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# Prenatal undernutrition increases the febrile response to lipopolysaccharides in adulthood in male rats

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ABSTRACT

It has been reported that prenatal undernutrition affects the development of the peripheral immune system. In this study, the effects of prenatal undernutrition on the febrile response and hypothalamic innate immune system were evaluated in male rats. Pregnant rats were divided into normally nourished (NN) and undernourished groups (UN). The febrile and anorectic responses to lipopolysaccharides (LPS) were evaluated in the offspring of NN and UN dams. The hypothalamic expression levels of pro-inflammatory cytokines, toll-like receptor 4 (TLR4), and neuropeptide Y (NPY) were also evaluated. The UN rats exhibited significantly lighter body weights than the NN rats at birth; however, their mean body weight was the same as that of the NN rats by postnatal day 10. In adulthood, the UN rats exhibited significantly stronger febrile responses than the NN rats, and the anorectic responses of the UN rats also tended to be stronger than those of the NN rats. On the other hand, no differences in hypothalamic interleukin (IL)-1β, IL-6, tumor necrosis factor-α, TLR4, or NPY mRNA expression were detected between the NN and UN rats. These results suggest that prenatal undernutrition has long-lasting effects on the febrile response to LPS. However, the precise mechanism underlying these effects and their pathophysiological significance remain unclear.



## 1. Introduction

Epidemiological and experimental evidence suggests that prenatal undernutrition affects the development of metabolic and physiological functions after birth and that such changes are associated with the pathogenesis of conditions such as type 2 diabetes, hypertension, and ischemic heart disease in adulthood (Godfrey and Barker, 2000; Breier et al., 2001; Gluckman and Hanson, 2004). These changes have also been reproduced in animal models, e.g., in mice the offspring of undernourished mothers tend to exhibit obesity and insulin resistance in adulthood (Yura et al., 2005). It has been established that changes in hypothalamic function play pivotal roles in the metabolic and physiological alterations induced by prenatal undernutrition (Yura et al., 2005; Breton et al., 2008; Delahaye et al., 2008).

It has also been reported that prenatal undernutrition affects the development of the immune system (Chandra, 2002). For example, humans with intrauterine growth retardation (IUGR) and animals that experience prenatal undernutrition exhibit persistently impaired immunological function (Ferguson, 1978; Chandra, 1981). In addition, small-for-gestational age (SGA) infants are at increased risk of infection-induced sepsis during the neonatal period and childhood (Smichen et al., 2000). Most of the abovementioned studies focused on the effects of IUGR or SGA on adaptive immunity, and little data is available about the effects of such conditions on the innate immune system. In 2005, Equils et al. suggested that IUGR rats exhibit increased innate immune system activity (Equils et al., 2005). They revealed that hepatic inflammatory cytokine expression was increased under basal and lipopolysaccharide (LPS)-injected conditions in IUGR rats and that dysregulation of the LPS receptor, toll-like receptor (TLR) 4, might be involved in these changes. On the other hand, TLR4 gene expression was not altered in other peripheral tissues; i.e., the spleen and intestine, in these rats. These findings indicate that the alterations in the innate immune system observed in prenatally undernourished IUGR rats are site-specific.

As noted above, the hypothalamus plays an essential role in the pathophysiological changes induced by prenatal

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undernutrition (Yura et al., 2005; Breton et al., 2008; Delahaye et al., 2008). Therefore, we evaluated the effects of prenatal undernutrition on the hypothalamic innate immune system in male rats. In addition, we assessed the febrile and anorectic responses induced under inflammatory conditions because the hypothalamic immune system plays a pivotal role in these inflammatory responses (Plata-Salaman, 2001; Mouihate and Pittman, 2003; Kim et al., 2007; Iwasa et al., 2014a,b). The hypothalamic expression of neuropeptide Y (NPY) was also assessed because NPY is a representative appetite-regulating factor.

## 2. Materials and methods

### 2.1. Animals

Sixteen pregnant Sprague–Dawley rats were purchased (Charles River Japan, Inc., Tokyo, Japan) and housed individually under controlled lighting (14 h light, 10 h darkness) and temperature (24 °C) conditions. All animal experiments were conducted in accordance with the ethical standards of the University of Tokushima. The pregnant rats were divided into the normally nourished (NN dams) ($n = 8$) and undernourished (UN dams) ($n = 8$) groups. The UN dams received about 50% (11 g/day) of the daily food intake of the NN dams from days 15 to 21 of pregnancy and were then allowed to feed ad libitum during the lactation period. The body weights of the dams were measured every other day during pregnancy. The day when the pups were delivered was defined as day 0. To control litter size to 10–12 per dam, pups were culled or moved to other dams and were fostered until weaning. The pups were weighed at various postnatal ages and weaned at postnatal day 21. Both male and female body weight data were collected at day 0 and 10 because it was slightly hard to distinguish male from female in this time periods. After being weaned, the pups were separated by sex and housed 3–4 animals per cage. Only male rats were used in the examinations in adulthood.

