rats fed the control diet were the lowest among those three groups (22).

It has been well known that the quantities of lipo-genic enzymes such as ATP citrate-lyase (EC 4.1.3.8), acetyl-CoA carboxylase (EC 6.4.1.2), fatty acid synthase (EC 2.3.1.85), malic enzyme (EC 1.1.1.40), and glucose-6-phosphate dehydrogenase (EC 1.1.1.49), mRNA concentrations and the activities of these enzymes in the liver are increased by a high carbohydrate diet (23–27). Therefore, it was assumed that the intake of the LFD increased the fatty acid synthesis in the liver and it appeared to involve the increased liver lipid concentration and liver weight of dams fed the LFD (Table 3). As the synthesized lipids in the liver were transferred to blood as VLDL, the plasma lipid concentration was higher in the dams fed the LFD than that in the dams fed the two-choice diet.

On the other hand, perirenal fat tissue weight and plasma lipid concentration were higher in dams fed the HFD than in the dams fed the two-choice diet (Table 3). Smith et al. have demonstrated that preferential consumption of a HFD led to an accumulation of body fat without hyperphagia or an increase in body weight (10). Many clinical studies have also demonstrated that high-fat consumers tend to have a higher body mass index and body fat content than low-fat consumers (28–30). Therefore it was assumed that dams ate an adequate ratio of the LFD and the HFD when they were placed on a self-selection regimen during pregnancy and lactation. Because these dams had the lowest accumulation of body fat, the lowest lipid synthesis in the liver and the lowest concentration in plasma triglycerides and total-cholesterol, we considered that a fat energy ratio of 31% was adequate for dams during pregnancy and lactation.

On week 1 after weaning, all pups were fed a large amount of the HFD (Fig. 1). However, after 2 wk, the ratio of the HFD intake for pups nursed by dams fed the two-choice diet was significantly lower than that of pups nursed by dams fed the LFD or the HFD. In week 5 after weaning, the ratio for male and female pups nursed by dams fed the two-choice diet was 67 and 69%, respectively and the fat energy ratio of the two-choice diet that they ate was calculated at about 32%. Thus, the fat energy ratio of pups nursed by dams fed the two-choice diet was similar to that of their dams. On the other hand, the fat energy ratio of both groups of pups nursed by dams fed the LFD and HFD was over 40%. Therefore, in our previous paper, we concluded that pups preferred the HFD immediately after weaning because both groups of pups nursed by dams fed the LFD or the HFD ate a large amount of the HFD (18). However, in the present study, if dams ate more than one diet during pregnancy and lactation, their pups also ate similarly more than one diet after weaning. One reason for this finding appeared to be that the pups were nibbling their dam’s diet before they were weaned. The finding that pups of dams fed the two-choice diet continued to eat both the HFD and the LFD after weaning may possibly be explained by the adequate fat-feeding of their dams during lactation. In this fashion, nibbling might have affected the pups’ dietary preferences. Moreover, the difference of the plasma lipid
concentration of dams fed the two-choice diet and that of dams fed the LFD and the HFD may have influenced the composition of their milk. Further, another reason for this finding was considered to be the difference in plasma lipids level of these groups of dams. A range of epidemiological and experimental studies suggest that anatomy, physiology and metabolism may be programmed in part through exposure to an adverse nutritional environment in utero (5, 6). Bellinger et al. suggested that early prenatal undernutrition leads to a preference for fatty foods and maternal nutrition may promote changes in systems that are involved in the control of appetite or the perception of palatability (4). However, physiological factors possibly contributing to the high fat diet and the low fat diet selection remain unknown.

In both the male and female pups the high concentration of plasma triglyceride concentration of the 10-d-old suckling pups decreased during infancy. Furthermore, the plasma triglyceride concentration of the suckling pups was higher than that of their dams in these three groups. It appeared that the high level in plasma lipid concentration was due to the feeding of high fat-low carbohydrate milk to suckling pups. Kees et al. reported that the protein, carbohydrate and fat concentrations in rat milk were 8.85–12.05, 2.46–3.72 and 10.91–17.54%, respectively, and that no clear differences were observed in the patterns of change in ingestion of these macronutrients during lactation (31). From these values, the fat energy ratio of rat milk was calculated to be about 70%. Del Prado et al. reported that the fat energy ratio of milk from rats fed a low fat diet (25 g/kg diet) and a high fat diet (200 g/kg diet) was about 74 and 76%, respectively (32). It thus appeared that all pups fed high fat milk during infancy self-selected a large amount of the high fat diet immediately after weaning. Although both groups of pups nursed by dams fed the HFD or the LFD self-selected a large amount of the HFD after weaning, the serum lipid concentration decreased rapidly in all pups. It appeared that the fat level of the diet which weaning pups self-selected was lower than that of their mothers’ milk since the serum lipid level is easily affected by dietary fat level.

