The Fat:Carbohydrate Energy Ratio of the Weaning Diet Programs Later Susceptibility to Obesity in Male Sprague Dawley Rats¹–³

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Abstract

Dietary fat intake, which is high during suckling, is markedly reduced when food and drinks are introduced into the diet. We investigated whether alterations in the fat:carbohydrate (CHO) content of the weaning diet influenced the later development of adiposity and insulin sensitivity. Three groups of male rats (24/group) were fed from age 16–37 d (phase I) with weaning diets varying in their fat:CHO energy (E) ratios, 10:70 low-fat, high-CHO (LFHC); 30:50 medium-fat, medium-CHO (MFMC), and 60:30 high-fat, high-CHO (HFLC), on an isocaloric basis. Then, all groups consumed ad libitum first a low-fat diet (13% fat E) for 30 wk (phase II) and subsequently a high-fat diet (45% fat E) for another 18 wk (phase III). At the end of phase I, the group fed the HFLC diet demonstrated higher plasma glucose and insulin responses to an oral glucose tolerance test (P < 0.05), but this effect was transient and did not persist into adulthood (phases II and III). By contrast, when challenged with a high-fat diet later in life (age 35.3–53.3 wk), the LFHC group had greater gains in weight (as percent initial weight) and body fat (as absolute and percent body weight) than the other 2 groups that had been weaned with diets higher in fat (P < 0.04 for all). These results provide evidence that metabolic programming by altering the dietary fat:CHO ratio can occur during the weaning period and emphasizes the importance of the fat:CHO ratio of the complementary diet and its relation to the susceptibility to develop adiposity later in life. J. Nutr. 141: 81–86, 2011.

Introduction

The increasing pandemic of obesity and the lack of an effective treatment has driven research interests further toward preventative therapy, especially during early life when metabolic processes may be defined or reset. Indeed, it is now well recognized that suboptimal nutrition during critical periods of development may induce long-term alterations in organ structures or functions, which can predispose humans to later chronic diseases (1–5). Such early programming by suboptimal pre- or postnatal nutrition has repeatedly been shown in animal models to affect body size, metabolism, and later health outcomes that include elevated blood lipids, high blood pressure, insulin resistance, obesity, and reduced life span (6–13). In humans, the concept of early programming is strongly supported by several large epidemiological and clinical studies indicating associations between markers of early nutrition (size at birth, size in infancy, rate of early growth) and increased risks for later adult hypertension, diabetes, coronary heart disease, and obesity (4,14–20). The prenatal and suckling periods are now recognized as critical windows for early programming, with interest focused upon the size at birth (2,18,20), kinetics of early growth (14–17), breast feeding and its duration (21–25), and, more recently, the protein content of infant formula (26).

By contrast, it is unclear whether or not programming may also occur during the period of complementary feeding (complementing the intake of breast milk with other foods and drinks). The few studies that have been conducted have examined the impact of the time of complementary food introduction (27–29) and its protein content (30,31) but have not investigated other components of the complementary diet. In fact, the complementary feeding period is a period of rapid growth characterized by a relatively low fat intake in affluent countries (~30% energy (E)) and is even lower in developing countries because of partial replacement of high-fat milk by carbohydrate (CHO)⁴-rich complementary foods such as fruits, vegetables, cereals, and fruit juices (32–35). Infant nutrition during the complementary feeding period is surrounded by uncertainties.

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³ Suppemental Tables 1 and 2 and Figures 1–3 are available with the online posting of this paper at j.nutrition.org.
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Abbreviations used: AUC, area under the curve; CHO, carbohydrate; E, energy; FAS, fatty acid synthase; HFLC, high-fat (60% E) low-carbohydrate (20% E); LFHC, low-fat (10% E) high-carbohydrate (70% E); MFMC, medium-fat (30% E) medium-carbohydrate (50% E); OGTT, oral glucose tolerance test; TG, triglyceride.
and there is little agreement about the optimal composition of the complementary diet, in particular what the optimal ratio of fat:CHO content of complementary diets should be. Nonetheless, most authorities emphasize that fat intake should not be restricted until 2 y of age and recommend gradual reduction of fat intake from 50−55% E at 6 mo of age to 30% E by the age of 2 y (36).

