

Food restriction in pregnant rat-like hamsters (*Cricetulus triton*) affects endocrine, immune function and odor attractiveness of male offspring

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Abstract

We studied the effect of intrauterine food restriction (FR) on the immune function, endocrine status and attractiveness of scents of male rat-like hamsters, *Cricetulus triton*. Work was conducted on field-caught parents from the North China plain and their laboratory-born progeny. Restricted pregnant dams were fed 70% of the mean daily intake of hamsters with free access to food. FR caused a marked and protracted weight reduction of the body, adrenal, testes and epididymides in males. During the refeeding period, the spleen and thymus, but not the adrenal weight of the malnourished offspring caught up with that of the control after about 60 days. The present results demonstrated that estrous females preferred the odors of control males to that of FR males. Males whose mothers were food restricted during gestation had lower testosterone concentrations, immune responses and reproductive organ mass but had higher circulating cortisol than did the males in the control group. Thus, the effect of maternal FR may be an important cause in population regulation in the rat-like hamster. The testosterone level was positively correlated with immune function in rat-like hamsters, but the lower immunity was not suppressed by higher level of testosterone, as previously suggested. We also found a negative relationship between cortisol and immune function in the rat-like hamster. © 2004 Elsevier Inc. All rights reserved.

Keywords: *Cricetulus triton*; Maternal effect; Food restriction (FR); Odor preference; Testosterone; Cortisol; Antihuman IgG

1. Introduction

Maternal effect is the effect of parental phenotype on offspring phenotype that does not have a Mendelian genetic basis [1]. Maternal effect has been suggested as a plausible explanation for density lows of cyclic rodent populations [2]. The adaptations of the fetal metabolism to the altered intrauterine environment have consequences for the offspring, persisting into adulthood and into the next generation [3]. Individuals that were conceived, born or raised during the peak and early decline phases of the population will continue to exhibit physiological and behavioral responses to the stressful environment within which they were born [2,4,5]. Some researchers have documented the decline, or even the destruction, of vegetation before or during peak phases [6]. Thus, maternal effect resulting from food shortage may be a potential proximate factor in

causing a delayed low density before the population begins to recover.

The fitness of an offspring can be affected by the food availability of a mother. If animals are food restricted prenatally, the reproduction of males may be more strongly impacted than the reproduction of females because it may decrease their ability to compete with other males and their attractiveness to females. For example, in wild house mice (*Mus musculus*), estrous females preferred the odor of the sons of well-nourished females [7]. In similar studies, gestational food restriction (FR) of the male offspring also resulted in lowered social dominance and body mass as adults when compared with control males. This may affect the ability of males to gain access to females [8].

Female mammals invest a large amount of energy in fetal growth, lactation and rearing of their young. Therefore, females are expected to be highly selective when choosing a mate. Male olfactory cues help females to distinguish among, and to evaluate, males during mate choice. Males whose odors are preferred by estrous female are usually those chosen as mates [9]. The attractiveness of male odors

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varies directly with plasma testosterone concentration [10]. According to the Hamilton–Zuk hypothesis, parasites are the selective agent that maintains female choice in populations [11]. Parasitized males will show decreased expression of the secondary sexual traits preferred by females [12]. Polygynous males that advertise high immune function via secondary sex traits should acquire more mating [13]. Hence, males with high testosterone concentrations, who do not have compromised immunity, are at a selective advantage.

Maternal undernourishment during gestation induces intrauterine growth retardation and disturbs steroidogenesis and the hypothalamo–pituitary–adrenal axis [14]. Intrauterine malnutrition also causes a marked and protracted reduction of immune function [15]. Although some studies have examined the relationship between steroid hormones and immunity in an ecological context, most have used indirect measures for immune functionality (e.g., parasite load; [11]), and little is known about the relationships among behavior, hormone, immunity and body growth in prenatal FR animals, as well as the underlying mechanism.

