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Maternal Immune Activation by Lipopolysaccharide During Late Gestation Reduces Body Weight and Food Intake in Male Offspring

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Abstract
Accumulating evidence indicates that early-life environmental exposures are important determinants of disease susceptibility later in life. In particular, maternal immune activation induced by lipopolysaccharide (LPS) has been shown to elevate proinflammatory cytokines, disrupt neuroendocrine homeostasis, and impair offspring development. In the present study, we investigated the effects of maternal LPS administration (800 µg/kg, on gestational days 15 and 17) on body weight and food intake in male offspring at postnatal day 35 (PND35). Prenatal LPS exposure significantly reduced both body weight and food intake compared with saline-treated controls. These findings suggest that maternal inflammatory challenge during late gestation can adversely affect postnatal growth and feeding in male offspring, supporting a role for prenatal immune stress in long-term somatic and metabolic dysregulation.

Keywords: Prenatal LPS challenge, Lipopolysaccharide, Late gestation, Body weight, Food intake.

Introduction

Lipopolysaccharide (LPS), an endotoxin present in the cell wall of gram-negative bacteria, induces a wide range of pathophysiological responses, including psychosis-like changes, suppression of reproductive function, and alterations in behaviours such as eating and drinking (Avitsur et al., 1997; Bachmanov et al., 2002; Battaglia et al., 1997; Battaglia et al., 2000; Coleman et al., 1993; Kraly, 1984; Saluk-Juszczak et al., 2005). Maternal exposure to LPS during pregnancy can increase the production of proinflammatory cytokines and glucocorticoids, which may affect the developing fetal brain and contribute to disruption of the reproductive axis in rodents (Gayle et al., 2004; Hava et al., 2006; Li et al., 2007). Because the hypothalamic–pituitary–adrenal (HPA) and hypothalamic–pituitary–gonadal (HPG) axes are closely interconnected, maternal immune activation and stress during pregnancy may have important consequences for offspring development and later reproductive function (Rivier & Rivest, 1991; Tilbrook et al., 2000).

A growing body of evidence suggests that maternal stress elevates circulating glucocorticoid levels, and these hormones can cross the placenta and adversely affect fetal growth, thereby reducing birth weight in prenatally stressed offspring (Su et al., 2015). In line with this, several studies have shown that maternal LPS exposure during pregnancy impairs reproductive development in male offspring, including reductions in body weight, testes weight, prostate and seminal vesicle weight, serum testosterone levels,

spermatogenesis, and sexual behaviour at puberty (Bernardi et al., 2009; Wang et al., 2014). In rodents and non-human primates, prenatal stress, including endotoxin exposure, has also been reported to alter HPA axis function and brain neurotransmitter systems in the offspring (Kapoor et al., 2006; Karrow, 2006). These effects are thought to be mediated, at least in part, by cytokine induction in the maternal circulation and placenta (Kirsten et al., 2010; Klein & Rager, 1995; Kofman, 2002).

Feeding, drinking and body weight are closely interrelated traits influenced by both genetic and environmental factors. Therefore, inflammatory, neurochemical, and hormonal disturbances induced by prenatal LPS exposure may also affect postnatal growth and eating-related behaviours (Bachmanov et al., 2002; Kraly, 1984). In the present study, we examined whether maternal exposure to LPS during pregnancy alters postnatal growth and food intake in male offspring, with particular emphasis on body weight–related outcomes at PND 35.