During this transition from suckling to complementary feeding in infants, or weaning period in animals (post suckling solid food intake), many important hormonal and enzymatic changes occur that affect CHO and lipid metabolism (37). In particular, a high-CHO, low-fat weaning diet increases plasma insulin and decreases plasma glucagon concentrations in the newborn of different species, including rats, rabbits, sheep, pigs, and humans (37). These changes do not occur when rats are weaned with a high-fat diet (37,38). Furthermore, the synthesis of lipids by de novo lipogenesis, which is very low during the suckling period of high-fat milk intake, remains low if rats are weaned onto a high-fat diet but increases markedly if they are weaned with a high-CHO diet (37,39−41). These effects are also observed when rats are prematurely weaned with a high-CHO diet (37,39−41), suggesting that the observed hormonal and enzymatic changes during the transition from suckling to the weaning period are linked to the fat:CHO ratio of the weaning diet rather than to an age-related process (37). Whether earlier initiation of these hormonal and metabolic changes can have a long-lasting impact on later health is not known. In the present study, we investigated the extent to which alterations in the fat and CHO content of the weaning diet in rats may influence the later development of obesity and affect whole-body insulin sensitivity.

In this article, the term weaning diet or period refers to the post suckling diet or period when the intake of breast milk is stopped completely by separating the pups from their dams and the pups are fed with only solid food and water.

On the other hand, the term complementary diet or period, defined as the intake of other drinks and foods in addition to breast milk, is used for describing infant studies.

Materials and Methods

Animals and experimental design. The study was approved by the Office Vétérinaire Cantonale Vaudois. Seventy-two male Sprague-Dawley rats were separated from their dams and weaned at 16 d of age. The rationale for early weaning in this study (16 d rather than the usual 21 d) was to mimic the early introduction of complementary foods commonly observed in infants and also to prevent the pups nibbling their dam’s diet during the last few days of lactation (age 15–21 d). The same number of male pups from each litter with similar body weights were assigned to 1 of the 3 study groups (n = 24/group). Thus, all groups had similar mean body weights and SD and the same number of pups from each litter. Rats were caged individually in a room at 23 ± 2°C with 55% relative humidity and a 12-h-light:12-h-dark cycle and had free access to water during the study.

Diets and feeding procedure. The rats were pair-fed on an isocaloric and iso-protein basis (20% E) using one of the following weaning diets with fat:CHO E ratios of: 10:70 (LFHC: low-fat, high-CHO); 30:50 (MFMC: medium-fat, medium-CHO), and 60:20 (HFLC: high-fat, low-CHO) % E for 3 wk (phase I, age: 16−37 d) (Fig. 1). The composition of the LFHC diet was based on the AIN-93G diet (42) with slight modifications as follows (g/100 g): 20 casein (90% protein, Schweizerhalle), 0.3 l-lysine, and 0.0014 tert-butylhydroquinone (Fluka), 51 corn starch and 0.25 chlorine bitartrate (Synopharm), 10 sucrose (Howeg), 5 lactose monohydrate (Meggle), 2 soybean oil and 2 corn oil (Nutriswiss), 1 AIN 93 G vitamin mixture and 3.5 AIN 93 mineral mixture (Socochim), and 5 cellulose (Christ water Technology). The composition of the MFMC and HFLC diets was similar to the LFHC diet, except for an isocaloric exchange of corn starch with the fat mixture (a mixture of corn oil and soy oil on a 50:50 weight basis) in the proportion described above. The LFHC group consumed food ad libitum and the daily intake of MFMC and HFLC groups was limited to that of the LFHC intake on an isocaloric basis. In this way, during the weaning period (phase I), all groups had similar intakes of E, protein, fiber, and micronutrients but not fat and CHO. All groups then consumed food ad libitum. They were first fed a commercial normal rat diet (Kliba 3434; Provimi, CH-4303 Kaiseraugst) with a macronutrient E composition of 25% protein (mainly from whey, poultry, and soybean), 13% fat (soybean, poultry), and 62% CHO (cereals) for 30 wk (phase II, age: 5.3−35.3 wk) and subsequently were challenged with a high-fat, obesogenic diet (Kliba 2126 Provimi, CH-4303 Kaiseraugst) with a composition of 21% protein (casein and t-cysteine), 45% fat (lard and soybean), and 24% CHO (corn starch, sucrose, and maltodextrin) for 18 wk (phase III, age: 35.3−53.3 wk) (Fig. 1). Food intake and body weight were measured 2–3 times/wk throughout the study, except during the weaning period when food intake was measured daily.

Killing and sample collection. Rats were killed at 53.3 wk of age after 6 h of daytime food deprivation (from 0730 to 1330 h) following anesthesia with isoflurane and blood collection. Blood samples taken from the abdominal aortal vein were collected into tubes containing EDTA. Plasma was separated (10 min centrifugation at 500 g), frozen in dry ice, and kept at −80°C until analysis. Organs (heart, liver, kidney, and spleen) were dissected, weighed, frozen in dry ice, and kept at −80°C. The nose-anus length was measured in the dead rats.

