High-protein nutrition during pregnancy and lactation programs blood pressure, food efficiency, and body weight of the offspring in a sex-dependent manner

C. Thöne-Reineke,1,2,* P. Kalk,1,3,† M. Dorn,1,2,‡ S. Klaus,2 K. Simon,1 T. Pfab,1 M. Godes,3 P. Persson,3 T. Unger,1 and B. Hocher1

1Center for Cardiovascular Research, Department of Pharmacology and Toxicology, Campus Mitte, Charité—Universitätsmedizin Berlin; 2German Institute of Human Nutrition, Potsdam-Rehbrücke, Germany; 3Institute of Vegetative Physiology, Charité, Campus Mitte, Berlin; and 4Department of Internal Medicine IV, Nephrology, Charité, Campus Benjamin Franklin, Berlin

Submitted 21 December 2005; accepted in final form 12 April 2006

Thöne-Reineke, C., P. Kalk, M. Dorn, S. Klaus, K. Simon, T. Pfab, M. Godes, P. Persson, T. Unger, and B. Hocher. High-protein nutrition during pregnancy and lactation programs blood pressure, food efficiency and body weight of the offspring in a sex-dependent manner. Am J Physiol Regul Integr Comp Physiol 291: R1025–R1030, 2006.—Maternal low-protein diet during pregnancy is a risk factor for cardiovascular disease of the offspring in later life. The impact of high-protein diet during pregnancy on the cardiovascular phenotype of the offspring, however, is still unknown. We examined the influence of a high-protein diet during pregnancy and lactation on the renal, hemodynamic, and metabolic phenotype of the F1 generation. Female Wistar rats were either fed a normal protein diet (20% protein: NP) or an isocaloric high-protein diet (40% protein: HP) throughout pregnancy and lactation. At weaning, the offspring were fed with standard diet, and they were allocated according to sex and maternal diet to four groups: normal-protein male (NPM, n = 25), normal-protein female (NPF, n = 19), high-protein male (HPM, n = 24), high-protein female (HPF, n = 29). During the experiment (22 wk), the animals were characterized by repeated measurement of body weight, food intake, blood pressure, glucose tolerance, energy expenditure, and kidney function. At the end of the study period histomorphological analyses of the kidneys and weight measurement of reproductive fat pads were conducted. There were no differences in birth weight between the study groups. No influence of maternal diet on energy expenditure, glucose tolerance, and plasma lipid levels was detected. Blood pressure and glomerulosclerosis were elevated in male offspring only, whereas female offspring were characterized by an increased food efficiency, higher body weight, and increased fat pads. Our study demonstrates that a high-protein diet during pregnancy and lactation in rats programs blood pressure, food efficiency, and body weight of the offspring in a sex-dependent manner.

fetal programming; hypertension; thrifty phenotype hypothesis; maternal diet

DAVID BARKER AND COLLEAGUES (2) described a close relation between mortality due to ischemic heart disease and infant mortality. Thus they proposed that poor living conditions, such as poor nutrition in early life, increase susceptibility to cardiovascular disease in adulthood. Moreover, Hales and Barker coined the “thrifty phenotype hypothesis” (8), which suggested that poor nutritional conditions lead to a lasting adaptive response of the fetus to enhance postnatal survival. This adaptive response of the fetus promotes a heightened sensitivity to hypernutrition and subsequent metabolic and cardiovascular disease in later life.

This novel concept of a fetal “programming” (19) of the susceptibility to metabolic and cardiovascular disease in adulthood has sparked extensive research in recent years. It has been established by a broad range of epidemiological studies that low birth weight is associated with cardiovascular death in adulthood (23); similar associations with risk factors, such as hypertension (11) and noninsulin-dependent diabetes (9) have been demonstrated. This concept has been proven via animal studies revealing that maternal low-protein nutrition leads to low birth weight in the offspring followed by higher blood pressure and altered insulin sensitivity (16, 24).