### 2.2. Effect of LPS injection on core body temperature

At 10 weeks of age, the offspring of the NN dams (NN) ($n = 8$) and UN dams (UN) ($n = 8$) had pre-calibrated temperature-sensitive radio transmitters (TA11TA-F10; Data Sciences International, New Brighton, MN, USA) implanted into their peritoneal cavities after the induction of anesthesia with sevoflurane. After 7 days' recovery, the rats were injected with LPS (500 μg/kg, intraperitoneal (i.p.)), and the resultant change in their core body temperature was measured. Frequency data (Hz) from each transmitter were recorded every 15 min by a receiver board antenna placed underneath each rat's cage and logged by a peripheral processor. The frequency data were converted to °C by the software DATAQUEST (Data Sciences). Body temperature was measured between 0900 on the day of injection and 2100 on the following day.

### 2.3. Effects of LPS injection on body weight and food intake

At 10 weeks of age, the NN and UN rats ($n = 6$ per each group) were injected with LPS (500 μg/kg, i.p.), and the changes in their body weight (% of initial body weight) and food intake (g/100 g body weight) were measured every 6 h until 36 h after the injection.

### 2.4. Effects of LPS injection on the mRNA expression of hypothalamic factors

At 10 weeks of age, the NN and UN rats were sub-divided into saline-injected and LPS-injected groups. The rats were injected with saline or LPS (500 μg/kg, i.p.), and their brains were collected 6 h later ($n = 6$–8 per each group). This time point was chosen based on our findings regarding the changes in the rats' body temperature after the injection of LPS. The brain sections were dissected via an anterior cut at 2 mm anterior from the optic chiasm and a posterior coronal cut at the posterior border of the mammillary bodies. Subsequently, these tissue blocks were subjected to two parasagittal cuts along the hypothalamic fissures and a dorsal cut at 2.5 mm from the ventral surface. Total RNA was isolated from the hypothalamus using a TRIzol reagent kit (Invitrogen, Carlsbad, CA, USA) and an RNeasy Mini kit (Qiagen, Hilden, Germany). cDNA was synthesized with oligo (deoxythymidine) primers at 50 °C using the SuperScript® III First-Strand Synthesis System (Invitrogen™) for the real-time polymerase chain reaction (RT-PCR). Real-time PCR analysis was performed using the StepOnePlus™ Real-time PCR System (PE Applied Biosystems, Foster City, CA, USA) and SYBR® green. Standard curves, which were generated from a 4 dilution series of an abundant sample, were used for the relative quantification of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-6, TLR4, and NPY. The mRNA expression levels of these molecules were normalized to the mRNA expression level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Dissociation curve analysis of each gene was also performed at the end of the PCR. Each amplicon generated a single peak. The forward and reverse primers used were as follows: IL-1β: F: 5′-GCT GTG GCA GCT ACC TAT GTC TTG-3′, R: 5′-AGG TCG TCA TCA TCC CAC GAG-3′; TNF-α: F: 5′-AGC CCT GGT ATG AGC CCA TGT A-3′, R: 5′-CCG GAC TCC GTG ATG TCT AAG T-3′; IL-6: F: 5′-TCC TAC CCC AAC TTC CAA TGC TC-3′, R: 5′-TTG GAT GGT CTT GGT CCT TAG CC-3′; TLR4: F: 5′-CAT GAA GGC CTC CCT GGT GTT-3′, R: 5′-TGC CAG AGC GGC TAC TCA GAA-3′; NPY: F: 5′-GGG GCT GTG TGG ACT GAC CCT-3′: R: 5′-GAT GTA GTG TCG CAG AGC GGA G-3′; GAPDH: F: 5′-ATG GCA CAG TCA AGG CTG AGA-3′, R: 5′-CGC TCC TGG AAG ATG GTG AT-3′. The PCR conditions were as follows: initial denaturation and enzyme activation at 95 °C for 20 s; followed by 45 cycles of denaturation at 95 °C for 3 s and annealing at 61 °C for 30 s (IL-1β), 64 °C for 30 s (GAPDH), 65.5 °C for 30 s (TNF-α), 67 °C for 30 s (IL-6), 66 °C for 30 s (NPY), or 68 °C for 30 s (TLR4); and a final extension step of 72 °C for 1 min.

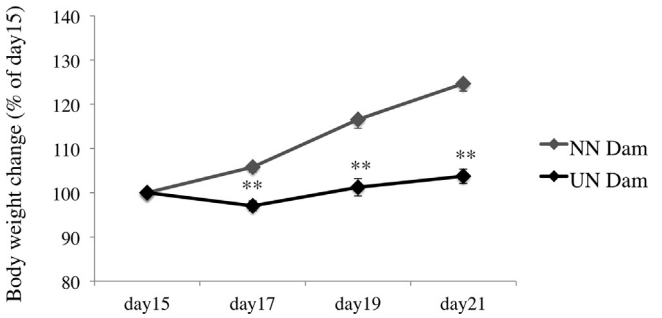


**Fig. 1.** Body weight changes (% of initial day of food control) seen in the normally nourished dams (NN dams) and 50% food-restricted undernourished dams (UN dams) ($n = 8$ per group) during pregnancy. Data are presented as mean ± SEM values. $^{**}P < 0.01$ vs. NN dams at same time point.

### 2.5. Statistical analysis

The body weight of the dams and their offspring at each time point were compared using the Student's *t*-test. The changes in body temperature, body weight, and food intake induced by the injection of LPS were compared between the NN and UN groups using two-way ANOVA. The hypothalamic mRNA levels of the examined molecules at key points were compared using the Student's *t*-test. Differences were considered significant at $P < 0.05$. Data are expressed as mean ± SEM values.