Exposure to Dams’ Low-Fat High-Carbohydrate Diet during Pregnancy and Lactation Establishes a Preference for Fat by Their Offspring

Yoko NAKASHIMA

Department of Human Nutrition, Seitoku University, 550 Iwase, Matsudo, Chiba 271–8555, Japan

(Received July 13, 2009)

Summary To investigate the causes why pups of dams fed a low-fat high-carbohydrate diet (LFD) showed strong preference for fat, two groups of pregnant rats were fed either the LFD or the control-diet (CTD) during pregnancy and lactation. After weaning, pups in both groups were divided into two groups. Two groups were offered a self-selection regimen of either a carbohydrate-protein diet (CPD) or a fat-protein diet (FPD) (the LFD-CF and the CTD-CF groups) and the remaining groups were fed the same diet as their dams (the LFD and the CTD groups) for 5 wk. Although the body weight of pups fed the LFD was lower than that of pups fed the CTD, the body weight of the LFD-CF group caught up with that of the CTD group. The ratio of FPD intake [FPD intake (g)/total intake (g)] by the LFD-CF group was higher than that of the CTD-CF group. In both dams and their pups, although no significant difference in the plasma glucose concentration was observed between the LFD and the CTD groups, the plasma insulin and triacylglycerol (TG) concentrations of the LFD group were higher than those of the CTD group. However, the self-selection diet appeared to prevent an increase in the plasma insulin and TG concentrations. Therefore, it was supposed that although pups of dams fed the LFD showed strong preference for fat in order to achieve optimal growth, the dietary selection pattern after weaning was associated with the depletion in plasma insulin.

Key Words fat preference, self-selection, fat-energy ratio, insulinemia

Although the physiological factors contributing to fat-diet self-selection are unknown, physiological homeostasis is maintained through a complex nutrient metabolic pathway regulated by hormones and the central nervous system (1–3). The large variations that exist in food choice, especially for fat preference, can be linked to animal strain, age, environment, genetic background or prenatal nutrition, feeding history and modifications of energy expenditure such as exercise and food deprivation (4–16). We were particularly interested in examining whether dams’ dietary fat types during pregnancy and lactation influence the preferential fat intake of their pups after weaning.

In a previous paper, we conducted an experiment in which each of three groups of dams was fed a low-fat high-carbohydrate diet (LFD), a control diet (CTD) or a high-fat low-carbohydrate diet (HFD) during pregnancy and lactation, then fed a two-choice diet program consisting of a fat protein diet (FPD) and a carbohydrate protein diet (CPD) after weaning for 3 wk (16). The ratio of the FPD intake [FPD intake (g)/total intake (g)] for male and female pups nursed by dams fed the LFD was higher than that for the two groups of pups nursed by dams fed either the CTD or the HFD. It was assumed that male and female pups nursed by dams fed the CTD and the HFD self-selected the FPD and the CPD in an adequate fat-energy ratio (F ratio) compared to that of AIN-93G. Therefore, although pups nursed by dams fed the CTD and the HFD had the ability to consume a FPD and a CPD in an adequate F ratio, if dams ate the LFD during pregnancy and lactation, their pups would be deprived of an ability to self-select the FPD and the CPD in an adequate F ratio after weaning. Thus, the purpose of the study is to clarify the causes why the preferential fat intake of pups nursed by dams fed the LFD is stronger than that of pups nursed by dams fed the CTD and the HFD.

Insulin and leptin are of particular importance in the long-term control of food intake and energy homeostasis (17). Moreover, insulin resistance in peripheral tissues increases with age in both humans and rodents (18, 19). Although glucose homeostasis may remain normal, both fasting and postprandial insulin concentrations tend to increase with age (18). On the other hand, adipokine leptin has been shown to affect food
intake and energy consumption through its receptors in the hypothalamus (20). Vickers et al. suggested that prenatal exposure to maternal malnutrition leads to changes in the regulation of the insulin-leptin endocrine axes that appear to predispose offspring to diet-induced obesity (21, 22). It has also been shown that dietary fat quantity (23) and quality (24) affect serum leptin concentrations in neonatal rats. As this phenomenon takes place early in development, diets reducing insulinemia in early life may be beneficial to later life.

Therefore, it seems important to investigate the effect of fat-feeding dams during pregnancy and lactation on spontaneous fat choice made by offspring and the relationship between their choice and plasma insulin and leptin concentrations.

In order to clarify the causes why the preferential fat intake of pups nursed by dams fed the LFD is stronger, two groups of pups nursed by dams fed the LFD were placed either on a self-selection regimen of the CPD and the FPD or the LFD, respectively. The same foods were supplied to two groups of pups nursed by dams fed the CTD and the preferential fat intake of the pups in these two groups were compared with the preferential fat intake of pups nursed by dams fed the LFD. Body and fat tissue weights and plasma glucose, lipid and hormone concentrations were also measured and analyzed in relation to their regimen and their observed dietary preferences.