However, in the contemporary Western world, the main health care issue is no longer hyponutrition, but hypernutrition. Therefore it is vital to address the question whether the corresponding phenomena of fetal programming can also occur in the offspring of mothers subjected to hypernutrition. The literature regarding this issue is limited (1), and especially, the effects of a maternal high-protein diet on the offspring phenotype remain poorly investigated. Human epidemiological data regarding this point are inconclusive for a number of reasons (22). But as we know that in the Western world, dietary protein intake exceeds the recommended levels among young people (25), it is crucial to investigate the effects of a high-protein diet on the phenotype of the offspring. Thus we designed this study to examine the impact of maternal isocaloric high-protein diet during pregnancy and lactation on metabolic and cardiovascular phenotype of the offspring in rats.

MATERIALS AND METHODS

Experimental design. The experiment was performed in accordance with the guidelines of the ethics committee of the Ministry of Agriculture, Nutrition and Forestry (State Brandenburg, Germany, Permission No. 32/48–3560/0/3). Rats were housed in a temperature-controlled room with 12:12-h light-dark cycle and free access to water and food. Food consumption and body weight were monitored twice a week.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Female and male adult rats (Wistar; Charles River, Hamburg, Germany) were switched from a commercial rat diet (Altromin GmbH 1321, Lage, Germany) to purified isocaloric diets containing either a high-protein (HP: 40% protein) or a normal protein concentration (NP: 20% protein) (Table 1). One male and one female rat were housed together for 3 days to mate. During the gestation and lactation periods, rats were housed individually. Only the original litter size of 8–12 pups/dam was considered. During the suckling period (21 days), the mothers continued to consume the diet they had been exposed to during pregnancy. From weaning on, the offspring were fed a commercial standard diet (Altromin) and water ad libitum. According to the maternal diet, the offspring were allocated to four groups: normal-protein male (NPm, n = 25), normal-protein female (NPf, n = 19), high-protein male (HPm, n = 24), high-protein female (HPf, n = 29).

The duration of the study was 22 wk, in which body weight of the pups was measured daily for the first 2 wk and afterward twice a week; food consumption was measured twice a week. During the study, blood pressure was measured by using a tail cuff device (Bouche, model FIBA/67-TARC) at week 4, 10, 16, and 22. Animals were placed in metabolic cages for a period of 16 h to obtain urine samples at week 8, 14, and 22, at the same time blood samples were taken from the retro-orbital vein plexus. Afterward, urine volumes were calculated for a 24-h period and glomerular filtration rate was derived by calculating endogenous creatinine clearance using standard formulae. At week 22, a glucose tolerance test was performed by injecting a 50% glucose solution at a dose of 2 g/kg of body weight into the peritoneal cavity. Once before injection and 15, 30, 60, and 120 min afterward, blood was obtained from the tail vein, and glucose concentration was measured. The food was removed from the cages on the day before measurement to ensure accurate fasting glucose values. At the end of the study, the animals were put under anesthesia and killed by decapitation; hearts, kidneys, and the epididymal/ovarian fat pads were weighed, and the kidneys were preserved for histological study.

### Measurement of metabolic parameters.

At week 12, energy expenditure of individual rats was measured using indirect calorimetry (12). Oxygen consumption and CO₂ production were determined every 6 min in an open respiratory system (O₂ and CO₂ analyzers: MagnoS 16 NS U14, Hartmann and Braun, Frankfurt/Main, Germany). The animals were housed in the system for a total period of 48 h subdivided in a 24-h adaptation period followed by 24-h period of data collection. Energy expenditure was calculated according to Weir (27). Total energy expenditure was calculated as a 24-h mean. Resting metabolic rate was calculated as a mean of the 20 lowest values during the measurement period according to a procedure previously described in mice (12).