Body composition (fat mass and lean mass). Body composition was measured with NMR using EchoMRI 2004 (Echo Medical Systems) at 27, 35, 47, and 52 wk of age.

Oral glucose tolerance test. The oral glucose tolerance test (OGTT) was performed in all rats after 6 h of daytime food deprivation (from 0730 to 1330 h) at 5, 27, and 52 wk of age after the NMR measurement. Two baseline blood samples (0.1 mL) were taken from the tail vein within at least 10 min (time −10 and 0 of study) before administration of
the glucose solution (1.67 mol/L) by gavage at a dose of 11 mmol glucose/kg body weight. Further blood samples were collected (0.1 mL) from the tail vein at 15, 30, 45, and 60 min during the first hour (age 5 wk) and then at 30-min intervals during the second hour (age 27 wk) and 2.5 h (age 52 wk) after glucose administration.

**Biochemical analyses.** Glucose during the OGTT was measured in blood using a glucometer (Bayer, Ascensia ELITE XL). Plasma insulin and leptin were measured by an ELISA method using ultra-sensitive rat insulin ELISA and rat leptin ELISA kits from Crystal Chem. Triglyceride (TG) and total cholesterol were measured by enzymatic methods with a centrifugal analyzer (Cobas FARA, Roche Diagnostica) using TG PAP 150 and Cholesterol RTU kits and calibration solutions from BioMérieux. Plasma adiponectin was measured by an ELISA method using a rat adiponectin ACRP 30 ELISA kit (Linco Research). Plasma FFA were measured by an enzymatic colorimetric method with a centrifugal analyzer (Cobas FARA, Roche Diagnostica) using a NEFA C kit (Wako).

Liver triglycerides were extracted by hexane/isopropanol (3:2, v:v), as previously described (43), and measured by an enzymatic colorimetric method using TG PAP 150 kit from BioMérieux following saponification with KOH in ethanol (0.5 mol/L) at 70°C for 1 h and neutralization with MgSO4 (0.15 mol/L). Fatty acid synthase (FAS) activity was measured in liver and epididymal fat pads of the LFHC and HFLC groups (i.e., those with the biggest differences in fat:CHO E ratio of the weaning diet), as described by Guichard et al. (44).

**Statistical analysis.** Results were checked for normality and homoscedasticity. Based on data distribution, blood variables were analyzed by 1-way ANOVA followed by post hoc pairwise comparisons. For all these analyses, the Bonferroni correction was applied. The threshold value for all the analyses was set at $P < 0.05$. Results are expressed as means or medians with SE. Analyses were performed with NCSS 2004 (NCSS Statistical Software) and SAS 9.1 (SAS Institute).

**Results**

**E intake, body weight, and body composition.** E intake did not differ among groups during any of the dietary phases (Supplemental Fig. 1) and there were no differences in body weight (Supplemental Fig. 2A–C) or weight gain in phases I and II among the groups. However, during the 4-mo high-fat challenge diet (phase III), the group fed the weaning diet with the lowest fat:CHO content (LFHC) had a greater weight gain than the MFMC and HFLC groups (Fig. 2A). Indeed, during the 4-mo high-fat diet (phase III), all groups gained a considerable amount of weight and fat while consuming the obesogenic diet but not all did so to the same extent (Fig. 2A–D). In fact, during this phase, the LFHC group gained more absolute weight and fat relative to the MFMC (P < 0.03) and HFLF groups (P = 0.08 and P < 0.04, respectively) (Fig. 2A, C), with no differences in lean mass (Supplemental Table 1). Furthermore, these differences in weight and fat between the LFHC and the MFMC and HFLC groups during the obesogenic diet (phase III) were significant when expressed as fractional gains (i.e., per 100 g weight at the start of phase III) (P < 0.02) (Fig. 2B, D).

**OGTT.** Basal glucose and insulin concentrations of all of the groups were similar at 5, 27, and 52 wk of age (Supplemental Fig. 3A–F). At the end of the weaning diet (5 wk, phase I), the peak values and area under the curve (AUC) for glucose and insulin were significantly higher in the HFLC group than in the LFHC and MFMC groups, which were fed the weaning diets with lower fat:CHO ratios (P < 0.05) (Supplemental Fig. 3A, D; Fig. 3A, B). However, these effects did not persist into older age. When the different groups were fed similar low-fat (phase II) or high-fat (phase III) diets, they all had similar glucose and insulin responses to the OGTT at 27 wk (phase II) and 52 wk (phase III) of age (Supplemental Fig. 3B, C, E, F; Fig. 3A, B).

