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Moderate maternal food restriction in mice impairs physical growth, behavior, and neurodevelopment of offspring

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ABSTRACT

Intrauterine growth retardation (IUGR) occurs in 3% to 7% of all pregnancies. Recent human studies have indicated that neurodevelopmental disabilities, learning disorders, memory impairment, and mood disturbance are common in IUGR offspring. However, the interactions between IUGR and neurodevelopmental disorders are unclear because of the wide range of causes of IUGR, such as maternal malnutrition, placental insufficiency, pregnancy toxemia, and fetal malformations. Meanwhile, many studies have shown that moderate food restriction enhances spatial learning and improves mood disturbance in adult humans and animals. To date, the effects of maternal moderate food restriction on fetal brain remain largely unknown. In this study, we hypothesized that IUGR would be caused by even moderate food restriction in pregnant females and that the offspring would have neurodevelopmental disabilities. Mid-pregnant mice received moderate food restriction through the early lactation period. The offspring were tested for aspects of physical development, behavior, and neurodevelopment. The results showed that moderate maternal food restriction induced IUGR. Offspring had low birth weight and delayed development of physical and coordinated movement. Moreover, IUGR offspring exhibited mental disabilities such as anxiety and poor cognitive function. In particular, male offspring exhibited significantly impaired cognitive function at 3 weeks of age. These results suggested that a restricted maternal diet could be a risk factor for developmental disability in IUGR offspring and that male offspring might be especially susceptible.

1. Introduction

Intrauterine growth retardation (IUGR), which occurs in 3% to 7% of all pregnancies [1], leads to low birth weight. Intrauterine growth retardation has been suggested not only to cause a significant increased risk of perinatal and neonatal morbidity [2,3] but also to increase the risk of several chronic adult diseases, including obesity, type 2 diabetes, and cardiovascular

Abbreviations: ANOVA, analysis of variance; BrdU, bromodeoxyuridine; DCX, doublecortin; DI, discrimination index; IUGR, intrauterine growth retardation; PBS, phosphate-buffered saline; PFA, paraformaldehyde; PND, postnatal day.

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disease [4]. Furthermore, recent human studies have shown that IUGR is associated with neurodevelopmental disorders such as learning disability, memory impairment, and mood disturbance [5-9].

The major cause of IUGR is a deficient supply of nutrition and oxygen to the embryo. In animal studies, IUGR models are induced by food restriction, low-protein diet, drugs, or ligation of the uterine artery. Studies using IUGR models have clearly shown that prenatal and early postnatal malnutrition induces abnormalities such as obesity and changes in endocrine systems [10-13]. By contrast, there have been very few studies on neurodevelopment and behavior in IUGR models. One study showed IUGR induced by uterine artery ligation transiently delayed oligodendrocyte maturation and myelination in utero [14]. Another study demonstrated that IUGR induced by maternal passive smoking caused impaired spatial learning and memory [15]. Maternal smoking is a risk factor of IUGR because uterine arteries are constricted by the nicotine in cigarettes. These studies suggest that a failure of uterine blood supply causes fetal neurodevelopmental disabilities in IUGR offspring.

Because fetal development is dependent on the placental supply of nutrition, malnutrition in childbearing or pregnant women is a critical risk factor for IUGR. The fetal brain is developing rapidly and, therefore, needs a wide variety of nutrients and growth factors. Thus, maternal nutritional status directly affects fetal neurodevelopment. However, the effects of prenatal malnutrition on neurodevelopment of IUGR offspring remain controversial. One report by Tonkiss et al [16] showed no effect of prenatal protein undernutrition on performance in a water maze test. By contrast, a recent animal study demonstrated that 50% global food restriction from mid-pregnancy to the lactation period mildly impaired spatial cognitive function of the offspring in a water maze test and also heightened anxiety behavior in an elevated plus maze test [17]. Another report showed that prenatal protein restriction impaired learning and motivation behavior of the IUGR offspring in an operant conditioning test [18]. These results imply that severe malnutrition or nutrient imbalance in the prenatal period might result in behavioral abnormalities in offspring with IUGR.

Recently, a large number of studies have shown an association between food intake and mental health, learning, and memory ability, in addition to longevity and peripheral health. For example, a high-calorie diet (eg, high-fat or high-sugar diet) can impair learning and memory [19-23]. Meanwhile, other reports have shown that moderate food restriction, that is, calorie restriction or intermittent fasting, enhances spatial learning and ameliorates mood disturbance in adult humans and animals [24-26]. The beneficial effects of moderate food restriction have been partly attributed to increased neurogenesis in the adult hippocampus [27-30]. At present, the effects on fetal neurodevelopment of moderate food restriction during pregnancy remain largely unknown.

We hypothesized that IUGR would be caused by even moderate food restriction in pregnant females and that offspring would have neurodevelopmental disorders such as learning disability, hyperactivity, or mood disturbance throughout the lifespan. Such impairments in offspring might be related to disruption of regulation of neurogenesis. The purpose of this study was to determine if there is a link between maternal diet, IUGR, and neurodevelopmental disorders in a mouse model of IUGR. To test this hypothesis, we subjected mid-pregnant mice to moderate food restriction. The rodent central nervous system is immature at birth; therefore, from the standpoint of neurodevelopment, birth plus 7 to 14 postnatal days in rodents correspond to the prenatal period in human infants [31,32]. Based on this evidence, moderate maternal food restriction was continued through day 7 of lactation. We examined physical development, coordination of movement, locomotor activity, emotional state, cognitive function, and hippocampal development of the offspring. Furthermore, it has long been recognized that low-birth-weight infants exhibit sex differences in regard to immaturity and clinical prognosis. For example, cerebral palsy and mental retardation are more common in males than in females [33]. Therefore, we also examined sex differences in the offspring.

2. Methods and materials

2.1. *Mice and treatments*

All experiments were approved by the Animal Ethics Committee of the National Cerebral and Cardiovascular Center Research Institute and were conducted in accordance with the guidelines of the Physiological Society of Japan. All mice were maintained under a 12-hour/12-hour light/dark cycle, with lights on at 0700. The study design is illustrated in Fig. 1.

Seven-day–pregnant ddY mice were purchased from Japan SLC, Inc (Shizuoka, Japan). The dams were individually housed and given ad libitum access to standard feed (CE-2; CLEA Japan, Tokyo, Japan) with free access to drinking water. CE-2 is a good laboratory practice–compliant, standard rodent food consisting mainly of vegetable protein (soybean waste) with a proper balance of animal protein (whitefish meal), and it is frequently used for rearing and breeding of laboratory animals. The Table provides the nutritional information for CE-2. The next day after arrival, the dams were randomly separated into a control group and a food restriction group. The dams of the control group were allowed free access to standard food, and daily food intake was recorded. The dams of the food restriction group (IUGR group) were allowed 70% of the food provided to the control group through day 7 of lactation (Fig. 2A and B). On the morning of parturition, dams of the IUGR group were supplied with 10 g of food to avoid maternal killing of the pups. Both groups had free access to drinking water for the entire period. At postnatal day (PND) 2, pups were culled or boarded out to another dam of the same group to control litter size to 8 to 10 pups per dam until weaning day (PND 21). At PND 7, mice were tagged individually using an indelible marker. After weaning from the mother, each pup was housed in a same-sex group of 5 to 6 mice per cage and allowed free access to standard food and drinking water. The study schedule is shown schematically in Fig. 1. Body weight was assessed at PND 0, 7 (end date of food restriction), 9, 16, 23, 30, 37, 44, and 51, and eyelid openings of the pups were checked each day on a schedule.