Comparative Biochemistry and Physiology, Part A 182 (2015) 38–44

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Maternal dietary protein supplement confers long-term sex-specific beneficial consequences of obesity resistance and glucose tolerance to the offspring in Brandt's voles

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 1 December 2014
Accepted 1 December 2014
Available online 8 December 2014

Keywords:
Body fat
Glucose tolerance
Leptin
Maternal protein supplement
Offspring

ABSTRACT

Maternal under- or over-nutrition not only alters neonatal body mass but also increases the risk of metabolic disorders in adulthood. Little is known about how maternal dietary protein affects offspring fitness in wild rodents. The present study was conducted to test the hypothesis that maternal dietary protein supplement has a long-term beneficial effect on offspring fitness in Brandt's vole (*Lasiopodomys brandtii*), a herbivorous rodent model. The vole dams were fed either a control (18% protein) or high-protein (36% protein) diet throughout pregnancy and lactation. After weaning, all offspring received a control diet till 14 weeks old. Energetic parameters, serum leptin concentration and glucose tolerance were measured. The adult offspring were fed high-fat diet for 8 weeks, and body weight and food intake were measured. No difference was observed in litter size, litter mass or pup mass before weaning. Maternal protein supplement increased body mass and the mass of reproductive organ but decreased digestibility and fat deposition and alleviated HFD-induced obesity especially in the males. Glucose tolerance was elevated in the offspring from maternal protein supplement, especially in the females. The accelerated growth may be associated with high serum leptin concentration at weaning, a state of leptin resistance, and the low digestibility may predispose obesity resistance especially in male offspring from maternal high-protein diet. These data demonstrate that maternal protein supplement confers the long-term sex-specific beneficial consequences of accelerated growth and improved obesity resistance and glucose tolerance of their offspring.

1. Introduction

The hypothesis of fetal origins (also called 'fetal programming') proposes that the perinatal period is critical for mammals, when most life history characteristics are shaped (Barker, 1998). The natural (such as cold, under- or over-nutrition) and social (such as social isolation) stresses during perinatal period would confer the long-term effects on behaviors, physiology and psychology in adulthood, including energy metabolism (Almond et al., 2012; Metges, 2001; Rebel et al., 2006), blood pressure and renal function (Ritz et al., 2011), immune activity (Kankova et al., 2014; Triggs and Knell, 2012) and reproductive maturity (Milner et al., 2013).

Many studies have focused on the effects of maternal protein restriction or supplement on the offspring due to the critical role of protein for growth and development. The change in maternal dietary protein content may lead to variations in the yield and composition of milk, which affect the offspring growth (Derrickson and Lowas, 2007). Laboratory studies suggest that low maternal protein intake during gestation and/or lactation can result in offspring with lower birth mass, reduced growth rate and higher blood pressure in adulthood (Derrickson and Lowas, 2007; Passos et al., 2000; Zambrano et al., 2006). In contrast, maternal high-protein diet can induce increased growth and improve glucose homeostasis (Maurer and Reimer, 2011; Thone-Reineke et al., 2006). However, other studies found that maternal low-protein diet increased abdominal fat deposition in mice (Han et al., 2012). Therefore, the impacts of maternal dietary protein on offspring phenotypes are still controversial.

Wild or wild-derived rodents are good models to study the natural environmental factors on life-history characteristics since they exhibit adaptive strategies to seasonal environment in life (Li and Wang, 2005; Zhang and Wang, 2006). Brandt's voles (*Lasiopodomys brandtii*)

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http://dx.doi.org/10.1016/j.cbpa.2014.12.002
1095-6433/

are the dominant species in typical steppe in the Inner Mongolian of China, Mongolia and the region of Baikal in Russia. It is cold in winter, which lasts for 5 months. The voles are herbivores, and the food habit, especially the protein content, showed a higher level during breeding season in the wild (Wang et al., 1992). In the previous studies, we found that the lactating voles increased digestible energy intake at peak lactation in response to high-protein diet (Lou et al., 2013), indicating that the dams had high demands for improving the reproductive output. Whether the changes in maternal dietary protein have a long-term effect on offspring fitness is rarely addressed in wild rodents. Therefore, the aim of this study was to explore how maternal dietary protein complement would affect the reproductive output and offspring metabolic phenotypes in Brandt's voles. We predicted that maternal dietary protein complement would improve the reproductive output and offspring fitness.

2. Materials and methods

2.1. Animals and experimental design

Brandt's voles were the offspring of the laboratory colony trapped from Inner Mongolian grasslands in 1999. They were housed in groups with the same sex after weaning in plastic cages (30 × 15 × 20 cm) with sawdust as beddings, and maintained at 23 ± 1 °C and under a 16L:8D photoperiod (lights on at 04:00 h). The voles were fed *ad libitum* commercial rabbit pellets (Beijing HFK Bioscience Co.) and water. All experimental procedures were in accordance with the guidelines of the Animal Care and Use Committee of Institute of Zoology, the Chinese Academy of Sciences.