Rat-like hamsters (*Cricetulus triton*) are an asocial, promiscuous species living in the farmland of North China [16,17]. Males have a pair of flank glands and a midventral gland; females are philopatric and have no stable mating association [17–19]. Except on the day of sexual receptivity, which is marked by copulation, females aggressively attack approaching males and chase them away [17]. Our recent work has indicated that there is an extended low density after the population crash in rat-like hamster populations [20]. The potential factors for this prolonged low density may involve social pressure, predation or food shortage. Food shortage during the peak years of rat-like hamsters has been observed (unpublished data). Thus, the effects of maternal food shortage might be potentially caused density lows after population crash in the rat-like hamsters.

The purpose of the study is to investigate the potential role of the maternal effect of FR in population regulation of rat-like hamsters. We also focus on the relationship among endocrine, reproductive activity and immune function of the male offspring in rat-like hamsters under prenatal FR. The male pup's serum testosterone, cortisol and antihuman IgG antibodies levels were determined, and the body, adrenal, spleen, thymus, testes, epididymides and epididymal fat pad masses of male offspring were recorded. The odor preference of estrous females to male pups was monitored to determine the effect of intrauterine FR on the odor attractiveness of male offspring.

2. Methods

2.1. Animals and maintenance

Subjects (Generation 1) were captured in farmland at the center of Hebei Province, North Plain China. Forty-three

females weighing 109–210 g (mean = 148.9 ± 24.3 g) were used. After mating to satiety with 2–3 males, as evidenced by the presence of sperm in vaginal smears, the subjects were placed into $40 \times 25 \times 15$ cm cages. The colony was maintained on a 16L:8D light cycle (lights on at 2100 h) at approximately 23 °C. Tap water was available ad libitum. Rat chow (Keaoxieli, Beijing, China) was provided as described below during the pregnancy period. The assessment of 24-h food intake was made by weighing the pellets daily. The animals were weighed daily prior to food allocation.

As previously ascertained, restricting the food intake of pregnant female *C. triton* to approximately 70% of the average food intake in hamsters fed ad libitum for 1–18 days can still lead to reproduction. Hence, during the pregnancy period, the food-restricted animals ($n=21$) were presented with 70% of the mean daily intake of ad-lib-fed animals ($n=22$). The amount of food feed to the experimental females was determined daily to reflect the fluctuation of food intake for the hamsters fed ad libitum.

All animals delivered on Day 18. The day of delivery was considered the first day of lactation. Generation 2 pups were removed from their mothers at 20 days of age and were housed individually in polycarbonate cages with wood shavings and cotton nesting as bedding. Food (rat chow) and tap water were provided ad libitum.

To measure a specific immune response, each individual was immunized with an intraperitoneal injection (200 μ g) of human IgG (DingGuo Biotech., Beijing, China) emulsified in complete Freund's adjuvant (DingGuo). Fourteen days later (at the age of 60 days), the animals were killed and anatomized, and blood was sampled. Blood samples were allowed to clot at 4 °C for 1 h and, then, were centrifuged at 3500 rpm at 4 °C for 30 min. The samples were stored at –80 °C until the assay of antibodies, testosterone and cortisol. Paired testes, epididymides, epididymal fat pad masses, as well as thymus, adrenal glands and spleens were removed, cleaned of connective tissue and weighted.

2.2. Odor preference test

The odor preferences of estrous females to the nesting materials of male offspring scent donors were recorded in the Y maze olfactometer. The control and FR scent donors included 15 males in each group. All scent donors were sexually naive. The female subjects ($n=15$) were in estrous and had raised at least one litter previously. The estrous females were judged by vaginal secretion and their behavioral responses to males. The bedding (15 ± 0.5 g) of the donor animals was in use for a 2-week period before use in the Y maze apparatus. The odors of the males presented in the maze were unfamiliar and had no kin relationship to the females whose preferences were being tested.

Odor preferences were tested in a covered Plexiglas Y maze, as described by Zhang et al. [17,19]. A 15-cm acclimation area was attached to the base of the Y maze,

connected by the common arm of 70 cm before the two choice arms of 60 cm each. Soiled bedding was placed in a mesh basket at the distal end of each choice arm. These baskets allowed air passage and the movement of volatile odors through the maze. A fan at the base of the Y maze pulls the air through the maze from both choice arms down to the acclimation area. The females were placed in the acclimation area for 1 min and then allowed to spend 10 min moving freely through the maze. The total time spent in each arm was recorded. In every test, an experimental subject was presented with two odor stimuli from the control versus the FR group, respectively.