In the fifth week after weaning, the plasma triglyceride concentration of male and female pups nursed by dams fed the two-choice diet was significantly lower than that of the other two groups, because the ratio of the HFD intake by the group was lower than that of the other two groups (Table 5). Similarly, the liver total lipid and triglyceride concentration of male and female pups nursed by dams fed the two-choice diet was significantly lower than for the other two groups. Therefore, the liver weight of the male pups nursed by dams fed the two-choice diet was significantly lower than in the other two groups. These results were similar to our previous study using growing male rats in which serum triglyceride and cholesterol concentration, liver total lipid, triglyceride and cholesterol concentration and liver weight of rats fed LFD and HFD for 9 wk were higher than those of rats fed the control diet, because rats fed HFD and LFD consumed a larger amount of fat or carbohydrate, respectively, than rats fed the control diet (22). Although no significant difference in liver weight was observed among these three female groups, the exact reason was unknown. However, liver weight is largely affected by the amount of glycogen that is contained as a hydrate compound in liver. Jean et al. observed that in general, females showed a preference for fat that increased continuously with age (33). Therefore, it was considered that glycogen content in the liver of female pups may be lower than that of male pups and the amount of glycogen may affect liver weight.

The protein content of AIN93G and AIN93M is 20 g/100 g and 14 g/100 g, respectively. The protein energy ratio of the LFD and HFD was 20.8 and 17.4, respectively (Table 1). The ratio of dams fed the two-choice diet during pregnancy and lactation was calculated to be 18.6. As the protein content of AIN93G was higher than that of AIN93M and the amount of protein of dams fed the two-choice diet was higher than that of dams fed the HFD, in the present study, we did not especially consider the protein content in the diets. Bellinger et al. reported that prenatal exposure to a maternal low-protein diet programs a preference for high-fat foods in young adult rats (4). In their experiment, the pregnant rats were fed a control diet (18 g casein/100 g diet) and a low-protein diet (9 g casein/100 g). Therefore, the protein content of diets used in the present study was not lower than that of the low-protein diet and was not consistent with the undernutrition reported by Bellinger et al. However, amino acids and oligopeptides have various tastes and may also influence appetite control systems.

The present findings suggest that a HFD has food properties preferable to those of a LFD, and that this contributes to greater consumption of a HFD compared to a LFD. However, from this study, it is likely that if dams eat more than one diet during pregnancy and lactation, their pups will also eat similarly after weaning. Because appropriate nutrition in infancy is essential for optimal growth and development, infants are particularly vulnerable to inappropriate nutrition. Therefore, infants need to be offered a variety of foods from all food groups. However, physiological factors possibly contributing to HFD and LFD selection remain unknown.

Jean et al. performed a study in which pregnant Wistar dams were fed a standard pregnancy and bleeding diet during pregnancy and lactation, and after weaning their pups received three macronutrient choice diets placed in separate cups. Fat intake in the self-selecting pups was reduced during the 3 wk after weaning, and reached a plateau from weeks 3 to 6 after weaning. The fat intake in male and female pups during the plateau period was 28 and 34% of energy intake, respectively (33). The fat energy ratio was almost similar to that of the pups nursed by dams fed the two-choice diet in this study. These results suggested that if dams ate a two-choice diet in an adequate range of fat energy ratio, their pups would have the ability to eat...
more than one diet in an adequate ratio. As the plasma triglyceride concentration in dams fed the two-choice diet was significantly lower than those in dams fed the LFD and the HFD, it is likely that if plasma triglyceride concentration in dams is higher during pregnancy and lactation, the HFD preference of their pups will become intense after weaning and if dams eat both a HFD and a LFD during pregnancy and lactation, their pups will also eat both diets after weaning. Further study is needed to clarify the mechanism by which culture-specific fat preferences are initiated early in life, and whether such preferences remain as children become adults and pass on their food habits to the next generation.

REFERENCES


