High-Protein Exposure during Gestation or Lactation or after Weaning Has a Period-Specific Signature on Rat Pup Weight, Adiposity, Food Intake, and Glucose Homeostasis up to 6 Weeks of Age

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Abstract

Background: Early-life nutrition has a programming effect on later metabolic health; however, the impact of exposure to a high-protein (HP) diet is still being investigated.

Objective: This study evaluated the consequences on pup phenotype of an HP diet during gestation and lactation and after weaning.

Methods: Wistar rat dams were separated into 2 groups fed an HP (55% protein) or normal protein (NP) (control; 20% protein) isocaloric diet during gestation, and each group subsequently was separated into 2 subgroups that were fed an HP or NP diet during lactation. After weaning, male and female pups from each mother subgroup were separated into 2 groups that were fed either an NP or HP diet until they were 6 wk old. Measurements included weight, food intake, body composition, blood glucose, insulin, glucagon, leptin, insulin-like growth factor I, and lipids.

Results: Feeding mothers the HP diet during gestation or lactation induced lower postweaning pup weight (gestation diet \times time, \(P < 0.0001\); lactation diet \times time, \(P < 0.0001\)). Regardless of dams’ diets, pups receiving HP compared with NP diet after weaning had 7% lower weight (NP, 135.0 ± 2.6 g; HP, 124.4 ± 2.5 g; \(P < 0.0001\)), 16% lower total energy intake (NP, 777 ± 4 kcal; HP, 649 ± 13 kcal; \(P < 0.0001\)) and 31% lower adiposity (\(P < 0.0001\)). Pups receiving HP compared with NP diet after weaning had increased blood glucose, insulin, and glucagon when food deprived (\(P < 0.0001\) for all). The HP compared with the NP diet during gestation induced higher blood glucose in food-deprived rats (NP, 93.2 ± 2.1 mg/dL; HP, 91.2 ± 2.1 mg/dL; \(P = 0.046\)) and increased plasma insulin in fed pups receiving the postweaning NP diet (gestation diet \times postweaning diet, \(P = 0.02\)).

Conclusion: Increasing the protein concentration of the rat dams’ diet during gestation, and to a lesser extent during lactation, and of the pups’ diet after weaning influenced pup phenotype, including body weight, fat accumulation, food intake, and glucose tolerance at 6 wk of age. J Nutr 2016;146:21–9.

Keywords: high-protein diet, gestation, lactation, rat model, programming

Introduction

The early-life period, starting even before conception, is a key determinant of adult health. Environmental exposure, particularly nutrition, during this period can have long lasting effects (1–3). Dietary proteins during the perinatal period are an important nutritional driver of growth, but also have been associated with an increased risk of metabolic diseases later in life (4). In humans, energy or protein restriction during pregnancy, followed by catch-up growth after birth, is associated with an increased risk of obesity (5, 6). In addition, a high-protein (HP) diet8

8 Abbreviations used: BCFA, branched-chain fatty acid; HP, high-protein; HPgest, pups born to mothers fed the high-protein diet during gestation; HPw, pups fed the high-protein diet after weaning; IGF-I, insulin-like growth factor I; NP, normal-protein (control); NPgest, pups born to mothers fed the normal-protein (control) diet during gestation; NPw, pups fed the normal-protein (control) diet after weaning; PND, postnatal day.

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diet during gestation may be harmful to the fetus (7). However, studies have reported conflicting results: an HP diet during gestation has been associated with lower (8), higher (9), or unmodified (9, 10) birth weight, as well as a higher BMI at 20 y of age, with a stronger effect in female subjects (11). Some studies in animal models concluded that an HP diet during gestation reduces the birth weight of pups (12–14), whereas others showed no effect on birth weight (15–17). After birth, formula-fed infants have been shown to have faster weight gain (18, 19) and an increased risk of obesity later in life (20, 21). Generally, infant formulas provide higher concentrations of proteins to compensate for the protein quality differences between cow and breast milk (22). This higher protein intake tentatively has been associated with the slight, but consistent, increase in obesity risk related to formula feeding (23, 24), although a causal relation has yet to be clearly established (25, 26). In animal models, HP diets provided immediately after birth have been shown to induce either a higher (27–29) or lower body weight (30, 31). Early-life programming can also be gender specific, depending on the timing and the environmental conditions (32). In rats, for instance, an HP diet during both gestation and lactation results in a predisposition to increased fat mass in adulthood, with a stronger effect on the female offspring (33).