Materials and Methods

Animals and experiment deigns

Swiss albino mice (weight: 29±1 g) obtained from Indian Institute of Toxicology Research, Lucknow, India were kept in polyvinyl chloride cages (290×320×390 mm^3) in standard laboratory conditions (12 h light–dark cycle; temperature 21±2 °C and humidity 55±5%) with free access to mice feed and water. After 2 weeks of acclimatization, mice were kept for mating. Females with vaginal plugs were designated as on gestational day (GD) 0 and were divided into two groups (8/group). Group I/Control were given intraperitoneal injection (i.p.) of physiological saline, Group II mice received i.p. injection of LPS (800 µg/kg; Escherichia coli serotype 026:B6, L-2654, Sigma-Aldrich, India) at GD 15 and GD 17. It is important to note that although maternal exposure to LPS causes some disturbances, it can vary depending on doses of LPS, potency of LPS, age, sex, and interactions with environmental and genetic risk factors (Bell & Hallenbeck, 2002; Urakubo et al., 2001). Body weight was monitored on weekly basis and food intake was measured daily. Bodyweight and food intake were recorded regularly till the day of dissection. The food intake was measured the next day by subtracting the left or uneaten food manually.

The experimental protocol followed the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India. The experimental protocols were approved by the Institutional Animal Ethical Committee of the University.

Analysis of data

Statistical analyses were performed using GraphPad Prism 5.0. Data are expressed as mean ± S.E.M., with eight mice in each group. Differences between groups were analyzed using an unpaired Student's t-test. A p value of ≤ 0.05 was considered statistically significant.

Results and Discussion:

To examine the effects of maternal LPS exposure during pregnancy on offspring growth and feeding, pregnant mice were administered intraperitoneal injections of LPS (800 µg/kg) on gestational days 15 and 17. As shown in Fig. 1A and B, prenatal LPS exposure significantly reduced both food intake and body weight in male offspring at PND 35 compared with saline-treated controls.

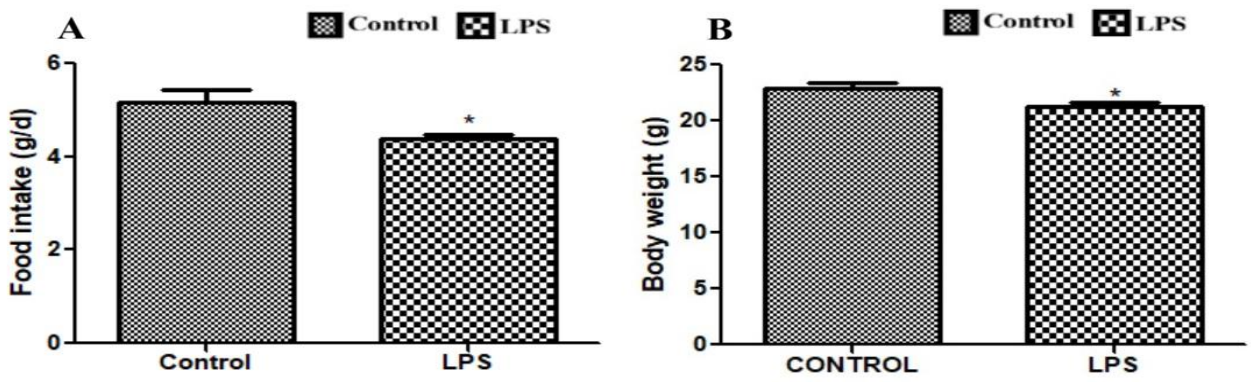

Fig. 1. Maternal immune activation induced by lipopolysaccharide during late gestation reduces food intake and body weight in male offspring at postnatal day 35. Bar graphs showing (A) food intake and (B) body weight in male offspring following prenatal exposure to lipopolysaccharide (LPS) compared with controls. Data are presented as mean ± SEM (n = 8 per group) and were analyzed using an unpaired Student's *t*-test. *P, 0.05 as compared with controls.

Food intake was significantly lower in the LPS-treated group than in the control group. The mean food intake was 5.16 ± 0.27 in controls and 4.39 ± 0.08 in the LPS group, and this difference was statistically significant ($t(14) = 2.74$, $p = 0.016$). Similarly, body weight was significantly reduced in the LPS-exposed group compared with controls. The mean body weight was 22.88 ± 0.58 in the control group and 21.23 ± 0.37 in the LPS group, indicating a significant effect of prenatal LPS exposure ($t(14) = 2.39$, $p = 0.031$). These results indicate that maternal inflammatory exposure during gestation impairs postnatal growth and feeding in male offspring.