### Histological evaluation.

For histological evaluation, kidneys were embedded in paraffin, cut in 3-μm sections, and submitted to hematoxylin and eosin, Sirius Red (SR), peroxide acid-Schiff (PAS), and Elastica-van Gieson staining. Glomerulosclerosis was defined by the presence of PAS-positive material within the glomeruli. To quantify the amount of glomerulosclerosis, a semiquantitative score was used, as recently described (10). Two investigators who were blinded to the groups to which the kidneys belonged judged the results.

The severity of renal interstitial fibrosis was evaluated after SR staining using computer-aided histomorphometry devices. In brief, at least 30 microscopic pictures per kidney section were transferred to a PowerMAC via Hitachi-CCD camera. After manually setting a threshold using a randomly chosen subset of the pictures, we measured the relationship of SR-stained area (connective tissue) to total area of the picture using the National Institutes of Health (NIH) Image program, version 1.61.

Accordingly, microscopic pictures of kidney sections after Elastica-van Gieson staining showing intrarenal arteries were generated. We measured the area contents of the media and the lumen of intrarenal arteries using the NIH Image program; afterwards media/lumen ratio was calculated. Renal perivascular fibrosis was judged after SR staining using a semiquantitative score by two independent investigators blinded to the groups to which the animals belonged.

### Statistical analysis.

All data were analyzed by using SPSS for Windows (SPSS, version 11.5, Chicago, IL). The data were expressed as means ± SE. ANOVA was used to screen for significant variances among the study groups; Student’s t-test was used to detect significant differences between two groups of interest. Statistical significance was assumed with a value of $P < 0.05$.

### RESULTS

#### Growth.

Birth weight was not different between diet groups; neither was body weight during the suckling period. After weaning, body weight gain was not different in male HP and NP rats. But the female HP group developed a slightly, but significantly, higher body weight at the beginning of puberty, persisting until the end of the experiment compared with the female NP group (Fig. 1).

#### Food consumption and food efficiency.

The development of food consumption was different in male and female HP groups vs. NP groups. After the suckling period, the female HPs started with a significantly higher food intake than the female NPs (Fig. 2).

This changed after puberty, and during the rest of the study period, female HP showed a persistently reduced food intake compared with female NP (Fig. 2). The cumulative food intake from weeks 5–20 was significantly lower in the female HPs vs. female NPs (Fig. 3). If this fact is viewed in the context of the slightly higher body weight in the female HPs vs. the female NPs (see above), this indicates significantly enhanced food efficiency in female HPs (Fig. 4).

The male HP group had a significantly increased food intake only in weeks 3, 4, 13, 15, and 16 compared with NPm (Fig. 2).

#### Laboratory results.

No significant differences between HP and NP study groups regarding blood electrolytes, Ca, phosphate, ALT, AST, albumin, creatinine, urea, triglycerides, or cholesterol was detected at weeks 8, 14, and 22. A glucose tolerance test performed at the end of the study period did not show any significant differences between NP and HP study groups (data not shown).

### Table 1. Composition of semisynthetic diets with NP or a HP protein concentration

<table>
<thead>
<tr>
<th>Components, g/kg diet</th>
<th>NP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat starch(^a)</td>
<td>630</td>
<td>430</td>
</tr>
<tr>
<td>Casein(^b)</td>
<td>197</td>
<td>393</td>
</tr>
<tr>
<td>L-Methionine(^c)</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Sunflower oil/lard(^d)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Cellulose(^e)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mixture(^f)</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamin mixture(^g)</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