The objective of the present study was to determine the consequences of an HP diet in early life on pup phenotype and gut microbiota activity. To this end, mothers were fed an HP or a normal-protein (NP) (control) diet during gestation and/or lactation, and pups were fed an HP or an NP diet after weaning until late childhood (6 wk of age). To assess possible gender-specific sensitivity in each group, both male and female offspring were studied.

**Methods**

**Diets.** Two isoenergetic diets were used: a control growth NP diet with 20% of energy from proteins, 10% of energy from fat, and 70% of energy from carbohydrates; and an HP diet with 55% of energy from proteins, 15% of energy from fat, and 35% of energy from carbohydrates. The NP diet was based on the AIN-93G diet composition requirements for pregnant, lactating, or growing rats (34). The composition of the NP diet was 200 g cow milk protein/kg, 570 g corn starch/kg, and 92.7 g sucrose/kg, whereas that of the HP diet was 530 g cow milk protein/kg, 287 g corn starch/kg, and 45.7 g sucrose/kg. Both diets also contained 40 g soybean oil/kg, 35 g mineral mix/kg, 10 g vitamin mix/kg, 50 g cellulose/kg, and 2.3 g choline/kg.

**Experimental design.** The protocol was approved by the local animal ethical committee [Animal Experimentation Ethics Committee (COMETHEA) at Jouy-en-Josas, France, no. 11/047]. Sixteen female Wistar Han rats (Haran) were maintained under controlled conditions (22°C, 12 h:12 h dark-light cycle; lights on at 0800) with free access to their food and water. After 1 wk of habituation, the 16 females were mated and randomly assigned to 2 groups of 8 females fed either the NP diet or the HP diet during gestation. Dams were housed individually. After the birth of the pups, pup litters were standardized to 8 pups (4 males and 4 females), and each previous group of 8 dams was randomly assigned to 2 groups of 4 females fed either the NP diet or the HP diet during lactation and housed individually with their pups. At weaning, pups were housed individually in tunnel-enriched cages. Each litter was randomly assigned to 2 groups of 4 pups (2 males and 2 females) that were fed either the NP diet or the HP diet. This design produced 8 groups of 16 pups (8 groups of females and 8 groups of males), with all possible combinations of the NP or HP diets over the three 3-wk periods that included the gestation, lactation, and postweaning periods (Figure 1). Pup groups included pups born to mothers fed the high-protein diet (HPgest group), pups born to mothers fed the normal-protein (control) diet (NPgest group), pups suckled by mothers fed the high-protein diet during lactation (HPlact group), pups suckled by mothers fed the normal-protein (control) diet during lactation (NPlact), pups fed the high-protein diet after weaning (HPw), and pups fed the normal-protein (control) diet after weaning (NPw). Dams were killed at weaning [postnatal day (PND) 21], and pups were killed at the age of 6 wk (PND 42) with pentobarbital sodium injection. Each pup was weighed daily from birth to PND 42. After weaning, food consumption was monitored every 2 d by the weight of the food. On PND 42, after a night of ad libitum food consumption, pups were anesthetized with pentobarbital sodium. Body composition was assessed by dissection and weighing of each organ, individual adipose tissue pads (subcutaneous and visceral adipose tissues), and carcass.

**Plasma hormones, metabolites, and hepatic TGs.** Blood samples were obtained from the pups after 12 h of food deprivation, around PND 40, via tail venous sampling. Blood samples were also collected on PND 42 by vena cava puncture in fed rats. Blood was collected in EDTA-coated tubes and centrifuged at 3000 × g for 10 min at 4°C. Plasma was stored at −20°C until analysis. Blood or plasma glucose was measured with a glucose meter and reactive strips (Accu-Check Go, Roche Diagnostics). Plasma insulin was assayed by ELISA. Standard kits were used for the sera collected in fed rats (Rat Insulin ELISA kit, 10–1250–01, Merodia). Ultrasensitive kits (Ultradsensitive Rat Insulin ELISA kit, 10–1251–01, Merodia) were used for samples collected in food-deprived rats in which insulin concentrations were too low and below the detection limit of the standard kits. The kits were used according to the manufacturer’s instructions. Plasma insulin-like growth factor I (IGF-I) was assayed by ELISA (Mouse/Rat IGF-I Immunooassay MG100 ELISA kit, R&D Systems) according the manufacturer’s instructions. Assays of plasma TGS, total cholesterol, and HDL cholesterol were performed by colorimetric enzymatic assays with the use of 10 μL samples for TGS and 5 μL samples for total and HDL cholesterol, and following the manufacturer’s instructions adapted for microplates (cholesterol: RTU, BioMérieux; TGS: TR210, and direct HDL cholesterol: CH2653,