2.3. Immunological assay

Plasma levels of antihuman immunoglobulin (IgG) were determined by the enzyme-linked immunosorbent assay (ELISA), as described by Li et al. [21]. A 96-well immunoplate (Nunc MaxiSorp) was coated with antigen (0.1 mg human IgG in 0.1 ml sodium bicarbonate buffer, pH 9.6), washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T, pH 7.4), blocked with 5% calf serum in PBS and then washed again. Thawed sera samples from *C. triton* were diluted 1:100 with PBS, and then, 0.1 ml of each serum dilution was added in duplicate to the wells of the antigen-coated plate. Negative control samples were also added in duplicate to the wells. The plate was sealed and incubated at 37 °C for 1 h and then washed with PBS-T. A secondary antibody (horseradish-peroxidase-conjugated antimouse IgG) diluted by 1:1000 was added to the plate. The plate was sealed, incubated for 1 h at 37 °C and washed with PBS-T, and 0.1 ml of the enzyme substrate (a mixture of 3,3',5,5' - tetramethylbenzidine and urea hydrogen peroxide in the proportion of 1:1 by volume) was added to each well. The plate was kept at 37 °C, away from light. After 20 min, the reaction was terminated by adding 0.05 ml of 2 M H₂SO₄ to each well. The optical density (OD) of each well was measured using a plate reader equipped with a 450-nm wavelength filter, and the average OD of each sample was calculated. Because no appropriate standard sample was available in this study, we used OD to represent the relative levels of serum antibodies instead of the absolute concentrations.

2.4. Testosterone and cortisol radioimmunoassay

Radioimmunoassays (RIA) were run using the reported methods [22,23]. Serum testosterone and cortisol concentrations were determined using ¹²⁵I RIA kits provided by the Beiming Institute of Biotechnology (Beijing, China). For cortisol, the detectable range of the assay was 10–500 ng/ml. The intra- and interassay coefficients of variation were <5% and <10%, respectively. For testosterone, the detectable range of the assay was 10–2000 ng/dl. The intra and interassay coefficients of variation were 5.4–7.4% and <3.1–6.1%, respectively.

2.5. Statistical analyses

We used the Wilcoxon matched-pairs signed-ranks test the significance of the differences in time spent investigating each pair of odor stimuli. Serum antihuman IgG specific antibodies, cortisol and testosterone concentrations, as well as reproductive organs and other tissues weights, were analyzed using an independent-samples *t* test. In all statistical analyses, the level of significance was set at $\alpha=.05$.

3. Results

In the odor preference trials, the estrous females spent significantly more time investigating the scents from the control males than scents from FR males ($Z=2.27$, $P=.023$; Fig. 1).

Relative body mass [$t(18)=2.877$, $P=.01$], testes [$t(18)=3.834$, $P<.01$] and epididymides masses [$t(18)=2.284$, $P<.05$] were lower in FR males than they were in the control males. Epididymal fat pad mass was not affected by the amount of food intake of the mother [$t(18)=1.483$, $P=.155$]. The adrenal mass in FR animals was significantly lower than that in the control animals [$t(18)=2.215$, $P<.05$]. FR had no significant effect on the spleen [$t(18)=0.800$, $P=.434$] or thymus masses [$t(18)=-0.588$, $P=.564$; Table 1].

The control male offspring had significantly higher serum testosterone concentrations [$t(18)=2.457$, $P<.05$] and lower cortisol concentrations than did the FR male offspring [$t(18)=-2.266$, $P<.05$]. Additionally, the control male offspring exhibited higher antihuman IgG responses than did the FR male offspring 14 days following immunization [$t(18)=2.745$, $P<.05$; Fig. 2].