NP, normal-protein diet; HP, high-protein diet; \(^a\)Kröner, Ibbenbüren/Westfalen, Germany; \(^b\)Dauermilchwerk Petling, Landshut, Germany (87.5% crude protein, 0.5% fat); \(^c\)Serva, Feinbiochemica, Germany; \(^d\)Europe, Hamm, Germany; J. Rettenmaler und Soehne, Rosenberg, Germany; and A'ltromin, Lage, Germany. Mineral and vitamin mixtures in mg/kg diet: minerals: 9500.8 Ca, 750.8 Mg, 7500.5 P, 2500.5 Na, 7042.7 K, 2810.6 S, 180.1 Fe, 100.8 Mn, 30.4 Zn, 12.2 Cu, 0.45 F, 4.19 F, 0.314 Se, 0.13 Co, 0.002 Ni; vitamins: 5.16 A, 0.013 cholecalciferol, 163.95 E, 10.0 K-3; 20.04 thiamine, 20.32 riboflavin, 15.3 B-6, 0.041 B-12, 50.0 niacin, 50.1 pantothenate, 10.01 folic acid, 0.20 biotin, 1011.5 choline chloride, 100.0 p-aminobenzoic acid, 111.0 inositol, 20.0 C.
Blood pressure and heart rate. Blood pressure monitored during the study period in the male offspring of rats fed NP or HP diets is illustrated in Fig. 5.

In the male study groups, the HP group developed a significantly higher blood pressure than the NP group at the age of 4 wk, and this effect persisted through the entire study period. In the female groups, no differences in blood pressure were detected.

Heart rate was assessed together with the blood pressure. We did not detect any difference between the study groups regarding the heart rate at any measurement; for example, heart rates at the end of the study were 399 ± 8.9 (NPf), 413 ± 8.6 (HPf), 400 ± 8.4 (NPm), and 416 ± 9.8 (HPm).

Metabolic parameters and organ weight. At the end of the study period, the weight of the ovarian fat pad was significantly increased in the female HP group compared with the NPf. The male groups showed no significant difference regarding the epididymal fat pad weight (Fig. 6).

The weight of heart or liver was not influenced by the diet, but in both sexes, the kidney weight of the HP group was slightly increased (data not shown).

Total energy expenditure (NPf 162.1 ± 4.8 kJ/days; HPf 172.0 ± 8.1 kJ/days; NPm 220.2 ± 3.5 kJ/days; HPm 218.6 ± 6.2 kJ/days) and resting metabolic rate/metabolic body mass (NPf 409.2 ± 21.0; HPf 398.4 ± 10.5; NPm 388.5 ± 11.8; HPm 385.6 ± 15.0) were similar in all study groups and were not influenced by high-protein diet during pregnancy or sex.

Kidney function and morphology. No significant differences regarding glomerular filtration rate, urine electrolyte and urinary protein excretion were detected during the study (Table 2).
Histological evaluation of the kidneys did not reveal any differences between the study groups regarding the following parameters: perivascular fibrosis, interstitial fibrosis, media-to-lumen ratio of intrarenal arteries, the total number and volume of glomerula (Table 2). However, there was a slight but significant increase of glomerulosclerosis in the male HP group vs. NPm.

DISCUSSION

Our animal study was designed to investigate the impact of a maternal high-protein diet on the renal, hemodynamic, and metabolic phenotype of the offspring, as hypernutrition, and its consequences are widespread phenomena and major health care issues throughout developed countries (14). Our study demonstrated that a high-protein diet during pregnancy and lactation in rats programs blood pressure, kidney morphology, food efficiency, and body weight in a sex-dependent manner. Male offspring were characterized by significantly elevated blood pressure and a slightly higher degree of glomerulosclerosis. Our data are in agreement with a human study showing that a maternal high-protein, low-carbohydrate diet is linked to elevated blood pressure in the offspring (26). However, Zimanyi et al. (30) did not find an influence of maternal high-protein diet on blood pressure, kidney weight, and kidney histology in male rats (30). To explain these conflicting results, one has to take into account that the difference in blood pressure that we observed was about 10 mmHg, and the study groups of Zimanyi et al. (30) were about half the size of our study groups, so their study was probably underpowered to detect the difference. In addition, there seemed to be technical problems, because baseline systolic blood pressure values in their healthy control group were very high (170 ± 14 mmHg; see Ref. 30). Furthermore, there were important differences in the study design used by Zimanyi et al (30). Their high-protein diet consisted of 54% protein, whereas in our study, the protein content was 40% and, unlike in our study, they did not continue the maternal high-protein diet during the lactation period. In addition, blood pressure values given in their publication were measured at week 30, whereas our last measurement was performed at week 22; therefore, we cannot exclude a possible convergence of the groups beyond that point.