### METHODS

**Animals and diets.** Ten sperm-positive pregnant rats of the Sprague-Dawley strain on day 3 of pregnancy (10 wk-old) were commercially obtained from Clea Japan, Inc. (Tokyo, Japan). They were housed individually in plastic cages with paper chip (ALPHA-DriTM, Shepherd Specialty Papers, Inc., Michigan) bedding. They were maintained in a room kept at a constant temperature (22±1°C) and illuminated with a 12-h light/12-h dark cycle with lights on at 7:00 a.m. They had free access to food and water. They were weighed and food intake was measured every day between 10:00 and 12:00 a.m. to determine daily intake during the experimental period. Dams and pups were sacrificed and their blood was collected between 10:00 and 12:00 a.m.

The composition of the CTD shown in Table 1 was based on an AIN-93G diet (25). Basically, the amount of soybean oil in the LFD was reduced to 1/2 that in the AIN-93G diet, replaced by cornstarch. During the self-selection period, pups were maintained on two separate diets: the CPD and the FPD. The protein-energy ratio of both the CPD and the FPD was prepared to be the same as the AIN-93G diet.

**Table 1. Composition of experimental diets.**

| Ingredient | LFD | CTD | CPD | FPD |
|------------|-----|-----|-----|-----|
| Casein     | 20.0| 20.0| 18.0| 33.0|
| l-Cystine  | 0.3 | 0.3 | 0.27| 0.5 |
| Cornstarch | 56.95| 52.95| 62.48| —   |
| Sucrose    | 10.0| 10.0| 10.0| —   |
| Soybean oil| 3.0 | 7.0 | —   | 7.0 |
| Lard       | —   | —   | 43.7| 8.2 |
| Cellulose  | 5.0 | 5.0 | 4.5 | 5.6 |
| Mineral mixture | 3.5 | 3.5 | 3.5 | 5.6 |
| Vitamin mixture | 1.0 | 1.0 | 1.0 | 1.6 |
| Choline bitartrate | 0.25| 0.25| 0.25| 0.4 |
| tert-Butylhydroquinone | 0.0014| 0.0014| 0.0014| 0.0014 |
| Energy (kcal/g) | 3.4 | 3.7 | 3.4 | 6.1 |
| Fat energy ratio (%) | 9.2 | 17.2| — | 77.6 |
| Carbohydrate energy ratio (%) | 68.8| 62.2| 79.4| 1.8 |
| Protein energy ratio (%) | 22.0| 20.6| 20.6| 20.6 |

The composition of all ingredients is given in grams per 100 g of diet. The mineral mixture and the vitamin mixture were based on AIN-93G formulation (25).
CPD or the FPD. Dietary ingestion and body weight were measured every day for 5 wk. On day 35 after weaning, all pups were sacrificed by decapitation. Dams were sacrificed immediately after weaning their pups. They were anesthetized by exposure to diethyl ether and killed by cardiac puncture. Blood was collected with a heparinized syringe. After centrifugation (3,000 rpm × 30 min), plasma was removed, frozen immediately and stored at −80°C until analysis. Perirenal fat tissues were removed from dams and pups and weighed.

The studies were performed in accordance with the Animal Experimentation Guidelines of the Laboratory Animal Care Committee of Seiitoku University.

**Analytical methods.** Triacylglycerol (TG) and total-cholesterol (T-cho) concentrations in plasma samples were measured using test kits (TG: Triglyceride E-test Wako, T-cho: Cholesterol E-test Wako) (Wako Pure Chemical Industries, Osaka, Japan).

Insulin and leptin concentrations in plasma were determined using test kits (insulin: Rat Insulin ELISA Kit, leptin: Rat Leptin ELISA Kit) (Wako Pure Chemical Industries).

**Statistical analysis.** Values are presented as individual group mean ± SD. Comparison between groups was made by ANOVA (one-way ANOVA in Tables 2–4, and two-way ANOVA in Fig. 1). Differences in values between groups were tested utilizing Scheffe’s multiple-range test. Differences were considered significant at $p<0.05$.

**RESULTS**

Food and energy intake, body and perirenal fat tissue weights and plasma lipid and hormone concentrations in dams and their suckling pups

No significant difference in food or energy intake during pregnancy and lactation was observed between dams fed the LFD and the CTD (Table 2). At the end of lactation, no significant difference in body or perirenal fat tissue weights was observed between the two groups. Although no significant difference in the plasma T-cho concentration was observed between the two groups, the plasma TG concentration in dams fed the LFD was significantly higher than that in the CTD group ($p<0.05$). The plasma insulin concentration in dams fed the LFD was significantly higher than that in the CTD group ($p<0.05$). No significant difference in plasma leptin concentration in dams fed the LFD was significantly higher than that in the CTD group ($p<0.05$). No significant difference in the plasma leptin concentration was observed between the two groups.

