

Lepin Administration during Pregnancy Improves Maternal Undernourishment Induced Metabolic Abnormalities in Adult Offspring

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Abstract: Undernourishment in womb would lead to persistent changes in glucose response and a range of metabolic abnormalities. Leptin, secreted from adipocytes, closely participates in energy homeostasis. We investigated that glucose intolerance in the adult offspring which was caused by maternal undernourishment whether could be improved by leptin repletion. The results showed the mice derived from undernourished dams had lower birth weight, but they gained more body weight and body fat after then. In addition, these mice at 6 month of age had higher plasma levels of glucose, insulin and leptin than their age-matched controls. Leptin administration during gestation markedly reversed maternal undernourishment induced metabolic abnormalities.

Keywords: Fetal programming, Glucose intolerance, Insulin, Leptin

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I. Introduction

The interaction between overweight and the manifestation of type 2 diabetes mellitus has been well noted [1]. Furthermore, maternal undernourishment has also been shown to predispose adult offspring to impaired glucose response, which is known as thrifty phenotype [2,3]. It has been proposed that glucose intolerance is caused by maternal malnutrition which will impair normal pancreatic development and predispose the subject to the subsequent manifestation of the metabolic defects in later life [4]. However, it is still unclear nutritional manipulation or hormonal factor whether participates or to be critical in this phenomenon.

Leptin, a satiety factor which mainly secreted from the fat cells, takes part closely in appetite control and energy expenditure [5]. In general, leptin is produced in proportion to the amount of body fat. However, many hormonal and nutritional manipulations can influence its circulating level. For example, in fasted subjects, their plasma leptin levels fall at a rate that cannot be accounted for by the loss of body fat alone. In addition, plasma leptin level promptly rises after food intake [6]. Leptin is also known to participate in reproduction and immune action [7]. Based on the findings of high plasma leptin levels during pregnancy, it has been considered that leptin might modulate maternal nutrient partitioning in order to optimize the provision of nutrients for fetal growth [8,9]. Furthermore, chronic undernourishment causes hypoleptinemia. Thus, it seems reasonable to suppose that reduced leptin secretion might have some contributions to the manifestation of maternal undernourishment induced glucose intolerance in adult offspring. This study was designed to examine whether leptin repletion could ameliorate glucose intolerance in the adult offspring which was caused by maternal undernourishment.

II. Materials and Methods

C57BL/6 mice were obtained from the National Laboratory of Animal Breeding and Research Center (Taipei, Taiwan). Throughout the study, mice were housed in plastic non-galvanized cages and kept at a constant temperature (22±1°C)/humidity (50±5%) controlled room with a 12-h light/12-h dark cycle. A protocol for animal care procedure was approved by the OCU Research Management Committee (OCU90B010).

Pregnant mice, determined by the appearance of sperm plug after mating at next morning, were divided into 4 groups. Each group contained 6 mice. Two groups of mice (control and control+leptin) during gestation and lactation were allowed free access to the standard lab chow. The other two groups of mice (undernourished and undernourished+leptin) were given a half amount of chow as calculated from that of the control groups. According to previous studies [10-12], the mean nonfasting plasma leptin level of pregnant mice is 5 ng/ml at the beginning then increases in gradient to 50 ng/ml. A continuously exogenous leptin administration (10 mg/kg/d), via osmotic minipump implanted subcutaneous, was performed to elevated leptinemia of undernourished mice to the comparative range of control mice. Based on previous observations [10-12 and our unpublished data], this given

dose of leptin to normal mice did not markedly reduce their food intake. Pregnant mice produced 7 ± 2 pups per litter. Male pups from each group were weaned at 21 d of age and further separated into groups according to their original treatments. Each group contained at least 12 mice. All pups had free access to diet and deionized water. Food intake and body weight were recorded weekly.