Although literature regarding a high-protein setting is rare, similar sex-dependent effects on the offspring in the setting of a maternal low-protein diet are well known. Kwong et al. (15) described in male offspring of protein-restricted rats a signif-

![Fig. 4. Food efficiency expressed as body weight per food intake per week in female (A) and male (B) rats. All values are given as means ± SE. *P < 0.05; **P < 0.01.](image-url)

![Fig. 5. Systolic blood pressure in female (A) and male (B) rats. All values are given as means ± SE. *P < 0.05; **P < 0.01.](image-url)
Maternal high-protein diet programs offspring phenotype in rats

R1029

Significantly elevated blood pressure and an increased kidney weight, whereas in females, both effects were absent (15). Langley-Evans et al. (18) likewise described that protein restriction in early gestation increased blood pressure only in male descendants.

Hypertension in a maternal low-protein setting is known to be linked to impaired nephrogenesis with subsequent lower nephron number in the offspring (28). As those parameters are normal in our study (see Table 2), an impaired nephrogenesis is unlikely to account for the blood pressure elevation in a high-protein setting.

As the tail-cuff method is known to be stressful, and we neither performed blood pressure measurement by indwelling catheter nor assessed stress hormone levels, we cannot exclude that an altered stress response in the male HP group contributed to the elevated blood pressure. However, as heart rates did not differ between the groups and the blood pressure was lowest at the very first measurement in all groups, we consider a significant amount of stress to be unlikely in our setting.

Our study is the first to demonstrate that the female offspring phenotype of mothers subjected to high-protein diet during pregnancy and lactation is characterized by an increased food efficiency followed by slightly higher body weight and increased ovarian fat pad weight, which is a widely used marker for obesity (4, 6, 21). The only animal study, including female offspring phenotype parameters in the setting of maternal high-protein diet from Zhang et al. (29) reported no difference in body weight in adult female mice offspring; food efficiency or fat pads were not investigated in their study. The difference is most probably related to the fact that they used a high-protein, but also a high-unsaturated-fat diet. In addition, their high-protein diet consisted of only 28%, whereas in our study it was 40% protein.

Increased food efficiency can be explained by decreasing either energy expenditure or body core temperature or locomotor activity. As there were no differences in energy expenditure between our study groups we were able to exclude this as an explanation. As body core temperature and locomotor activity were not assessed in our study, further investigations are needed to elucidate this point.

In our study no differences in birth weight related to maternal diet were detected. This is in line with a recent meta-analysis of human studies addressing the issue of maternal protein supplementation and fetal outcome (13). The animal studies known to us using a maternal high-protein diet are not conclusive at this point: Daenzer et al. (5) found a lower birth weight, Zhang et al. (29) found a higher birth weight, and Zimanyi et al. (30) did not find a difference in offspring birth weight. This is most likely due to wide variations in diet composition and group sizes between the studies.

We did not detect an impact of maternal diet on blood lipids, which is in line with the findings by Zhang et al. (29); neither was there an impact on glucose tolerance.

The modulating effect of sex could best be demonstrated in the increased food efficiency in females: The decrease of food intake and the increase of body weight with subsequent enhanced food efficiency started directly after the sexual maturity (8–10th wk). Before that crucial point, there was no difference in weight, and the food intake was even inversed between the two female study groups (see Fig. 2).

A recent review regarding fetal programming in the setting of hyponutrition stated that there is an impact of sex in a range of animal species, but the physiological basis of this effect is not yet understood (20). One possible explanation is that mammalian male preimplantation embryos might exhibit a heightened sensitivity to maternal environment, as they develop faster than the female embryo (7).