**Liver FAS and TG content.** Liver FAS activity (999 ± 71 and 973 ± 70 U·mg⁻¹) and TG content (1.40 ± 0.10 and 1.22 ± 0.19 mmol) were similar in the LFHC and HFLC groups, respectively.

**Blood variables.** Plasma FFA concentrations were lower in the group fed the highest fat:CHO weaning diet (HFLC) than in the other 2 groups (P < 0.05) (Table 1). Plasma total cholesterol, TG, leptin, and adiponectin concentrations did not differ among the groups (Table 1).

**Organ weights and body length.** Organs weights (kidney, heart, liver, and spleen) and body lengths were similar in all groups at the end of the study (Supplemental Table 2).

**Discussion**

The deleterious effects of reducing the fat content and increasing the CHO content of milk during suckling period, with reference...
to later programming of obesity and hyperinsulinemia, has repeatedly been demonstrated in rats by Patel’s group (45–49). They demonstrated that neonatal pups who were artificially reared from 4 to 24 d of age with a low-fat (20% E), CHO-rich (56% E) milk substitute formula developed obesity, hypertrophy, and hyperplasia of the pancreatic β-cells with age and hyperinsulinemia as adults compared with pups fed dam milk or a formula with a similar composition to that of dam milk (68% fat E and 8% CHO E). Furthermore, these characteristics were transmitted to the next generation without any further dietary intervention. These investigations emphasize the potential of nutritional programming in the later development of obesity and diabetes in response to nutritional interventions with a low-fat, high-CHO diet from an early age of 4 d during the suckling period. The results of the present study further suggest that exposure to a low-fat, high-CHO diet at an older age during weaning (age 16–37 d), under an isocaloric food intake condition, can also program the later susceptibility to fatness in adult life.

We found that in groups of rats who were previously weaned with diets varying markedly in the fat:CHO E ratio, i.e. 10:70% E (LFHC group), 30:50% E (MFMC group), and 60:20% E (HFLC group), body weight and body composition did not differ when rats were fed a normal rat diet (13% fat E) (phase II),

However, those weaned with the LFHC diet demonstrated greater gains in body weight and body fat than either the HFLC or MFMC groups when subsequently exposed to an obesogenic high-fat challenge diet (45% fat E) (phase III). This suggests that the fat:CHO E distribution of the weaning diet does, under specific dietary conditions, affect the later development of excess adiposity such as a high-fat adult diet. Because the high-fat challenge was introduced later in life at 35 wk of age after the low-fat adult diet, one cannot exclude the importance of an age × diet interaction on the impact of fat:CHO intake during the weaning period on the later development of excess fat. These differential effects on body weight gain and fat gain in response to high-fat feeding are small but sustained (Fig. 2A–D) and may have affected body weight and body fat if rats were exposed to the obesogenic diet for longer. Further analysis of body weight and body composition over this 4-mo period of high-fat feeding also revealed that virtually all (90–100%) of the excess weight gain in the group with the lowest fat:CHO weaning diet (LFHC) could be explained by an excess of body fat gain, with little or no change in lean body mass relative to the MFMC (90%) and HFLC (100%), respectively (Fig. 2A,C; Supplemental Table 1). This lack of difference in total lean mass was also reflected in the masses of the various vital organs of lean mass such as the heart, liver, kidneys, and spleen (Supplemental Table 2).

The higher glucose and insulin responses (peak and AUC) in the HFLC group relative to other groups at the end of weaning period (age 5 wk) indicates lower insulin sensitivity with weaning diet higher in the fat:CHO ratio (60:20% E) relative to a weaning diet lower in fat:CHO (10:70% and 30:50% E), consistent with other reports in rats in euglycemic-hyperinsulinemic-clamp studies (38). In fact, the newborns of many species (human, lamb, dog, and rats) have insulin resistance when consuming their mother’s milk during the suckling period (37). During the weaning period, insulin sensitivity develops in rats if the weaning diet is rich in CHO but not when it is rich in fat, indicating the importance of the composition of the weaning diet in the development of insulin sensitivity (38).