Male and female litter size in the group receiving the LFD was $7.1±0.9$ and $6.8±1.5$, respectively and that in the group receiving the CTD was $7.0±1.4$ and $6.6±0.8$, respectively. Within 24 h of birth, litters were weighed. The body weight of male and female pups of dams fed the LFD was $6.3±0.5$ and $6.2±0.3$ g, respectively and that of male and female pups of dams fed the CTD was $7.1±0.6$ and $6.8±0.5$ g, respectively. No significant difference in male or female birth weight was...
observed between pups of dams fed the LFD and the HFD. Therefore, maternal diets did not affect litter size, ratio of female vs. male pups or birth weight of pups.

On day 9 after birth, no significant difference in body weight or plasma TG, T-cho, insulin or leptin concentrations was observed between suckling pups nursed by dams fed the LFD and the CTD (Table 3).

On day 21 after birth, the body weight of pups of dams fed the LFD was significantly lower than that of pups of dams fed the CTD (p<0.05). No significant difference in perirenal fat tissue weight was observed between the two groups. Although no significant difference in the plasma T-cho concentration was observed between the two groups, the plasma TG concentration in pups of dams fed the CTD was higher than that in pups of dams fed the LFD (p<0.05). The plasma insulin concentration in pups of dams fed the LFD was higher and plasma leptin concentration in pups of dams fed the LFD was lower than that in the other groups (p<0.05).

**Body and perirenal fat tissue weights and plasma lipid and hormone concentrations in weaning pups at the end of self-selection period**

No significant difference in growth was observed between male and female suckling pups. However, as it is known that growth of male pups is significantly larger than that of females after weaning (14–16), we showed data for male and female pups separately in Table 4 and Fig. 1.

In male and female pups, at the end of 5 wk after weaning, although the body weight of pups fed the LFD (LFD group) was lower than that of pups fed the two-choice diet of the CPD and the FPD (LPD-CF group) (p<0.05), no significant difference in body weight was observed between pups fed the CTD (CTD group) and pups fed the two-choice diet of the CPD and the FPD (CTD-CF group) (Table 4). The body weight gain of the LFD group during 5 wk after weaning was lower than that of the other three groups (p<0.05). However, body weight gain of male pups after weaning was significantly higher than that of females (p<0.05). In male and female pups, no significant difference in perirenal fat tissue weight was observed among these four groups. In male and female pups, although the plasma TG and the insulin concentrations in the LFD group were higher than in the other three groups (p<0.05), no significant difference in the plasma TG concentration was observed among the LFD-CF, the CTD and the CTD-CF groups. In male and female pups, no significant difference in the plasma T-cho, the glucose or the leptin concentrations was observed among these 4 groups.

### Table 4. Body and perirenal fat tissue weights and plasma lipid and hormone concentrations in weaning pups at day 56 after birth to dams fed the LFD and the HFD.

| Group: Dams | LFD | LFD-CF | CTD | CTD-CF |
|-------------|-----|--------|-----|--------|
| Male        |     |        |     |        |
| n           | 10  | 9*     | 10  | 10     |
| Final body weight (g) | 322±15a | 364±21b | 358±23b | 366±19b |
| Body weight gain (g/35 d) | 264±29a | 306±12b | 296±18b | 302±19b |
| Perirenal fat tissue weight (g/100 g) | 1.36±0.15 | 1.62±0.33 | 1.58±0.19 | 1.65±0.26 |
| Plasma concentration |     |        |     |        |
| Triacylglycerol (mg/dL) | 126±22a | 75±18b | 58±29b | 65±12b |
| Total-cholesterol (mg/dL) | 81±16 | 73±14 | 70±19 | 62±9 |
| Glucose (mg/dL) | 139±31 | 112±24 | 128±22 | 108±16 |
| Insulin (ng/mL) | 4.66±1.17a | 2.21±0.62b | 2.55±0.72b | 2.32±0.29b |
| Leptin (ng/mL) | 4.92±1.93 | 5.77±2.50 | 6.85±2.47 | 5.26±2.23 |