**Figure 1** Formation of rat pup HP and NP diet–exposed groups for study from gestation until the age of 6 wk. HP, high-protein diet; HPgest, pups born to mothers fed the high-protein diet; HPlact, pups suckled by mothers fed the high-protein diet during lactation; HPw, pups fed the high-protein diet after weaning; NP, normal-protein (control) diet; NPgest, pups born to mothers fed the normal-protein (control) diet; NPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation; NPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation (NPlact group), pups suckled by mothers fed the normal-protein (control) diet during lactation (NPlact), pups fed the high-protein diet after weaning (HPw), and pups fed the normal-protein (control) diet after weaning (NPw). Dams were killed at weaning [postnatal day (PND) 21], and pups were killed at the age of 6 wk (PND 42) with pentobarbital sodium injection. Each pup was weighed daily from birth to PND 42. After weaning, food consumption was monitored every 2 d by the weight of the food. On PND 42, after a night of ad libitum food consumption, pups were anesthetized with pentobarbital sodium. Body composition was assessed by dissection and weighing of each organ, individual adipose tissue pads (subcutaneous and visceral adipose tissues), and carcass.

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both Randox). The control serum used was Unitrol (BioMérieux). Plasma leptin and glucagon were assayed by Luminex with a multiplex kit (Bio-Plex 200 system; Biorad). TGs in the liver were measured by a method previously described by Arakawa et al. (35). Approximately 80 mg of a specific liver lobe was precisely weighed and mixed in buffer (150 mM NaCl, 10 mM tris, and 0.1% triton), and assayed by colorimetric enzymatic assays (TGs, TR210, Randox) in 10 μL of mixed sample.

**Intestinal microbiota activity.** The method from Kristensen et al. (36) was used to assay SCFA and branched-chain fatty acid (BCFA) concentrations. It is based on esterification of FAs by 2-chloroethyl-chloroformate in acid and a partially aqueous milieu. The esters are then separated by GC. The luminal contents of the cecum and colon were collected after the pups were killed, and the contents were weighed and stored at -20°C. Bacterial metabolites were extracted by vigorous homogenization from diluted contents with ultrapure water, followed by centrifugation (14000 × g for 15 min at 4°C). H$_3$PO$_4$ was added to a final concentration of 0.5%. SCFAs and BCFAs were derivatized by esterification and analyzed with a gas chromatograph equipped with a capillary column (30 m, 0.32 mm ID, Restek Rtx 502.2) as already described (37). The amount of SCFAs and BCFAs was determined by external standards with reference to internal standards.

**Statistical analysis.** Data were modeled with the use of mixed models, one (model 1) for the analysis of traits measured only at final timepoint, and one (model 2) for the analysis of traits measured throughout each day for the duration of the experiment. Both models 1 and 2 account for the (fixed) effects of diet during gestation and lactation and after weaning, gender, and their interactions. A random rat dam effect was added to account for correlations between measurements performed on related pups. Model 2 also includes a time effect and all interactions between time, diets, and gender. A random pup effect was also added to account for correlations between repeated measurements on the same rat (see Supplemental Methods for detailed statistical model). All tests presented in the present paper were performed within 1 of the 2 models. When testing for differences between groups at each time point, a Tukey post hoc procedure was performed to account for multiple testing. All statistical analyses were performed with the use of SAS 9.1.