An intraperitoneal glucose tolerance test was performed on mice at 6 month of age by using a dose of 1 g glucose per kg body weight. Glucose and insulin concentrations were measured by a blood glucose meter and the enzyme-linked immunoabsorbent assay, respectively. Fasting plasma leptin was also determined. The measurements of body fat content and plasma variables were performed by following the methods published elsewhere [13]. All data were presented as the mean \pm SD. Statistical analyses of the results were conducted by ANOVA and multiple comparison test by using a commercial package, StatWorks 1.2. The difference was considered to be significant when the P value was less than 0.05.

III. Results And Discussion

Although had higher plasma leptin levels, leptin-administered dams (5.8 and 2.9 g/d) had similar amount of food intake as their respective controls (6.1 and 3.0 g/d). The pups derived from undernourished dams had markedly lower body weights at birth than that of well-nourished dams (Table 1). Thereafter, they had increased food intake (data not shown), body weight and body fat accumulation. Although the body weight at adulthood did not differ among groups, mice derived from undernourished dams had significantly higher body fat contents.

Table 1. Body weight and body fat content of the pups at birth and 6 month of age

	Control	Under	Control+Leptin	Under+Leptin
Birth BW (g)	1.53 \pm 0.05 ^a	1.02 \pm 0.08 ^b	1.44 \pm 0.07 ^a	0.95 \pm 0.08 ^b
Adult BW (g)	27.8 \pm 1.9 ^a	26.7 \pm 2.3 ^a	27.3 \pm 2.0 ^a	26.4 \pm 2.2 ^a
Adult BF (%)	20.8 \pm 1.1 ^a	28.7 \pm 1.3 ^b	19.6 \pm 0.9 ^a	24.3 \pm 1.2 ^c

Mean \pm SD. Different superscript letters (a, b, c) in the same row indicates that there are significant difference (P<0.05).

At weaning period, plasma concentrations of glucose, insulin and leptin did not markedly differ among groups. However, mice derived from undernourished dams at adulthood had significantly higher plasma glucose, insulin and leptin levels than those of their age-matched controls (Table 2). In addition, these mice exhibited glucose intolerance (data not shown). In short, prenatal leptin repletion markedly attenuated body fat accumulation, hyperglycemia, hyperinsulinemia and hyperleptinemia of the mice derived from undernourished dams. However, leptin administration had no significant effect on the pups derived from well-nourished dams.

Table 2. Plasma glucose (mg/dL), insulin (ng/mL), and leptin (ng/mL) concentrations of male offspring at 21 d (W) and 6 month (A) of age

	Control	Under	Control+Leptin	Under+Leptin
W Glucose	113 \pm 6 ^a	111 \pm 9 ^a	121 \pm 8 ^a	117 \pm 6 ^a
W Insulin	1.4 \pm 0.3 ^a	1.6 \pm 0.5 ^a	1.5 \pm 0.4 ^a	1.6 \pm 0.4 ^a
W Leptin	6.9 \pm 0.8 ^a	6.2 \pm 1.0 ^a	6.6 \pm 0.7 ^a	6.5 \pm 0.8 ^a
A Glucose	120 \pm 10 ^a	145 \pm 10 ^b	119 \pm 9 ^a	133 \pm 8 ^c
A Insulin	1.5 \pm 0.4 ^a	2.8 \pm 0.6 ^b	1.3 \pm 0.5 ^a	2.2 \pm 0.3 ^c
A Leptin	7.2 \pm 0.9 ^a	15.3 \pm 0.9 ^b	7.8 \pm 0.6 ^a	13.7 \pm 0.6 ^c

Mean \pm SD. Different superscript letters (a, b, c) in the same row indicates that there are significant difference (P<0.05).