| Female | n | Final body weight (g) | Body weight gain (g/35 d) | Perirenal fat tissue weight (g/100 g) |
|--------|---|-----------------------|--------------------------|-------------------------------------|
|        | 10 | 241±9a                | 183±9a                  | 0.98±0.26                           |
|        | 10 | 268±23b               | 210±14b                 | 1.29±0.18                           |
|        | 10 | 273±19b               | 209±20b                 | 1.27±0.32                           |
|        | 10 | 281±15b               | 217±13b                 | 1.35±0.27                           |
|        |    |                       |                         |                                    |
|        | 10 | 118±12a               | 76±6                    | 122±35                              |
|        |    |                       |                         | 113±21                              |
|        |    | 118±12a               | 76±6                    | 122±35                              |
|        |    |                       |                         | 113±21                              |
|        |    |                       |                         |                                    |
|        | 10 | 59±13b                | 52±24b                  | 2.16±0.43b                          |
|        |    |                       |                         | 2.54±0.55b                          |
|        |    | 52±24b                | 66±8                    | 2.09±0.42b                          |
|        |    |                       |                         | 4.78±1.18                           |
|        |    |                       |                         | 4.61±2.13                           |

Values represent means±SD. Within a row, values not sharing a common superscript letter are significantly different at p<0.05.
No significant difference in food or energy intake during pregnancy and lactation was observed between dams fed the LFD and the CTD (Table 2). At the end of lactation, nor was any significant difference in body or perirenal fat tissue weights or the plasma T-cho, the glucose or the leptin concentrations observed between the two groups of dams. However, the plasma insulin concentration in dams fed the LFD containing high levels of carbohydrates was higher than that of dams fed the CTD. It has been well known that the blood glucose concentration is increased after feeding of a high carbohydrate diet. The plasma TG concentration of dams fed the LFD was also higher than that of dams fed the CTD. There are many reports that insulin affects fatty acid synthesis by altering gene expression of acetyl-CoA carboxylase in the rate-limiting step of fatty acid synthesis (26–28). Further, the increase in quantities of lipogenic enzymes in the liver are induced by a high carbohydrate diet (29–32). Therefore, it was assumed that the intake of the LFD increased the fatty acid synthesis in the liver. As the synthesized lipids in the liver were transferred to blood as VLDL, in this study, the plasma TG concentration was actually higher in dams fed the LFD than that in dams fed the CTD (Table 2).

Pups were sacrificed by decapitation and dams were anesthetized by exposure to diethyl ether and killed by cardiac puncture. However, no significant difference in the postprandial glucose concentration was observed between dams fed the LFD and the HFD, or 56-d-old male and female pups fed the LFD and the HFD, respectively. Therefore, in both dams and pups, although there was no significant difference between groups in the postprandial glucose response to a high-carbohydrate low-fat diet, plasma insulin concentration of the groups fed the LFD was higher than that of the groups fed the HFD (Tables 2 and 4).

Although maternal diets did not affect litter size or birth weight of pups, at the end of lactation, the average body weight of pups nursed by dams fed the LFD was lower than that of pups nursed by dams fed the CTD (Table 3). The same phenomenon was observed in our previous paper (16). During the pregnancy and nursing periods, no significant difference in energy intake or body weight was observed between dams fed the LFD and the CTD and no significant difference was observed in the growth of suckling pups on day 9 after birth was observed between the two groups (Table 3). However, at the end of lactation, the body weight of suckling pups nursed by dams fed the LFD was lower than that of the other group. Although no significant difference in the plasma TG or the T-cho concentrations was observed between the two groups on day 9 after birth, the plasma lipid concentration in 9-d-old suckling pups was higher than that in their dams (Tables 2 and 3). The high plasma lipid concentration of 9-d-old suckling pups decreased after weaning (Table 4). It appeared that the

**DISCUSSION**

The ratio of the FPD intake to the total diet intake by weaning male and female pups of dams fed the LFD and the CTD when they were placed on a self-selection regimen from a two-choice program of the FPD and the CPD. Values are mean±SD. Values not sharing a common letter are significantly different (p<0.05). LFD-CF: pups nursed by dams fed the LFD during pregnancy and lactation, then fed a two-choice diet comprised of the CPD and the FPD after weaning. CTD-CF: pups nursed by dams fed the CTD during pregnancy and lactation, then fed a two-choice diet comprised of the CPD and the FPD after weaning.

**Self-selection of the FPD and the CPD by weaning pups**

Immediately after weaning, pups of the LFD-CF and the CTD-CF groups were provided both the CPD and the FPD, placed in separate cups. The ratio of the FPD intake to total intake during 5 wk is illustrated in Fig. 1. In male pups, no significant difference in the ratio of the FPD intake was observed between the LFD-CF and the CTD-CF groups within the first week of the self-selection period. However, although no significant difference in the ratio by the CTD-CF group was observed during the self-selection period, the ratio of the LFD-CF group increased after the third week. Therefore, after the third week, the ratio of the CTD-CF group was higher than that of the LFD-CF group (p<0.05).