**Results**

**Body weight, food intake, and feed efficiency.** Birth weight of the pups was not significantly different between the HPgest and NPgest groups (HPgest, 5.58 ± 0.06 g; NPgest, 5.74 ± 0.08 g; FIGURE 2. Body weight during the lactation and postweaning periods of male (A) and female (B) rat pups fed the HP or NP diet after weaning, born to dams fed the HP or NP diet during gestation and/or lactation. Values are means, n = 8. *Weight of the pups in the HPgest and HPlact groups is significantly different from other groups (comparison corrected by Tukey post hoc test, P < 0.05). #Weight of the pups in the HPw group is significantly different from the weight of the pups in the NPw group. Effects of diets, gender, time, and their interactions were tested within mixed-model 2. HP, high-protein diet; HPgest, pups born to mothers fed the high-protein diet during gestation and/or lactation; HPlact, pups suckled by mothers fed the high-protein diet during lactation; HPw, pups fed the high-protein diet after weaning; NP, normal-protein (control) diet; NPgest, pups born to mothers fed the normal-protein (control) diet; NPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation; NPw, pups fed the normal-protein (control) diet after weaning; PND, postnatal day; P.-w., postweaning.
P = 0.39) despite a slightly lower food intake by the dams fed the HP diet during gestation (HP, 18.3 ± 0.78 g/d; NP, 19.9 ± 0.65 g/d; P = 0.04). During lactation, pup weight gain was affected by gestation as well as lactation diets. The HP diet during the 2 periods decreased pup weight gain after birth compared with the NP diet (gestation × time, lactation × time, and gestation × lactation × time effects: P interaction < 0.0001) (Figure 2).

After weaning, gestation and lactation diets had persistent effects and resulted in lower weight gains with HP feeding in early life compared with NP feeding (gestation × time effect, P-interaction < 0.0001; lactation × time effect, P-interaction < 0.0001) (Figure 2). Weight gain was also reduced in the HPw group compared with the NPw group (postweaning effect, P < 0.0001; postweaning × day effect, P-interaction < 0.0001). Maternal diet during gestation showed an interaction with postweaning diet. The reduced body weight gain after the HP diet during weaning was more pronounced in the HPgest group than in the NPgest group (gestation × postweaning effect, P = 0.019; gestation × postweaning × time effect, P-interaction = 0.002) (Figure 2). There was no significant difference in birth weight between males and females, but at 1 wk of age, males showed significantly higher weight than did females (females, 14.2 ± 0.3 g; males, 15.1 ± 0.3 g; P < 0.05). The postweaning diet had a stronger effect on males than on females with respect to weight gain after weaning (gender × postweaning effect, P-interaction = 0.005; gender × postweaning × time effect, P-interaction < 0.0001) (Table 1). Pup total food intake after weaning was significantly lower in the HPgest group than in the NPgest group (gestation effect, P = 0.041). The effect of gestation diet on food intake was stronger in the HPlact group than in the NPlact group (gestation × lactation effect, P-interaction = 0.049). Pup food intake was also reduced by the postweaning HP diet compared with the NP postweaning diet (P < 0.0001, Table 2). Furthermore, the postweaning diet effect on food intake was stronger in males than in females (gender × postweaning effect, P-interaction = 0.002) (Table 1). Feed efficiency is the ratio between weight gain after weaning (gender × postweaning effect, P-interaction = 0.005, respectively), without a significant effect on HDL cholesterol proportion (Table 2). Males showed higher plasma TG concentrations and total cholesterol but a lower proportion of HDL cholesterol (P < 0.0001 for all) (Table 1) than did females. Hepatic TGs were higher in females than in males (gender effect, P < 0.0005) (Table 1).

In addition, carcass absolute weight was influenced by diet but strongly related to total body weight. The differences in weight of the carcass relative to body weight were physiologically unimportant (Supplemental Table 1).

**Body composition and hepatic and plasma lipids.** After 3 wk of the postweaning HP diet, pups in the HPw group had a lower total adiposity than did pups in the NPw group (postweaning effect on fat pad absolute and relative weights, P < 0.0001) (Table 2). The effect of the postweaning diet was amplified by the HP diet during lactation (lactation × postweaning effect, P-interaction = 0.04). In addition to the lower overall adiposity, the HPw group had significantly reduced the proportion of visceral/subcutaneous adipose tissue compared with the NPw group (P < 0.0001) (Table 2). This effect was enhanced in the HPgest group compared with the NPgest group (gestation × postweaning effect, P-interaction = 0.046). Although there was no significant difference in adiposity between males and females, the ratio of visceral to subcutaneous adipose tissue was higher in females (gender effect, P = 0.0003) (Table 1). The postweaning diet had a stronger effect on this ratio in males than in females (gender × postweaning effect, P-interaction < 0.0001).