In this study, as expected, the baseline insulin and insulin responses to OGTT increased with age (phase II) and in response to the high-fat challenge with aging (phase III) (Supplemental Fig. 3). However, when the rats were older and were being fed diets with the same composition, they all had similar blood glucose and plasma insulin concentrations in the basal state and in response to the OGTT at 27 and 52 wk old. Thus, the results suggest that the higher glucose and insulin responses observed in the HFLC group at the end of the weaning period were transient and did not persist into adult life during phase II when the normal rat diet was fed, nor during phase III when the obesogenic diet was fed. The increase in weight and fat gains in the LFHC group during phase III therefore occurred in the absence of differences in blood

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**TABLE 1** Plasma cholesterol, TG, FFA, leptin, and adiponectin concentrations at age 53.3 wk in rats fed LFHC, MFMC, and HFLC diets only during the weaning period (age 16–37 d)¹

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Triglyceride</th>
<th>FFA</th>
<th>Leptin</th>
<th>Adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmol/L</td>
<td>mmol/L</td>
<td>µmol/L</td>
<td>µg/L</td>
<td>g/l</td>
</tr>
<tr>
<td>LFHC 2.5 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>526 ± 24³</td>
<td>36.3 ± 2.8</td>
<td>14.4 ± 0.7</td>
</tr>
<tr>
<td>MFMC 2.5 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>458 ± 45³</td>
<td>27.5 ± 3.7</td>
<td>15.2 ± 0.8</td>
</tr>
<tr>
<td>HFLC 2.5 ± 0.1</td>
<td>1.7 ± 0.2</td>
<td>380 ± 21³</td>
<td>28.0 ± 2.8</td>
<td>14.0 ± 0.5</td>
</tr>
</tbody>
</table>

¹ Values are median ± SE, n = 21–23. Medians in a column with superscripts without a common letter differ, P < 0.03.
glucose homeostasis and insulinemia. In contrast to our finding with fat:CHO intervention during the weaning period (age 16–37 d), later hyperinsulinemia was repeatedly reported when low-fat, high-CHO diets were consumed during the neonatal suckling period (age of 4–21 d) (45–49).

The present data also indicate that alterations in the dietary fat:CHO ratio during the weaning period, unlike that found during the suckling period by others (45–48), did not modify FAs activity, a key marker of de novo lipogenesis in the liver and epididymal fat tissue at the end of our study. Similarly, there were no between-group differences in hepatic lipids at 53 wk of age. Because high-fat diets are known to depress de novo lipogenesis (41), the 4 mo of high-fat feeding during phase III may have decreased de novo lipogenesis to a minimum in all groups and hence masked possible dietary effects of the weaning period on markers of de novo lipogenesis. Among the other variables assessed, plasma FFA were significantly lower in the HFLC group relative to the LFHC and MFMC groups, which may suggest better adipose tissue insulin sensitivity at this time in the group fed the highest fat:CHO weaning diet (HFLC). Nonetheless, this cannot explain the observed differences in later fat gain, because both HFLC and MFMC with different plasma FFA concentrations had similar fat gains during the high-fat challenge.

Although there were no significant differences in E intake among the groups in all periods (phase I, II, or III), the cumulative E intake during phase III was slightly greater (4–6%) in the LFHC group than in the 2 other groups (ANOVA, P = 0.2). Though small in magnitude, the possible impact of slight differences in E intake on body fat gain during the high-fat challenge period cannot be excluded. E expenditure as a plausible mechanism of action was not assessed in this study and whether the weaning diet with a higher fat content could condition the body’s metabolism to channel dietary fat into oxidative, rather than fat storage, remains to be investigated.

The overall results suggest that, at least in rats, the fat:CHO E ratio of the weaning diet, under the condition of equal E intake, plays a role in the susceptibility to develop adiposity later in life. A low-fat weaning diet appears to increase susceptibility to develop adiposity later in life if an unfavorable diet, such as a high-fat diet, is consumed. The mechanism of action is not clear but does not seem to be associated with lower basal or insulin responses to a glucose challenge.

To our knowledge, this is the first report of metabolic programming related to the fat:CHO ratio of the weaning diet. However, the effect induced during the weaning period in this study (age 16–37 d) was much smaller than that observed during the earlier suckling period (age 4–24 d) by Patel et al. (45–49).

The results of the present study suggest that a complementary diet with a higher vegetable fat content, under conditions of equal E intake, may reduce the gain in adiposity during exposure to an obesogenic diet later in life. Consistent with our findings, which highlight the potential consequences of low-fat weaning diets, are reports in humans demonstrating a negative association between fat intake at 2 y of age and BMI at 20 y old (50); furthermore, the age of introduction of complementary foods is negatively associated with BMI at 42 y of age (27). Our results, if applicable to infants, are aligned with the current dietary recommendations specifying that the fat intake should not be restricted until 2 y of age (36).

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**Literature Cited**