**Fig. 1.** The ratio of the FPD intake to the total diet intake by weaning male and female pups of dams fed the LFD and the CTD during pregnancy and lactation, then fed a two-choice diet comprised of the CPD and the FPD after weaning. Values are mean±SD. Values not sharing a common letter are significantly different (p<0.05). LFD-CF: pups nursed by dams fed the LFD during pregnancy and lactation, then fed a two-choice diet comprised of the CPD and the FPD after weaning. CTD-CF: pups nursed by dams fed the CTD during pregnancy and lactation, then fed a two-choice diet comprised of the CPD and the FPD after weaning.

Although maternal diets did not affect litter size or birth weight of pups, at the end of lactation, the average body weight of pups nursed by dams fed the LFD was lower than that of pups nursed by dams fed the CTD (Table 3). The same phenomenon was observed in our previous paper (16). During the pregnancy and nursing periods, no significant difference in energy intake or body weight was observed between dams fed the LFD and the CTD and no significant difference was observed in the growth of suckling pups on day 9 after birth was observed between the two groups (Table 3). However, at the end of lactation, the body weight of suckling pups nursed by dams fed the LFD was lower than that of the other group. Although no significant difference in the plasma TG or the T-cho concentrations was observed between the two groups on day 9 after birth, the plasma lipid concentration in 9-d-old suckling pups was higher than that in their dams (Tables 2 and 3). The high plasma lipid concentration of 9-d-old suckling pups decreased after weaning (Table 4). It appeared that the

**DISCUSSION**

No significant difference in food or energy intake during pregnancy and lactation was observed between dams fed the LFD and the CTD (Table 2). At the end of lactation, nor was any significant difference in body or perirenal fat tissue weights or the plasma T-cho, the glucose or the leptin concentrations observed between the two groups of dams. However, the plasma insulin concentration in dams fed the LFD containing high levels of carbohydrates was higher than that of dams fed the CTD. It has been well known that the blood glucose concentration is increased after feeding of a high carbohydrate diet. The plasma TG concentration of dams fed the LFD was also higher than that of dams fed the CTD. There are many reports that insulin affects fatty acid synthesis by altering gene expression of acetyl-CoA carboxylase in the rate-limiting step of fatty acid synthesis (26–28). Further, the increase in quantities of lipogenic enzymes in the liver are induced by a high carbohydrate diet (29–32). Therefore, it was assumed that the intake of the LFD increased the fatty acid synthesis in the liver. As the synthesized lipids in the liver were transferred to blood as VLDL, in this study, the plasma TG concentration was actually higher in dams fed the LFD than that in dams fed the CTD (Table 2).

Pups were sacrificed by decapitation and dams were anesthetized by exposure to diethyl ether and killed by cardiac puncture. However, no significant difference in the postprandial glucose concentration was observed between dams fed the LFD and the HFD, or 56-d-old male and female pups fed the LFD and the HFD, respectively. Therefore, in both dams and pups, although there was no significant difference between groups in the postprandial glucose response to a high-carbohydrate low-fat diet, plasma insulin concentration of the groups fed the LFD was higher than that of the groups fed the HFD (Tables 2 and 4).

Although maternal diets did not affect litter size or birth weight of pups, at the end of lactation, the average body weight of pups nursed by dams fed the LFD was lower than that of pups nursed by dams fed the CTD (Table 3). The same phenomenon was observed in our previous paper (16). During the pregnancy and nursing periods, no significant difference in energy intake or body weight was observed between dams fed the LFD and the CTD and no significant difference was observed in the growth of suckling pups on day 9 after birth was observed between the two groups (Table 3). However, at the end of lactation, the body weight of suckling pups nursed by dams fed the LFD was lower than that of the other group. Although no significant difference in the plasma TG or the T-cho concentrations was observed between the two groups on day 9 after birth, the plasma lipid concentration in 9-d-old suckling pups was higher than that in their dams (Tables 2 and 3). The high plasma lipid concentration of 9-d-old suckling pups decreased after weaning (Table 4). It appeared that the

**DISCUSSION**

No significant difference in food or energy intake during pregnancy and lactation was observed between dams fed the LFD and the CTD (Table 2). At the end of lactation, nor was any significant difference in body or perirenal fat tissue weights or the plasma T-cho, the glucose or the leptin concentrations observed between the two groups of dams. However, the plasma insulin concentration in dams fed the LFD containing high levels of carbohydrates was higher than that of dams fed the CTD. It has been well known that the blood glucose concentration is increased after feeding of a high carbohydrate diet. The plasma TG concentration of dams fed the LFD was also higher than that of dams fed the CTD. There are many reports that insulin affects fatty acid synthesis by altering gene expression of acetyl-CoA carboxylase in the rate-limiting step of fatty acid synthesis (26–28). Further, the increase in quantities of lipogenic enzymes in the liver are induced by a high carbohydrate diet (29–32). Therefore, it was assumed that the intake of the LFD increased the fatty acid synthesis in the liver. As the synthesized lipids in the liver were transferred to blood as VLDL, in this study, the plasma TG concentration was actually higher in dams fed the LFD than that in dams fed the CTD (Table 2).