At 6 wk of age and after a 3 wk postweaning HP diet period, hepatic storage of TGs and plasma TGs of food-deprived pups in the HPw group were lower than in the NPw group (P = 0.005 and P = 0.0002, respectively) (Table 2). Plasma total and HDL cholesterol measured higher in the HPw group than in the NPw group (P < 0.003 and P = 0.005, respectively), without a significant effect on HDL cholesterol proportion (Table 2). Males showed higher plasma TG concentrations and total cholesterol but a lower proportion of HDL cholesterol (P < 0.0001 for all) (Table 1) than did females. Hepatic TGs were higher in females than in males (gender effect, P < 0.0005) (Table 1).

**Plasma glucose, insulin, glucagon, leptin, and IGF-I.** Blood glucose concentrations of 6-wk-old food-deprived rats were affected by the diet of the pup and the mother. Pups in the HPgest group had increased blood glucose concentrations on PND 40 compared with pups in the NPgest group (P = 0.046)

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**TABLE 1** Phenotypic differences between male and female rat pups after weaning and interactions between gender and postweaning diet<sup>1</sup>

<table>
<thead>
<tr>
<th></th>
<th>Females (n = 64)</th>
<th>Males (n = 64)</th>
<th>Gender</th>
<th>Postweaning diet × gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight on PND 40, g</td>
<td>119 ± 2.13</td>
<td>139 ± 2.45</td>
<td>&lt;0.0001</td>
<td>0.009</td>
</tr>
<tr>
<td>Postweaning cumulative energy intake, kcal</td>
<td>671 ± 12.9</td>
<td>761 ± 16.6</td>
<td>&lt;0.0001</td>
<td>0.002</td>
</tr>
<tr>
<td>Feed efficiency, mg gain/g feed</td>
<td>398 ± 3.42</td>
<td>430 ± 4.90</td>
<td>&lt;0.0001</td>
<td>0.98</td>
</tr>
<tr>
<td>White adipose tissue, % total weight</td>
<td>6.41 ± 0.215</td>
<td>6.38 ± 0.202</td>
<td>0.45</td>
<td>0.54</td>
</tr>
<tr>
<td>Visceral/subcutaneous AT ratio, mg/g</td>
<td>691 ± 19.3</td>
<td>625 ± 13.5</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hepatic TGs, g/1000 g</td>
<td>16.9 ± 0.446</td>
<td>15.2 ± 0.373</td>
<td>0.0002</td>
<td>0.26</td>
</tr>
<tr>
<td>Plasma TGs, mg/dL</td>
<td>84.8 ± 4.76</td>
<td>119 ± 7.07</td>
<td>&lt;0.0001</td>
<td>0.80</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dL</td>
<td>41.5 ± 0.794</td>
<td>43.7 ± 0.823</td>
<td>0.03</td>
<td>0.10</td>
</tr>
<tr>
<td>Plasma HDL cholesterol, mg/dL</td>
<td>44.7 ± 0.634</td>
<td>42.4 ± 0.612</td>
<td>&lt;0.0001</td>
<td>0.32</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>86.8 ± 1.94</td>
<td>87.6 ± 2.34</td>
<td>0.37</td>
<td>0.033</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are means ± SEMs. Effects of gender and gender × postweaning were tested within mixed-model 1. The other studied variables not shown in this table are not significantly different between males and females. AT, adipose tissue; PND, postnatal day.
TABLE 2