Previous reports on the effects of prenatal stress on offspring body weight have been inconsistent. Some studies have shown that prenatal stress reduces pup body weight (Klein & Rager, 1995; Patin et al., 2002; Vallée et al., 1996), whereas others reported no significant effect (Fride et al., 1986; Glockner & Karge, 1991; Hughes & Beveridge, 1990). These discrepancies may reflect differences in the nature, timing, and intensity of the prenatal stressors used. In the present study, prenatal exposure to Escherichia coli endotoxin clearly reduced both body weight and food intake in male offspring at PND 35.

Our findings are consistent with previous evidence indicating that prenatal LPS exposure can impair postnatal growth. Male offspring exposed to LPS on gestational day 10 likewise exhibited reduced body weight later in life (Asiaei et al., 2011). However, findings from neonatal immune challenge models have been less consistent. Iwasa et al. (2010) reported that neonatal immune challenge increased body weight and food intake in rats, whereas Walker et al. (2006) found that neonatal LPS exposure on postnatal days 3 and 5 reduced body weight during adolescence and adulthood, possibly through impaired glucose tolerance and persistent insulin deficiency. These differences underscore the importance of developmental timing, as prenatal and neonatal inflammatory exposures may influence distinct physiological pathways.

Several mechanisms may explain the reduction in body weight observed in the present study. Prenatal immune activation can elevate maternal cytokine and glucocorticoid levels, which may impair fetal growth

and alter later behavioural and metabolic function in the offspring (Anisman et al., 2002; Anisman et al., 2002; Krieg et al., 1992; Levitt et al., 2000; Reynolds & Phillips, 2000). In addition, neonatal LPS exposure has been associated with reduced food intake, impaired glucose tolerance, and insulin deficiency (Doosti et al., 2013; Walker et al., 2006), suggesting that inflammatory programming may have long-term metabolic consequences.

Another possible explanation is that prenatal LPS exposure altered the maternal environment after birth. Cytokines are known to affect neuroendocrine function, including suppression of GnRH, LH, corticotropin-releasing factor, and growth hormone secretion, while stimulating prolactin release (Schanberg et al.,1984; Levine, 1994; Feleder et al., 1996; Yoo et al., 1997; He et al., 2003). Although these hormonal parameters were not measured in the present study, such alterations may have influenced lactation and postnatal nutrient availability. Moreover, our previous study (Kumar & Mohanty, 2015), together with the findings of Aubert et al. (1997), showed that LPS disrupts maternal behaviour. Therefore, it is possible that the pups received less milk, which may have contributed to reduced nutrient intake and lower body weight. This interpretation is consistent with earlier reports suggesting that reduced milk intake may underlie early postnatal growth deficits in pups (Bernardi et al., 2009).

Overall, the present findings demonstrate that prenatal LPS exposure on gestational days 15 and 17 is associated with reduced food intake and body weight in male offspring. These effects may result from a combination of fetal inflammatory programming, altered glucocorticoid exposure, metabolic dysfunction, and disruption of maternal care. Further studies measuring cytokine, hormone, and lactational parameters would help clarify the mechanisms underlying these alterations.

Conclusion

Prenatal LPS exposure on gestational days 15 and 17 significantly decreased food intake and body weight in male offspring at PND 35, suggesting that maternal immune activation during pregnancy can produce lasting effects on offspring growth and feeding behaviour. These effects may involve maternal inflammatory and glucocorticoid responses, altered metabolic regulation, disrupted lactational hormonal milieu, and impaired maternal care leading to reduced milk intake. The findings also raise the possibility that prenatal LPS exposure may influence neuroendocrine processes related to sexual masculinization, although this requires direct experimental confirmation. Overall, the study highlights a significant association between prenatal maternal sickness behaviour and subsequent alterations in offspring feeding and body weight later in life.

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