Pups were sacrificed by decapitation and dams were anesthetized by exposure to diethyl ether and killed by cardiac puncture. However, no significant difference in the postprandial glucose concentration was observed between dams fed the LFD and the HFD, or 56-d-old male and female pups fed the LFD and the HFD, respectively. Therefore, in both dams and pups, although there was no significant difference between groups in the postprandial glucose response to a high-carbohydrate low-fat diet, plasma insulin concentration of the groups fed the LFD was higher than that of the groups fed the HFD (Tables 2 and 4).

Although maternal diets did not affect litter size or birth weight of pups, at the end of lactation, the average body weight of pups nursed by dams fed the LFD was lower than that of pups nursed by dams fed the CTD (Table 3). The same phenomenon was observed in our previous paper (16). During the pregnancy and nursing periods, no significant difference in energy intake or body weight was observed between dams fed the LFD and the CTD and no significant difference was observed in the growth of suckling pups on day 9 after birth was observed between the two groups (Table 3). However, at the end of lactation, the body weight of suckling pups nursed by dams fed the LFD was lower than that of the other group. Although no significant difference in the plasma TG or the T-cho concentrations was observed between the two groups on day 9 after birth, the plasma lipid concentration in 9-d-old suckling pups was higher than that in their dams (Tables 2 and 3). The high plasma lipid concentration of 9-d-old suckling pups decreased after weaning (Table 4). It appeared that the
high level of plasma lipid concentration in suckling pups was due to the feeding of high fat milk. Keen et al. and Del Prado et al. reported that the F ratio in rat milk was 70–75% (33, 34). We also reported in a previous paper that the F ratio in the stomach content of 8-d-old suckling pups was 74–76% and that no clear difference was observed in the ratio among the groups of pups nursed by dams fed the low fat diet, the control diet and the high fat diet (16). As the plasma lipid concentration is easily affected by dietary fat level, the plasma lipid concentration in 9-d-old suckling pups was naturally higher than that in their dams and weaning pups.

On day 21 after birth, the plasma lipid concentration in suckling pups decreased and the plasma TG concentration in suckling pups of dams fed the LFD was lower than that in the suckling pups of dams fed the CTD. Moreover, the plasma insulin concentration in suckling pups nursed by dams fed the LFD was higher than that in the other group. One reason for this finding appeared to be that suckling pups were nibbling their dam’s diet before weaning. Therefore, it was assumed that in suckling pups of dams fed the LFD, due to the feeding of the LFD containing high levels of carbohydrates, the plasma TG concentration was lower and the plasma insulin concentration was higher than that in the suckling pups of dams fed the CTD.

No significant difference in the ratio of FPD intake was observed in either male or female pups of the CTD-CF group during the self-selection period. However, the ratio in male and female pups of the LFD-CF group increased from 20 to 40% and 19 to 35%, respectively, during the self-selection period. On the other hand, in both male and female pups, although no significant difference in the body weight gain was observed between the CTD group and the CTD-CF group, body weight gain of the LFD-CF group was higher than that of the LFD group (Table 4). Therefore, the body weight of the LFD-CF group caught up with the CTD and the CTD-CF groups by the self-selection of the CPD and the FPD. One of the reasons for this finding was likely that, as the LFD group needed a high energy density diet in order to catch their body weight up to that of the CTD group, the LFD-CF group spontaneously consumed larger amounts of the FPD than the CTD-CF group. Therefore, the pups nursed by dams fed the LFD during pregnancy and lactation showed a strong preference for fat after weaning.

In both male and female pups, as the ratios of the FPD intake were 20% (Fig. 1), the F ratios of the CTD-CF groups at the end of self-selection period was calculated to be 31.0 and 27.2%, respectively. However, although the plasma TG concentration of the LFD groups was higher than that of the LFD-CF group, no significant difference was observed between the concentrations of the LFD-CF group and the concentrations of the CTD and the CTD-CF groups. Therefore, when pups nursed by dams fed the LFD were placed on a self-selection regimen of the CPD and the FPD after weaning, they self-selected both diets to maintain an adequate plasma lipid level.