<table>
<thead>
<tr>
<th>Measure</th>
<th>NPw</th>
<th>HPw</th>
<th>NPw</th>
<th>HPw</th>
<th>NPw</th>
<th>HPw</th>
<th>NPw</th>
<th>HPw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postweaning energy intake, kcal</td>
<td>5.8</td>
<td>6.0</td>
<td>5.9</td>
<td>6.1</td>
<td>5.8</td>
<td>6.0</td>
<td>5.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Red AT, % of total weight</td>
<td>52.1</td>
<td>51.2</td>
<td>51.7</td>
<td>51.4</td>
<td>52.1</td>
<td>51.2</td>
<td>51.7</td>
<td>51.4</td>
</tr>
<tr>
<td>White AT, % of total weight</td>
<td>5.9</td>
<td>6.1</td>
<td>5.9</td>
<td>6.1</td>
<td>5.9</td>
<td>6.1</td>
<td>5.9</td>
<td>6.1</td>
</tr>
<tr>
<td>Visceral subcutaneous AT, mg/d</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Hepatic TGs, g/1000 g</td>
<td>4.5</td>
<td>4.6</td>
<td>4.5</td>
<td>4.6</td>
<td>4.5</td>
<td>4.6</td>
<td>4.5</td>
<td>4.6</td>
</tr>
<tr>
<td>Plasma total cholesterol, mg/dl</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
<td>6.1</td>
</tr>
</tbody>
</table>

Values are means ± SEMs. n = 16. Effects of diets and interactions were tested within mixed-model 1. AT, adipose tissue; G, gestation diet; HP, high-protein diet; HPgest, pups born to mothers fed the high-protein diet; HPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation; NPw, pups fed the normal-protein (control) diet after weaning; PND, postnatal day; PW, postweaning diet.

**Discussion**

In this study, an HP diet was given to rat dams during gestation and/or lactation and to pups after weaning up to 6 wk of age. First, the results showed that pups in the HPgest and HPlact diet groups had higher glucose concentrations and lower insulin concentrations than those in the NPw group. Additionally, the HPgest diet group had higher plasma IGF-I concentrations than those in the NPw group, which was observed in the male pups but not in the female pups. Furthermore, the HPgest diet group had lower plasma IGF-I concentrations than those in the HPw group, which was observed in the male pups but not in the female pups. The results also showed that the HPw group had lower plasma IGF-I concentrations than those in the NPw group, which was observed in the male pups but not in the female pups. These findings suggest that the HP diet during gestation has a positive effect on the metabolic profile of the offspring, particularly in males. **Microbiota metabolites.** The weight of the total content of the cecum and colon was significantly influenced by the postweaning HP diet (cecum, 1.66 ± 0.09 g in pups in the HPw group compared with 1.21 ± 0.07 g in pups in the NPw group; colon, 0.88 ± 0.04 g in pups in the HPw group compared with 0.62 ± 0.05 g in pups in the NPw group; P < 0.0001). Males had greater intestinal content than females (cecum, 1.66 ± 0.09 g in males compared with 1.22 ± 0.07 g in females; colon, 0.88 ± 0.04 g in males compared with 0.61 ± 0.05 g in females; P = 0.0001). In light of these results, both concentration (in millimoles) and total quantity (in micromoles in the total cecal or colonic lumen) of metabolites were studied to take into account the difference in total contents. Overall, metabolite concentrations were unaffected by diet, except for the cecal valerate (postweaning effect, P = 0.005) and some BCFA concentrations that were increased in the HPw group compared with the NPw group (cecal and colonic isobutyrate, P < 0.0001 and P = 0.03, respectively; cecal isovalerate, P = 0.002). In contrast, total quantities of metabolites in both total cecal and colonic contents were all increased in pups in the HPw group compared with pups in the NPw group, irrespective of the mother’s diet, with the exception of colonic butyrate, which was not significantly affected by diet. Nevertheless, the lactation diet had an interesting effect with the postweaning diet for colonic propionate, butyrate, and valerate concentrations, lowering the effect of the postweaning diet (Supplemental Table 2). A gender effect was also observed, with an overall increase in metabolite total quantities in the male cecum and, specifically, acetate and valerate in the male colon compared with those of females (Supplemental Table 2).
groups had a lower body weight compared with the NPgest and NPlact groups. Second, the results also showed that pups in the HPgest group compared with the NPgest group had lower postweaning food intake and higher plasma glucose concentrations when food deprived, and had higher plasma insulin concentrations in the fed state when fed a postweaning NP diet. Third, irrespective of the diet given to the mothers, the results showed that pups in the HPw group compared with the NPw group had lower body weight, food intake, adiposity, plasma and liver TGs, and plasma IGF-I concentrations at 6 wk of age; had lower plasma blood glucose concentrations in the fed state but higher plasma blood glucose concentrations when food deprived; and had higher insulin and glucagon concentrations, as well as an increased total amount of gut bacterial metabolites.

