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Prenatal undernutrition attenuates fasting-induced reproductive dysfunction in pre-pubertal male rats

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ABSTRACT

Prenatal undernutrition affects various physiological functions, such as metabolic and reproductive functions, after birth, and such changes are associated with the pathogeneses of certain diseases. It has been hypothesized that these changes are predictive adaptive responses that help individuals to endure similar conditions in the postnatal period. Thus, we evaluated the effects of prenatal undernutrition on the responses of the body weight (BW) regulation system and reproductive functions to fasting in the pre-pubertal period in male rats. Prenatally normally nourished and undernourished rats exhibited similar reductions in BW and visceral fat after 48 h fasting in the pre-pubertal period. Furthermore, these two groups displayed similar fasting-induced patterns of change in their hypothalamic levels of appetite regulatory factors; i.e., neuropeptide Y and pro-opiomelanocortin. These results indicate that prenatal undernutrition had no marked effects on BW regulation in male rats. On the other hand, serum luteinizing hormone and testosterone levels were decreased by 48 h fasting in the prenatally normally nourished rats, whereas the levels of these hormones did not change in the prenatally undernourished rats. However, the hypothalamic mRNA level of kisspeptin 1 (Kiss1), which is a positive regulator of gonadotropin-releasing hormone/gonadotropins, was reduced by fasting in both groups. These results indicate that prenatal undernutrition might attenuate fasting-induced reproductive dysfunction in the postnatal period; however, these changes might not be induced by alterations in the hypothalamic Kiss1 system. Further studies are needed to clarify the mechanisms involved in these changes in reproductive function.

1. Introduction

Epidemiological and experimental evidence has shown that prenatal undernutrition affects various physiological functions after birth and that such changes are associated with the pathogeneses of certain diseases, such as diabetes, in adulthood (Godfrey and Barker, 2000; Breier et al., 2001; Gluckman and Hanson, 2004). These phenomena can be reproduced in animal models, and it has been established that changes in hypothalamic functions play pivotal roles in such prenatal undernutrition-induced physiological changes (Yura et al., 2005; Breton et al., 2008; Delahaye et al., 2008). Although most previous studies into this topic focused on the effects of prenatal undernutrition on metabolic functions, some studies, including ours, have evaluated the effects of prenatal undernutrition on reproductive functions. Prenatal and/or perinatal undernutrition attenuates the hypothalamic mRNA expression of kisspeptin (Kiss1), which is a potent positive regulator of gonadotropin-releasing hormone (GnRH), and the serum gonadotropin level, which consequently retards the onset of puberty in female rats (Iwasa et al., 2010a; Castellano et al., 2011). It has been hypothesized that these changes are predictive adaptive responses that help individuals to endure similar environments during the postnatal period and that certain disorders might be induced if prenatally undernourished (UN) individuals unexpectedly encounter abundant nutritional conditions (Hayward et al., 2013). However, as far as we know only a few studies have evaluated this hypothesis; i.e., that predictive adaptive responses have beneficial effects under low-energy conditions in later life. In this study, we evaluated the effects of prenatal undernutrition on the responses of the body weight (BW) regulation system and reproductive functions to fasting in the pre-pubertal period. As noted above, changes in hypothalamic functions might play important roles in predictive adaptive responses (Yura et al., 2005; Breton et al., 2008; Delahaye et al., 2008); therefore, the effects of prenatal undernutrition on the hypothalamic expression of the factors that regulate appetite and reproduction were also examined. In this study, the responses to fasting during the pre-pubertal period were evaluated because predictive adaptive responses, if they exist, might confer great advantages that help individuals to endure poor nutritional conditions in this period.

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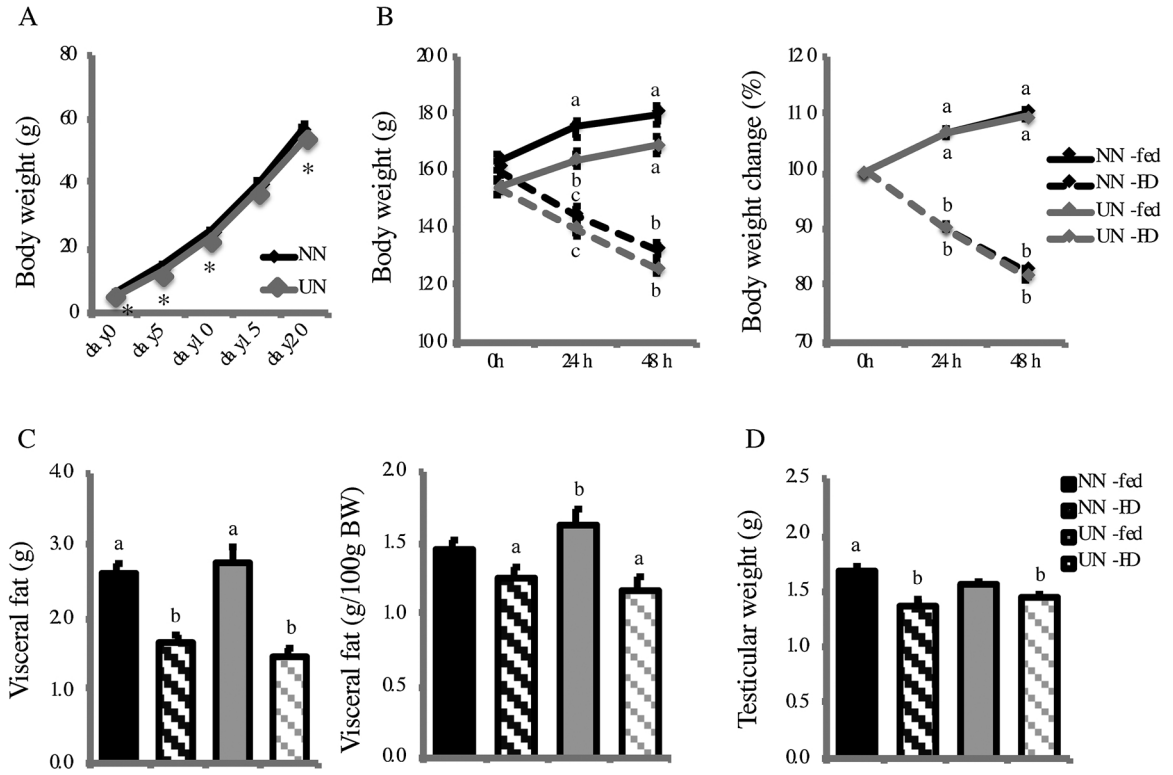