In both male and female pups, no significant difference in plasma glucose concentration was observed between the LFD groups and the CTD groups. However, the plasma insulin and the TG concentrations of the LFD group were higher than that of the CTD group. It was assumed that the increased plasma insulin concentration by the feeding of the LFD increased the fatty acid synthesis in liver and adipose tissues as well as increased the plasma TG concentration (26–28). However, although no significant difference in glucose concentration was observed between the LFD group and the LFD-CF group, the plasma insulin and the TG concentrations of the LFD-CF group were lower than those of the LFD group. Therefore, the self-selection diet appeared to prevent the increase in plasma insulin observed in pups fed the LFD. It was assumed that the dietary selection pattern of pups after weaning was associated with the reduced plasma insulin concentration.

It seems to be generally accepted that hyperinsulinemia does develop in rats fed high-carbohydrate diets while the basal plasma glucose level does not increase, mainly due to a loss of sensitivity to insulin with age together with high-carbohydrate diet ingestion (35). Insulin resistance in peripheral tissues increases with age. Although glucose homeostasis may remain normal, insulin concentrations tend to increase with age. Berthelot et al. demonstrated that a decrease in insulin sensitivity could take place early in development (36). In this study, at the end of the suckling period, the plasma insulin concentration in pups nursed by dams fed the LFD was already higher than that in pups nursed by dams fed the CTD. Hyperinsulinemia did continue in pups fed the LFD after weaning and the increased plasma insulin concentration increased the fatty acid synthesis in the liver as well as increased the plasma TG concentration (Tables 3 and 4). These data suggest that only pups fed the LFD might experience a loss of insulin sensitivity in later life. Thereafter, as pups become older, a high-carbohydrate diet may lead to insulin resistance. In conclusion, the choice of a high ratio of the FPD in pups nursed by dams fed the LFD could be related to improved insulin sensitivity and might prevent the onset of insulin resistance in later life. Additional studies are required to validate this supposition and to describe the precise mechanism involved in this differentiation.
REFERENCES

1) Campfield LA, Smith E, Gulseh Y, Devos R, Burm P. 1995. Evidence for a peripheral signal linking adiposity and central nervous networks. *Science* **269**: 546–549.

2) Schwartz MW, Woods CS, Porte D, Seeley RJ, Baskin DG. 2000. Central nervous system of food intake. *Nature* **404**: 661–671.

3) Little TJ, Horowitz M, Feinle-Bissinger C. 2007. Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. *Am J Clin Nutr* **86**: 531–541.

4) Leibowitz SE, Lucas DJ, Leibowitz KL, Jhanwar YS. 1991. Developmental patterns of macronutrient intake in female and male rats from weaning to maturity. *Physiol Behav* **50**: 1167–1174.

5) Larue-Achagiotis C, Martin C, Vergar P, Louis-Sylvestre J. 1992. Dietary self-selection versus complete diet. BW gain and meal pattern in rats. *Physiol Behav* **51**: 995–999.

6) Rieth N, Larue-Achagiotis C. 1997. Exercise training decreases body fat more in self-selecting than control fed rats. *Physiol Behav* **62**: 1291–1297.

7) Smith BK, Andrews PK, West DB. 2000. Macronutrient diet selection in thirteen mouse strains. *Am J Physiol* **278**: R797–R805.

8) Jean C, Fromentin G, Tome D, Laure-Achagiotis C. 2002. Wistar rats allowed to self-select macronutrients from weaning to maturity choose a high-lipid diet. *Physiol Behav* **76**: 65–73.

9) Bellinger L, Lilley C, Langley-Evans SC. 2004. Prenatal exposure to a maternal low-protein diet programs a preference for high-fat foods in the young adult rat. *Br J Nutr* **92**: 513–520.

10) Saito M, Ishii T, Takewaki T, Nishimura M. 2005. Preferential intake of high-fat diet with cellulose added to main- diet during growth period on the preferential fat intake in adult rats. *Jpn Soc Nutr Food Sci* **59**: 297–304 (in Japanese).

11) Nakashima Y, Yokoyama M, Kido T, Shimoda A. 2007. Effect of a lard diet and fish oil diet during the growth period on preferential fat intake in adult rats. *Jpn Soc Nutr Food Sci* **60**: 97–104 (in Japanese).

12) Nakashima Y, Yokoyama M, Kido T, Shimoda A. 2007. Effect of a lard diet and fish oil diet during the growth period on preferential fat intake in adult rats. *Jpn Soc Nutr Food Sci* **60**: 97–104 (in Japanese).

13) Nakashima Y. 2007. Effect of dams’ dietary fat type during pregnancy and lactation on the preferential fat intake in their pups after weaning. *Jpn Soc Nutr Food Sci* **60**: 241–247 (in Japanese).

14) Nakashima Y, Hiruoka K, Nomoto Y. 2007. Effect of dietary fat type by dams and weaning pups on preferential intake of high-fat diet with cellulose added to maintain same energy concentrations as low-fat diet. *Jpn Assoc Dietary Fiber Res* **11**: 57–66 (in Japanese).