According to the “early protein hypothesis,” HP intake in early life is associated with higher IGF-I plasma concentrations and faster growth in human infants (38). In contrast, with a rat model, our results showed that exposure to the HP diet in mothers during gestation, but also during lactation or after weaning, induced lower growth, food intake, plasma IGF-I, and adiposity in pups. Young rats appeared to be more sensitive to the satiating effects of the HP diet, because food intake was ~16% lower in rats in the HPw group than in those in the NPw group, and this difference remained significant during the 3 wk postweaning period (data not shown). This result differs from what can be found in adult rats that experience decreased food intake mainly during the first week after starting an HP diet (39–41). In another study that used young rats fed an HP diet, lower body adiposity was also observed, but no effect on either total weight or IGF-I concentrations were found (42). The lower hepatic TG content induced by the HP diet as observed in the present study also has been described in adult rats (43) and is likely related to reduced liver lipogenesis (44, 45). In contrast, higher plasma TG concentrations have been found in adult rats fed an HP diet (43), but no effects have been found on cholesterol, in contrast to the present study, in which total cholesterol was significantly increased. Interestingly, the HP diet differentially affected glucose metabolism in young rats compared with adult rats, and the results may suggest that the postweaning HP diet led to some early signs of insulin resistance.

**FIGURE 3** Blood glucose (A), insulin (B), glucagon (C), and HOMA-IR (D) on PND 40 of food-deprived rat pups fed the HP or NP diet after weaning, born to dams fed the HP or NP diet during gestation and/or lactation. Data are means ± SEMs, n = 16. Effects of diets were tested within mixed-model 1. *Effect of gestation diet, P < 0.05. HP, high-protein diet; HPgest, pups born to mothers fed the high-protein diet; HPlact, pups suckled by mothers fed the high-protein diet during lactation; HPw, pups fed the high-protein diet after weaning; NP, normal-protein (control) diet; NPgest, pups born to mothers fed the normal-protein (control) diet; NPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation; NPw, pups fed the normal-protein (control) diet after weaning; PND, postnatal day.

**FIGURE 4** Plasma glucose (A) and insulin (B) on PND 42 of fed rat pups that received the HP or NP diet after weaning, born to dams fed the HP or NP diet during gestation and/or lactation. Data are means ± SEMs, n = 16. Effects of diets were tested within mixed-model 1 and comparison between groups were corrected by Tukey post hoc test. HP, high-protein diet; HPgest, pups born to mothers fed the high-protein diet; HPlact, pups suckled by mothers fed the high-protein diet during lactation; HPw, pups fed the high-protein diet after weaning; NP, normal-protein (control) diet; NPgest, pups born to mothers fed the normal-protein (control) diet; NPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation; NPw, pups fed the normal-protein (control) diet after weaning; PND, postnatal day.
After a night of food deprivation, pups in the HPw group showed higher blood glucose after food deprivation, insulin, and glucagon concentrations and, consequently, the HOMA-IR index was increased in the rats in the HPw group compared with those in the NPw group, as has been reported previously (46). The elevated glucose concentration after food deprivation was previously hypothesized to result from enhanced gluconeogenesis (46) although the glucagon-to-insulin ratio did not differ significantly between groups. On the contrary, in the fed state, pups in the HPw group had lower plasma glucose concentrations than did pups in the NPw group, but this was likely explained by the lower carbohydrate content of that diet. An HP diet is known to promote insulin secretion (47), but there was no effect in the fed state from the postweaning diet on insulin concentrations than did pups in the NPw group, as has been reported previously (41). The transition toward a more putrefactive profile of the microbiota may contribute to the overall picture of phenotype changes, with relevant links having been shown recently with host metabolism (54), and even with host behavior (55). The relation between the HP diet and the microbiota putrefactive profile of the microbiota may contribute to the overall picture of phenotype changes, with relevant links having been shown recently with host metabolism (54), and even with host behavior (55).