4. Discussions

Our results showed that the maternal FR of rat-like hamsters inhibited their male pups' endocrine activity, immune function and reproductive development. FR in the

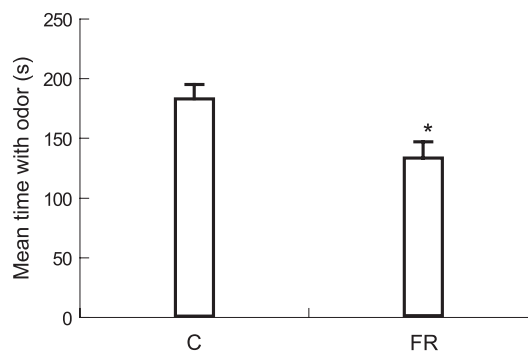


Fig. 1. Mean time (\pm S.E.) that the female rat-like hamsters investigated scents from the nesting material of control vs. FR male offspring ($n=15$). * $P<.05$, Wilcoxon's matched-pairs signed-ranks test.

Table 1
Mean (\pm S.E.) relative body mass (g/cm body length) and reproductive and immune organ masses (mg/g)

	Control group	FR group
Body mass	7.12 \pm 0.07	6.88 \pm 0.04 *
Testes	28.4 \pm 2.30	15.20 \pm 2.57 *
Epididymides	3.90 \pm 0.34	2.70 \pm 0.39 *
Epididymal fat pad	17.20 \pm 1.74	14.10 \pm 1.14
Spleen	0.70 \pm 0.07	0.60 \pm 0.11
Thymus	3.60 \pm 0.23	3.80 \pm 0.21
Adrenal	0.20 \pm 0.02	0.10 \pm 0.01 *

* Significantly different at $P < .05$.

mother during gestation induced lowered testosterone levels, reproductive organ masses and immune responses, and increased the circulating cortisol in the male offspring. FR also resulted in a protracted weight reduction in the body and adrenal gland of the male, but the weights of the spleen and thymus were not affected. Estrous females preferred the odors of the control to that of the FR males. Therefore, the maternal effect of FR may be an important factor in regulating rat-like hamster populations. Future work should focus on the measurement of endocrine, immunity and reproductive performance of rat-like hamsters during different phases of the population cycle in the field.

Maternal effect occurs commonly in rodents [1]. It was reported that maternal food deprivation affects sons only if they engage in agonistic encounters [8], but our data demonstrated that the maternal effect of FR occurred even without social encounters. The terminal body mass was dependent on the level of food availability. Body mass may be of significance to male reproductive success because it can influence males to establish social dominance and to protect resources, which, in turn, affect males' ability to gain access to females [24]. FR during gestation may have reduced the nutrients received by male fetuses, and this may subsequently have inhibited steroidogenesis [25] and accessory sex glands in males [26]. In this study, lower levels of testosterone and immunity and smaller body mass and reproductive organs were observed simultaneously in the male offspring of FR females. Thus, we also support the view that FR negatively affects the biological process of endocrine hormones (e.g., testosterone, corticosteroid), which, in turn, reduces the mass of organs (e.g., testes, adrenal and body).

In solitary *C. triton*, females lack a long association with mates and contribute all paternal care [17]. It is important for female hamsters to discriminate among mates through olfactory cues associated with the male's quality to insure their own reproductive success. The attractiveness of male odors to females varies directly with the plasma testosterone concentration [10]. The growth of some scent glands in male mammals is positively correlated with testes size and testosterone level [27]. Estrous females were able to discriminate between the odors of males with different gonad and endocrine conditions [28]. Similarly, the control male offspring had heavier testes and levels of plasma testosterone than did the FR male offspring, and estrous females prefer

scent from control male pups in the rat-like hamster. During the breeding season, interactions with mates occur often, and parasite loads are high. According to the Hamilton–Zuk hypothesis, parasites are the selective agent that maintains female choice in populations [11]. Maternal malnutrition induces neuroendocrine immune dysfunction on male pups at weaning [15,29]. Males that have higher immune function may gain more mating than will males that have lower immune function [30]. In this study, females preferred the odors of male hamsters with high immunity. This, in general, supports the immunity hypothesis. But how the immunity of males is discriminated by females is still unknown. Based on previous studies, females preferred odors from males that