15) Nakashima Y. 2008. Fish-oil high-fat diet intake of dams after day 5 of pregnancy and during lactation guards against excessive fat consumption of their weaning pups. *J Nutr Sci Vitaminol* **54**: 46–53.

16) Nakashima Y, Tsukita Y, Yokoyama M. 2008. Preferential fat intake of pups nursed by dams fed low fat diet during pregnancy and lactation is higher than that of pups nursed by dams fed control diet and high fat diet. *J Nutr Sci Vitaminol* **54**: 117–123.

17) Baskin D, Breininger J, Schwartz M. 1999. Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. *Diabetes* **48**: 824–833.

18) Fraze E, Chiu M, Chen Y. 1987. Age related changes in postprandial plasma glucose, insulin and FFA concentrations in non-diabetic individuals. *J Am Geriatric Soc* **35**: 224–228.

19) Gommers A, De Gasparo M. 1972. Variation of insulinemia in relation to age in the untreated male rats. *Geron tologia* **18**: 176–184.

20) Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. *Nature* **395**: 763–770.

21) Vickers MH, Ikenasio BA, Breier BH. 2001. IGF-I Treatment reduces hyperphagia, obesity and hypertension in metabolic disorders induced by fetal programming. *Endocrinology* **142**: 3964–3973.

22) Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, Breier BH, Harris M. 2005. Neonatal leptin treatment reverses developmental programming. *Endocrinology* **146**: 4211–4216.

23) Trottier G, Koski KG, Brun T, Tourfexis DJ, Richard D, Walker CD. 1998. Increased fat intake during lactation modified hypothalamic-pituitary-adrenal responsiveness in developing rat pups: a possible role for leptin. *Endocrinology* **139**: 3704–3711.

24) Cha MC, Jones PJH. 1998. Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats. *J Lipid Res* **39**: 1655–1660.

25) American Institute of Nutrition. 1993. AIN-93G purified diets for laboratory rodents: final reports of the American Institute of Nutrition ad hoc writing committee on the reformation of the AIN-76A rodent diet. *J Nutr* **123**: 1939–1951.

26) Pape ME, Lopez-Casillas F, Kim K-H. 1988. Physiological regulation of acetyl-CoA carboxylase gene expression: Effect of diet, diabetes and lactation on acetyl-CoA carboxylase mRNA. *Arch Biochem Biophys* **257**: 63–68.

27) Katsurada A, Iritani N, Fukuda H, Matsumura Y, Nishimoto N, Noguchi T, Tanaka T. 1991. Effects of nutrients and hormones on transcriptional and post-transcriptional regulation of acetyl-CoA carboxylase in rat liver. *Eur J Biochem* **190**: 435–441.

28) Dai ZH, Xing YZ, Boney CM. 1994. Human insulin-like growth factor-binding protein-1 (hIGFBP-1) in transgenic mice: Characterization and insights into the regulation of IGFBP-1 expression. *Endocrinology* **135**: 1136–1137.

29) Iritani N. 1992. A review. Nutritional and hormonal regulation of lipogenic enzyme expression in rat liver. *Eur J Biochem* **205**: 433–442.

30) Prostko CR, Frits RS, Kletzien R. 1989. Nutritional regulation of hepatic glucose-6-phosphate dehydrogenase. *Biochem J* **258**: 295–299.

31) Towle HC, Kaytor EN, Shih H-M. 1997. Regulation of the expression of lipogenic enzyme genes by carbohydrate. *Am J Physiol* **273**: 405–413.

32) Fukuda H, Katsurada A, Iritani N. 1992. Effect of nutrients and hormones on gene expression of ATP citrate- lyase in rat liver. *Eur J Biochem* **209**: 217–222.

33) Keen CL, Lommerdal B, Clegg M, Hurley LS. 1981. Developmental changes in composition of rat milk: Trace elements, mineral protein, carbohydrate and fat. *J Nutr* **111**: 226–230.
34) Del Prado M, Delgado G, Villalpando S. 1997. Maternal lipid intake during pregnancy and lactation alters milk composition and production and litter growth in rats. *J Nutr* **127**: 458–462.

35) Bezerra RMN, Ueno M, Silva MS, Tavares DQ, Cavalho CRO, Saad MJA. 2000. A high fructose diet affects the early step of insulin action in muscle or liver of rats. *J Nutr* **130**: 1531–1535.

36) Berthelier C, Kergoat M, Portha B. 1997. Lack of deterioration of insulin action with aging in the GK rats: a contrasted adaptation as compared with non diabetic rats. *Metabolism* **46**: 890–896.