Fig. 1. Body weight (BW) during the postnatal period (A) and BW, BW changes (B), visceral fat weight (C), and testicular weight (D) under fed and food-deprived (FD) conditions in prenatally normally nourished (NN) and undernourished (UN) rats. n = 8 per each group. BW changes are expressed as percentages of the initial BW. Data are expressed as mean ± SE values. * $P < 0.05$ vs. each other (Student's unpaired t-test, or the Mann-Whitney U test); a, b: groups with different letters are significantly different (Tukey-Kramer test).

2. Materials and methods

2.1. Animals

Pregnant Wistar rats were purchased (Charles River Japan, Inc.; Kanagawa, Japan) and housed individually under controlled lighting (12 h light, 12 h darkness; lights turned on at 0800 and turned off at 2000) and temperature (24 °C) conditions with free access to water. All animal experiments were conducted in accordance with the ethical standards of the University of Tokushima. The pregnant rats were divided into prenatally normally nourished (NN, n = 4) and UN (n = 4) groups. The UN dam received about 50% of the daily food intake of the NN dam from days 14 to 21 of pregnancy and then was allowed to feed ad libitum during the lactation period. The day when the pups were delivered was defined as postnatal day 0. On postnatal day 1, male pups were randomly assigned to NN dams (11-12pups per each dam), and then fostered until weaning. The pups were weaned at postnatal day 21 and housed at 3–4 animals per cage. Only male rats were used in this study.

2.2. Effects of food deprivation in the pre-pubertal period on body weight, visceral fat weight, testicular weight, serum hormone levels, and the hypothalamic levels of various factors

At 34 days of age, the offspring of the NN dam (the NN group) and UN dam (the UN group) were divided into two groups each; i.e., into fed and food-deprived (FD) groups (n = 8 per each group). In the fed groups, the rats were given ad libitum access to food and water. In the FD groups, the rats were deprived of food for 48 h (starting between 0900–1000 h), but were allowed free access to water. These food-deprivation conditions were chosen because previous studies indicated that they were sufficient to downregulate reproductive functions in pre-pubertal rats (Iwasa et al., 2010b). BW was measured at 24 h and 48 h after the initiation of the food-control measures, and samples (brain and blood) were harvested between 0900–1000 h. Visceral fat weight and testicular weight were also measured during the sampling procedures.

2.3. Hormone assays

Serum leptin levels were measured using radioimmunoassay kits (multi-species leptin RIA kit; Linco Research Inc., MO, USA). The sensitivity of the assay was 1.0 ng/mL, and its inter- and intra-assay coefficients of variation (CV) were 3.2% and 7.8%, respectively. The serum luteinizing hormone (LH) level was measured using a radioimmunoassay (rLH [I-125] RIA kit; Institute of Isotopes Co., Ltd., Tokyo, Japan). The sensitivity of the assay was 0.8 ng/ml, and its inter- and intra-assay CV were 7.7% and 6.5%, respectively. Serum testosterone levels were measured by a commercial laboratory (SRL, Tokyo, Japan) using an electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH, Mannheim, Germany).

2.4. Quantitative real-time polymerase chain reaction

Whole hypothalamic explants were dissected from the frozen brains, as described previously (Iwasa et al., 2016). Total RNA was isolated from the hypothalamic explants and visceral fat using the TRIzol® reagent kit (Invitrogen Co., Carlsbad, CA, USA) and the RNeasy® mini kit (Qiagen GmbH, Hilden, Germany). Then, cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript III first-strand synthesis system for the real-time polymerase chain reaction (PCR; Invitrogen Co.). The PCR analysis was performed using the StepOnePlus™ real-time PCR system (PE Applied Biosystems, Foster City, CA, USA) and FAST SYBR® green. The mRNA levels of appetite regulatory factors; i.e., the leptin receptor (OBRb), neuropeptide Y (NPY), and pro-opiomelanocortin (POMC), and reproductive factors; i.e., Kiss1 and its receptor (Kiss1r), were measured. The mRNA expression level of