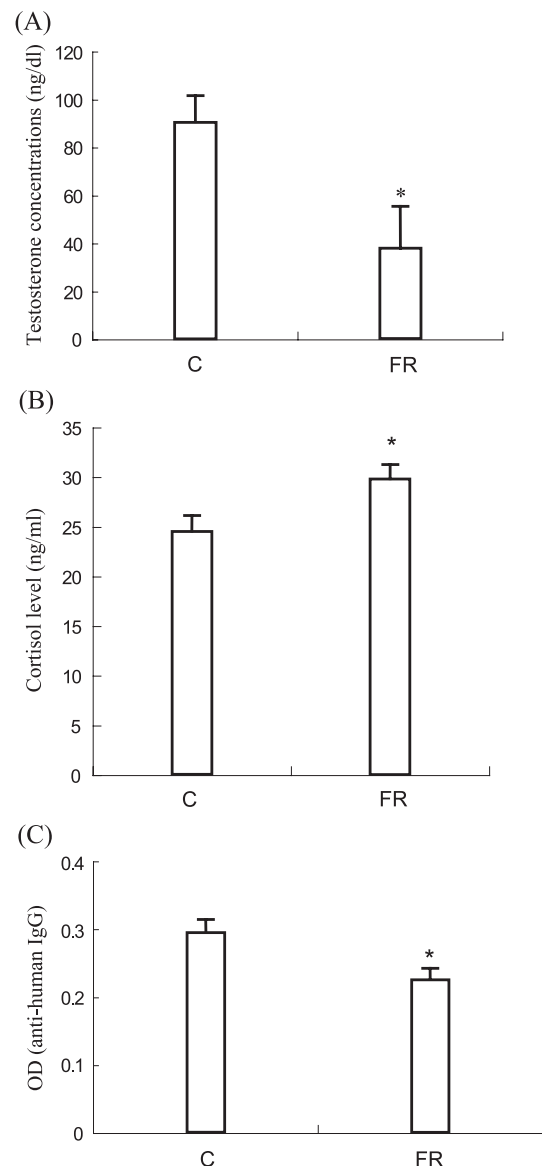


Fig. 2. (A) Serum testosterone concentrations (mean \pm S.E.), (B) serum cortisol concentrations (mean \pm S.E.) and (C) serum antihuman IgG antibodies levels (mean \pm S.E.) in male offspring between the control and FR groups ($n = 10$). Columns with different symbols are significantly different at $P < .05$.

had a high level of testosterone, which affects the flank and midventral glands in rat-like hamsters [28].

The relationship between testosterone and immune responses are complex. Although testosterone is generally considered immunosuppressive [31], some studies suggest that high circulating testosterone can increase resistance to infection [32,33]. Recently, studies suggest that the immune system may function in mediating the costs of various energetically demanding activities [34]. If low-quality males have less available energy, then balancing an energy budget between the essential bodily functions becomes challenging [35]. We found that the testosterone level was positively linked to immunity in rat-like hamsters, indicating that lower immunity was not suppressed by a higher level of testosterone. It was reported that androgens might be costly by suppressing the immune system. Only high-quality male offspring would be able to withstand the high concentration of androgens [36]. Our data seem to support this reasoning because we did find a positive link between the concentration of androgens and the level of immunity.

Traditionally, the level of glucocorticoids is positively related to adrenal gland weight [14]. But our discovery in rat-like hamster does not support this opinion. Few studies have shown that the level of glucocorticoids is negatively related to adrenal gland weight [37,38]. It has been demonstrated that the steroidogenesis rate increases in undernourished rats, and such increase is associated with a concomitant, significant decrease in adrenal gland weight [38]. It may be due to the slower rate of disappearance and degradation of corticosteroid in tissues [39]. On the other hand, by elevating the neuroendocrine stress hormone, corticosterone in adolescent rats attenuated weight gain and reduced adrenal weights [40]. Generally, high circulating glucocorticoids suppress immune responses in rodents [41]. In this study, we found that FR males had higher serum cortisol and lower antibodies levels, which seem to support the view. However, the higher concentration of glucocorticoid may not be the only key factor in reducing immunity in prenatal food-restricted hamsters.

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