Both in rodent and human studies, prenatal exposure to various hormonal factors, such as cytokines and glucocorticoid, have been shown to be followed by permanent metabolic abnormalities during adulthood [1-4]. Exposure to famine during early gestation did not affect birth size, but resulted in low birth weight and instead correlated with obesity and resulted insulin resistance, as well as other features of the metabolic syndrome in adulthood [2-4]. Leptin is an important regulator of energy balance through the various effects it has on food intake and energy expenditure [5]. For example, it can inhibit the activation of HPA axis at hypothalamic and peripheral levels. Leptin has also been found to suppress insulin secretion and gene expression in pancreatic islets [6]. Since insulin is an adipogenic factor and leptin stimulant, a feedback loop between fat tissue and pancreas has thus been considered as the adipoinsular axis [1-3]. According to this hypothesis, insulin stimulates adipogenesis and leptin secretion whilst leptin inhibits insulin production. As body fat accumulated, hyperleptinemia reduces insulinemia and directing less energy to the formation of fat tissue. On the other hand, when fat tissue became depleted, hypoleptinemia permits increased insulin secretion and resulted in the deposition of additional energy. In this study, multiple leptin administrations during gestation were used to effectively expose the fetus to exogenous leptin repletion and to cover the sensitive period when brain development is most predominant in the mouse fetus [10-12].

By using the paradigm of fetal programming, some investigators have developed a rodent model that displays the phenotype of obesity and metabolic abnormalities commonly seen in humans [1-3]. These investigators apply maternal undernourishment throughout gestation, generating a nutrient-deprived intrauterine environment to induce fetal programming [2-4]. Maternal undernourishment results in fetal growth retardation and markedly reduced body weight at birth. Programmed offspring develop obesity, hyperinsulinemia, and hyperleptinemia during adulthood, and postnatal hypercaloric nutrition amplifies the metabolic abnormalities induced by fetal programming.

In this study, the birth weights of pups from undernourished dams were markedly lower than that from normal controls. Since all pups were fed on a standard lab chow, programmed offspring showed apparent catch-up in body weight gain when they reached at 6 month of age. Prenatal leptin administration did not influence the values of body weight at birth and at adulthood. The elevated fat accumulation in mice derived from undernourished dams indicated a positive energy balance (increased food intake and decreased energy expenditure) had been established. In addition, prenatal leptin repletion markedly attenuated body fat accumulation and hyperglycemia which caused by fetal programming.

Both hyperinsulinemia and hyperleptinemia were found in the adult mice which were derived from undernourished dams. In these mice, euglycemia was observed at weaning period but not at adulthood. In addition, prenatal leptin administration significantly reduced hyperinsulinemia and hyperleptinemia of these programming mice. However, this result also suggested that the elevation in plasma insulin was not sufficient to maintain normal glycemia control. Fetal programming's metabolic abnormalities might be attributed to both leptin resistance and insulin resistance. Our results showed that an undernourished intrauterine environment could cause permanent alterations of the adipoinular axis and leading to increased adiposity, as well as hyperleptinemia and hyperinsulinemia. However, this study still cannot answer the predominant defect is in fat accumulation, leptin response or insulin activity. Nevertheless, the appearance of leptin and/or insulin resistance in the offspring from undernourished dams might be a mechanism induced by the expression of a nutrient-deprived intrauterine environment to store large amount of fats when food supply is plentiful. Thus, concomitant hyperinsulinemia and hyperleptinemia would create a competitive advantage in preparation for a nutrient-deprived environment to store as much as fat as possible when food becomes available. However, hyperinsulinemia and hyperleptinemia would lead to metabolic syndrome like obese diabetes. Interestingly, a recent study has shown that micronutrient zinc could influence plasma leptin concentration by modulating leptin gene expression during pregnancy [14]. Zinc nutrition during pregnancy is known to be critical for embryo survival and fetal development. Our previous works have clearly indicated that zinc participates in mediating leptin production [15]. Nevertheless, to further elucidate the regulatory mechanisms as well as the role that dietary zinc deficiency might play, additional studies will be required.

IV. Conclusion

Leptin administration significantly reduced body fat accumulation, hyperglycemia, hyperinsulinemia, and leptinemia of the adult mice that were derived from undernourished dams. Our data suggest that fetal-programmed abnormalities were partly attributed to leptin resistance. In addition, exogenous leptin administration might be beneficial to undernourishment induced metabolic defects.

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Declaration of interest

No conflict of interest.

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