We are well aware that the most important limitation in interpreting results dealing with fetal programming is the

Table 2. Synopsis of urine parameters and kidney histology at the end of the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPI</th>
<th>HPi</th>
<th>NPM</th>
<th>HPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR, ml/min</td>
<td>0.9±0.05</td>
<td>0.9±0.04</td>
<td>2.0±0.09</td>
<td>2.3±0.13</td>
</tr>
<tr>
<td>Proteinuria, mg/24 h</td>
<td>0.1±0.02</td>
<td>0.1±0.01</td>
<td>0.8±0.05</td>
<td>0.8±0.05</td>
</tr>
<tr>
<td>Albuminuria, mg/24 h</td>
<td>4.4±2.42</td>
<td>2.6±0.53</td>
<td>2.7±0.68</td>
<td>1.9±0.32</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>11.4±1.17</td>
<td>10.0±0.84</td>
<td>12.2±1.02</td>
<td>12.2±1.15</td>
</tr>
<tr>
<td>Sodium, mmol/l</td>
<td>53.0±6.73</td>
<td>52.6±4.24</td>
<td>60.4±6.33</td>
<td>63.8±6.35</td>
</tr>
<tr>
<td>Potassium, mmol/l</td>
<td>98.3±6.05</td>
<td>109.9±6.28</td>
<td>113.4±6.17</td>
<td>115.5±6.88</td>
</tr>
<tr>
<td>Chloride, mmol/l</td>
<td>48.1±7.39</td>
<td>45.7±4.55</td>
<td>47.0±6.15</td>
<td>48.3±4.36</td>
</tr>
<tr>
<td>Glomerulosclerosis</td>
<td>2.6±0.13</td>
<td>2.6±0.11</td>
<td>2.5±0.09</td>
<td>2.7±0.11*</td>
</tr>
<tr>
<td>Perivascular fibrosis</td>
<td>2.4±0.10</td>
<td>2.6±0.09</td>
<td>2.7±0.08</td>
<td>2.8±0.08</td>
</tr>
<tr>
<td>Number of glomeruli</td>
<td>364.4±7.22</td>
<td>377.7±9.30</td>
<td>360.7±9.18</td>
<td>369.5±7.73</td>
</tr>
<tr>
<td>Media to lumen ratio</td>
<td>2.0±0.09</td>
<td>1.9±0.10</td>
<td>1.9±0.10</td>
<td>1.8±0.12</td>
</tr>
<tr>
<td>Intersitial fibrosis</td>
<td>6.7±1.37</td>
<td>7.0±1.15</td>
<td>6.5±1.44</td>
<td>6.0±1.00</td>
</tr>
</tbody>
</table>

All values are given as means ± SE. NPM, normal-protein male offspring; NPI, normal-protein female offspring; HPi, high-protein male offspring; HPi, high-protein female offspring. *P < 0.05 vs. male NP.
maternal diet interdependency. In our case, the high-protein diet is at the expense of the carbohydrate content (see Table 1). It is well known that relationships between nutritional contents are complex, for example, a low-protein, high-starch diet produces hypertension in the offspring, while a low-protein, high-glucose diet does not (17). From human studies, we know that the impact on hypertension in the offspring is determined interdependently by protein and carbohydrate contents of maternal diet (3).

As a conclusion, our study demonstrates that a high-protein diet during pregnancy and lactation in a rat model programs blood pressure, food efficiency, and body weight in a sex-dependent way.

ACKNOWLEDGMENTS

The technical assistance of Norma Schulz, Elisabeth Meyer, Carola Plau, Elke Thom, Elvira Steinnmeyer, and Jeanette Krause is appreciated.

GRANTS

This study was supported by a grant from the Deutsche Forschungsgemeinschaft to Dr. B. Hocher (DFG Ho1665–5/2). The work of Dr. P. Kalk was supported by a grant from the Dr.-Werner-Jackstaedt-Stiftung.

REFERENCES