Experiment 1 was designed to detect the short- and long-term effects of maternal protein supplement on offspring fitness. Weight-matched virgin female voles were housed individually at least 2 weeks and then fed with either a control diet (Con, 17.5 kJ/g), which consists of 18% protein, 3% fat, 12% fiber and 47% carbohydrate, or a high-protein diet (HP, 18.9 kJ/g), which consists of 36% protein, 3% fat, 12% fiber and 29% carbohydrate. One female and one male were housed together for a week to mate. Only the original litter size of 5–10 was selected. During pregnancy and lactation, the mothers consumed their respective diet. The pups were weaned at day 18 and kept together with their littermates. After 4 weeks of age, one male and one female from each litter were randomly selected and housed individually until 14 weeks of age. The offspring were fed control diet after weaning. According to maternal diets, the offspring were divided into four groups: male offspring from maternal control diet (ConM, $n = 12$) or high-protein diet (HPM, $n = 9$), and female offspring from maternal control diet (ConF, $n = 10$) or high-protein diet (HPF, $n = 10$). The pup mass was measured once every 3 days during lactation (18 days) and then once every 2 weeks. Food consumption was measured once every 2 weeks from 4 to 12 weeks of age.

Experiment 2 was designed to test whether the adult offspring from control and HP diets exhibited different responses to high-fat diet (HFD). Adult male and female offspring (4–5 months of age) from maternal control and HP diet were fed HFD (26% fat) for 8 weeks. They were allocated to four groups (ConM, $n = 10$; HPM, $n = 6$; ConF, $n = 10$; HPF, $n = 10$). Body mass and food intake were measured once a week.

2.1.1. Energy intake

Food intake was measured in metabolic cages as previously described (Liu et al., 2003). Food residues and feces were collected, oven-dried at 60 °C to constant mass and separated manually. Dry food intake was calculated from the difference between the food given and food residue. Energy contents of the food and feces were determined by a Parr 1281 oxygen bomb calorimeter (Parr Instrument, USA). Digestible energy intake (DEI) and apparent digestibility of energy (hereafter referred to as digestibility) were calculated as follows:

$$\begin{aligned}\text{DEI (kJ/d)} = {} & \text{dry food intake (g/d)} \\ & \times \text{energy content of food (kJ/g)} - \text{dry mass of feces (g/d)} \\ & \times \text{energy content of feces (kJ/g)}\end{aligned}$$

$$\begin{aligned}\text{Digestibility (\%)} = {} & \text{DEI (kJ/d)} \\ & \times 100\% / [\text{dry food intake (g/d)} \\ & \times \text{energy content of food (kJ/g)}].\end{aligned}$$

2.2. Body composition

All subjects were weighed and sacrificed by CO_2 asphyxiation between 09:00 and 11:00 h at the end of experiment 1. Blood samples were collected, and the interscapular brown adipose tissue (BAT) was carefully dissected, weighed, frozen in liquid nitrogen and stored at −80 °C. The visceral organs, including heart, lung, liver, kidneys, spleen, male testes, epididymis, seminal vesicle or female uterus and ovary, were dissected and weighed. The digestive tract (including stomach, small intestine, caecum and colon) was removed and weighed (±1 mg). The stomach and intestines were rinsed with saline to eliminate the contents before being dried and weighed. Mesenteric fat, epigonadal fat, retroperitoneal fat and inguinal fat were also dissected carefully and weighed (±1 mg). The remaining carcass and all the organs were dried in an oven at 60 °C to constant mass and then weighed again to obtain the dry mass. The difference between the wet and dry carcass mass was the water mass of carcass (body water).

2.3. Serum leptin assay

As described previously, serum leptin concentration was determined by radioimmunoassay with the ^{125}I multi-species Kit (Cat. No. XL-85 K, Linco Research Inc., St. Charles, USA) (Li and Wang, 2005; Zhang and Wang, 2006). The lower and upper limits of the assay were 1 and 50 ng/ml when using a 100 μl sample. The intra- and inter-assay variations were < 3.6 and 8.7%, respectively.

2.4. Uncoupling protein 1 (UCP1) measurement

The total protein concentration of BAT was determined by the Folin phenol method using bovine serum albumin (500 μg/ml) as the standard. UCP1 content was measured by western blotting (Li and Wang, 2005; Zhang and Wang, 2006). Total BAT protein (80 μg per lane) was separated in a discontinuous sodium dodecyl sulfate–polyacrylamide gel (10% running gel and 4% stacking gel) and transferred onto PVDF membrane (Millipore, USA, IPVH00010) (200 mA, 2 h), and the membrane was blocked in 5% nonfat dry milk in Tris-buffered saline–Tween for 1 h at room temperature. The membrane was detected using a polyclonal rabbit anti-hamster UCP1 (1:5000) as the primary antibody overnight at 4 °C and with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:5000) as the secondary antibody. Protein bands were detected with enhanced chemiluminescence (Amersham), and quantified using Quantity One Software (BioRad, USA, Ver.4.4.0). UCP1 content was expressed as relative units (RU).

2.5. Intraperitoneal glucose tolerance test

Intraperitoneal glucose tolerance test was conducted between 08:00 h and 11:00 h after the voles were fasted overnight at 11 weeks of age. Blood samples were taken by tail veni-puncture, and blood glucose was measured immediately before, 15, 30, 60 and 120 min after intraperitoneal glucose injection (glucose dose, 2 g/kg body mass) by FreeStyle Blood