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Metabolic adaptations to early life protein restriction differ by offspring sex and post-weaning diet in the mouse

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Abstract *Background and aims:* Low birth weight affects 1 in every 7 babies born globally and can predict a lifetime of increased risk for adverse health outcomes, including cardiovascular disease, hypertension, obesity, diabetes, and metabolic syndrome. Maternal low protein diet during pregnancy and lactation is a well-characterized rat model for low birth weight and the subsequent increase in chronic disease risk. However, mice have been relatively understudied in this paradigm and represent a critical resource for investigating the underlying molecular mechanisms that link adverse early life experience and the development of chronic disease.

Methods and results: The present manuscript describes a mouse model of low birth weight (maternal consumption of low protein diet (8% protein) through pregnancy and lactation) and characterizes metabolic adaptations (food intake, locomotor activity, oxygen consumption, and glucose tolerance) in male and female offspring. At weaning, mice were maintained either on the control diet or a high fat diet. Notable sex differences were observed, with male mice from the low protein pregnancies showing increased food intake, hyperactivity and increased metabolic rate only when weaned to the high fat diet, while female mice consistently showed increased food intake and were hypometabolic, regardless of post-weaning diet.

Conclusion: These data identify offspring sex and post-weaning diet as critical variables in the metabolic adaptations to early life protein deficiency, and suggest that females may be more vulnerable to the adverse long-term health consequences of low birth weight

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Introduction

The "fetal origins" hypothesis developed by Barker and colleagues noted the relationship between inadequate maternal nutrition and the eventual development of metabolic disease (hypertension, insulin resistance, and dyslipidemia) in adulthood [1]. This relationship has been seen across diverse examples of human famine, including the Dutch Hunger Winter [2] and the 1950s Chinese Famine [3,4]. To varying degrees, this finding has been replicated in animal models, as aspects of the metabolic syndrome (hypertension, atherosclerosis, dyslipidemia, glucose/insulin imbalance, central adiposity) can be seen in the adult offspring born to dams that consume limited nutrition during pregnancy and/or lactation. Maternal protein restriction (typically restricted to half the normal protein levels) has been extensively studied as a valid rodent model for the development of low birth weight (growth-retarded) offspring [5–9]. The vast majority of the protein-restriction experiments have been done using rats, and relatively few studies to date [10–12] have examined the effects of maternal protein restriction in a mouse model. Further, significant sex differences have been noted in the offspring's response to prenatal undernutrition [13–17]. For example, male mice from undernourished dams were adversely affected with respect to weight gain, adiposity and glucose tolerance [13], effects which were all absent in females. From these and other studies, it is clear that species, sex and the timing of the restriction (pregnancy and/or lactation) exhibit critical interactions that impinge on the outcome of study. Given the power of mouse genetics, it is critically important to fully characterize the effect of maternal protein restriction in a mouse model; therefore, current experiments were designed to examine the effect of maternal protein restriction on metabolic endpoints in male and female mouse offspring.

Methods

Animals and experimental model

Eight to twelve weeks old C57BL/6J females (The Jackson Labs, Bar Harbor, ME) were mated with DBA/2J males (The Jackson Labs, Bar Harbor, ME) and fed either a control (18% protein) or isocaloric 8.5% low protein (LP) diet during breeding, pregnancy and lactation (diet details below, and see Supplemental Table). Breeding pairs were housed in standard mouse cages with the addition of nestlets and breeding houses; males were removed from the pair just prior to the birth of the pups. Litters with fewer than 6 or greater than 8 pups were excluded from analysis. At weaning, half the litters were fed the control diet, and half were fed a high fat diet (60% calories from fat). One animal per litter from 6 to 8 different dams/group was randomly chosen for use in individual experiments, to control for any litter effect. There was no difference in litter size between LP and control pregnancies. Body weights were recorded weekly, and male and female mice ($n = 4–6$/group) were used in all experiments. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Pennsylvania.

Diet composition

Total energy content of control diet (Test Diet 5755, Richmond, IN) was 4.09 kcal/g with 18% of total energy calories from protein, 22% from fat, and 60% from carbohydrate. The isocaloric, low protein diet (Test Diet 5769) was 8.5% protein purified diet with total energy content of 4.13 kcal/g with 8.5% of total energy calories from protein, 22% from fat, and 69.5% from carbohydrate. High fat diet (Test Diet 58G9) had a total energy content of 5.21 kcal/g with 18% of total energy calories from protein, 60% from fat, and 22% from carbohydrate.

Metabolic measurements

Animals were tested in the Comprehensive Lab Animal Monitoring System (CLAMS, Columbus Instruments, Columbus, OH), which monitors food and water intake, indirect calorimetry, and *x*-axis activity. Animals had unrestricted access to powdered chow through a feeder located in the middle of the cage floor, which was directly connected to a precision scale. At least one week prior to testing, animals were housed in the CLAMS overnight to acclimate to powdered food and a novel cage. While in the CLAMS cages, animals had *ad libitum* access to powdered diet and water. Food intake was normalized to body weight. Animals were placed in the cages 1 h prior to lights out and were removed 20 h later. Data from the 12 h dark period and the first 6 h of the light period were analyzed and reported. Data in Fig. 2D were previously published in [18].

Glucose tolerance test

An intraperitoneal glucose tolerance (IPGTT) test was performed at 3 months (males) or 6 months (females) of age after an 18-h overnight fast ($n = 5$/group). A glucometer (Ascensia, Bayer) was used to determine blood glucose levels using blood from a tail nick. After determination of fasting blood glucose levels, each animal received a 2 g/kg intraperitoneal injection of 20% glucose. Blood glucose levels were detected after 15, 30, 60, and 120 min. Glucose tolerance was calculated by adding the areas under the glucose-time curve (AUC-GTT) for the intervals 0–15, 15–30, 30–60, and 60–120 min.

Statistical analyses

Data, presented as means ± S.E.M., were analyzed using Prism 4 (GraphPad) and Excel tools for statistical analysis. Student's *t*-test was used to analyze differences between groups in Fig. 1B–E. Two-way repeated measures ANOVA (maternal diet × time) was used to analyze all other data sets. A *p*-value of 0.05 or lower was considered significant.

Results

Weights

Dams were fed either a low protein (LP) diet or an isocaloric control diet through breeding, pregnancy and lactation.