### TABLE 3

| Table 3 | Plasma leptin and IGF-I on PND 40 of food-deprived rat pups fed the HP or NP diet after weaning, born to dams fed the HP or NP diet during gestation and/or lactation, and ratios relative to adiposity and weight gain, respectively

<table>
<thead>
<tr>
<th></th>
<th>NPgest</th>
<th>HPlact</th>
<th>NPgest</th>
<th>HPlact</th>
<th>NPgest</th>
<th>HPlact</th>
<th>Gestation</th>
<th>Lactation</th>
<th>Postweaning</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, pg/mL</td>
<td>61.9±11.9</td>
<td>72.8±17.4</td>
<td>42.5±15.7</td>
<td>25.0±7.71</td>
<td>81.9±15.8</td>
<td>61.7±15.2</td>
<td>63.7±15.0</td>
<td>21.4±9.84</td>
<td>0.086</td>
<td>0.022</td>
</tr>
<tr>
<td>Leptin/body fat, (pg/mL)/% of total weight</td>
<td>807±128</td>
<td>1170±274</td>
<td>470±148</td>
<td>407±127</td>
<td>972±211</td>
<td>921±294</td>
<td>1010±227</td>
<td>407±259</td>
<td>0.69</td>
<td>0.05</td>
</tr>
<tr>
<td>IGF-I, pg/mL</td>
<td>785±43.9</td>
<td>638±39.9</td>
<td>723±53.4</td>
<td>650±52.6</td>
<td>630±22.6</td>
<td>592±21.1</td>
<td>691±46.1</td>
<td>599±36.2</td>
<td>0.22</td>
<td>0.90</td>
</tr>
<tr>
<td>IGF-I/weight gain on PND 38, (pg/mL)/g</td>
<td>8.91±0.584</td>
<td>8.23±0.475</td>
<td>8.40±0.586</td>
<td>8.08±0.567</td>
<td>7.59±0.339</td>
<td>7.85±0.289</td>
<td>8.49±0.612</td>
<td>8.96±0.747</td>
<td>0.61</td>
<td>0.87</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs; n = 16. Effects of diets and interactions were tested within mixed-model 1. HP, high-protein diet; HPgest, pups born to mothers fed the high-protein diet; HPlact, pups fed the high-protein diet during lactation; HPw, pups fed the high-protein diet after weaning; IGF-I, insulin-like growth factor I; NP, normal-protein (control) diet; NPgest, pups born to mothers fed the normal-protein (control) diet; NPlact, pups suckled by mothers fed the normal-protein (control) diet during lactation; NPlact, pups fed the normal-protein (control) diet after weaning; PND, postnatal day.
signature and the rat phenotype needs to be further explored. Higher metabolite quantities and concentrations were found in overweight compared with lean subjects (56), whereas in the present study, the HP diet increased metabolite quantities but maintained most measured metabolite concentrations in the intestinal lumen and decreased adiposity. Future studies that further examine the long-term effects of early HP exposure or other dietary challenges after weaning on pup metabolism and microbiota will be key to help elucidate the impact of early-life diet on adult health.

This study describes a rat model of perinatal exposure to an HP diet during periods of gestation and lactation and after weaning up to 6 wk of age. The effects of the diets were sequentially characterized during the 3 periods. This study aims to represent reference data to test other experimental conditions. First, the model showed that an HP diet decreased pup weight regardless of the moment, and that the longer the period under an HP diet, the greater the effect. The postweaning HP diet also decreased food intake, adiposity, and fed blood glucose and IGF-I concentrations. In contrast with previously published results from human studies, this study did not show that protein excess in early life increased weight or IGF-I concentrations. The low level of significance of some effects could be explained by the rather short-term follow-up of the pups. In contrast, the gestational HP diet had a specific effect on food intake reduction and also led to an alteration in glucose metabolism; this effect was enhanced by the postweaning NP diet. In addition, the model also showed differences between males and females as differences in body weight and food intake. HDL cholesterol plasma concentrations were increased in females compared with males, comparable to results previously described in humans (57). In contrast with humans (58), female pups had a higher ratio of visceral to subcutaneous adipose tissue. This gender effect was also observed for gut microbial metabolite contents that were higher in males. Furthermore, males and females were differently affected by diet. The effect of the diet after weaning was enhanced in males compared with females. The present results did not show a gender-specific effect from the early-life diet, indicating that the maternal diet has the same programming effect on phenotype of males and females, yet these results differ from the findings of previous studies (11, 33). One explanation could be that the pups were followed until only 6 wk of age, i.e., before full sexual maturation, which is known to be a critical step in revealing possible gender differences.

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References
