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Maternal High-Fat-Diet Leads to Intrauterine Growth Restriction and the Development of Type 2 Diabetes

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Abstract: Intrauterine Growth Retardation (IUGR) has been linked to the development of type 2 diabetes. In this study, researchers developed two models of IUGR, one is maternal malnourishment and the other one is maternal high cholesterol environment. In both models, researchers measured maternal body weights, offspring body weights and offspring body lengths. To further assess whether these two models develop type 2 diabetes, Glycosylated Serum Protein (GSP) concentrations and fasting insulin levels were examined once a month. Body weights and body lengths of IUGR animals induced by high fat diet group were significantly lower than those of controls. Moreover, both two models of IUGR rats showed hyperglycemia and high serum insulin concentration indicating rats in these two models possess the potential of forming type 2 diabetes. Comparison of these two models demonstrated that hypercholesterol intake induced more severe hyperglycemia and serum insulin levels. Furthermore, rats in high fat diet group showed significant lower body weight and body length compared with controls. In conclusion, maternal high cholesterol food could induce intrauterine growth retardation and develop type 2 diabetes.

Key words: IUGR, offspring, hyperglycemia, type 2 diabetes, growth retardation

INTRODUCTION

Intrauterine Growth Retardation (IUGR) is a common complication of pregnancy and a significant cause of prenatal morbidity and mortality. Perturbations in the intrauterine environment resulting in poor fetal growth increase the susceptibility for age-related diseases, particularly Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease. Barker *et al.* (1993) and Barker and Osmond (1986) first proposed the thrifty phenotype hypothesis that an adverse intrauterine environment could induce disease in later life. His hypothesis postulates that the lack of adequate nutrients in the intrauterine environment programs the offspring for survival in a nutrient-poor world. It follows that if the actual postnatal environment is not nutrient poor but instead nutrient rich, metabolic pathways will have been mal-programmed, leading to adult-onset metabolic syndrome diseases including atherosclerosis and diabetes. Therefore, in his hypothesis, low-birth weight infants were at increased risk for developing obesity, hypertension and type 2 diabetes. Moreover, a great deal of subsequent studies provides sufficient support to Barker's hypothesis (Ravelli *et al*., 1976; Valdez *et al*., 1994; Hales *et al*., 1991; Rich-Edwards *et al*., 1999).

Many types of maternal stresses in different animal models have been used to produce Intrauterine Growth Restriction (IUGR) (Armitage *et al*., 2004). Earlier research using the rat model of maternal protein restriction has shown that it results in offspring with IUGR, low muscle mass (Toscano *et al*., 2008), adult-onset glucose intolerance (Ozanne and Hales, 1999), hypertension (Langley-Evans *et al*., 1994; Petry *et al*., 1997) and early aging (Aihie Sayer *et al*., 2001; Martin-Gronert *et al*., 2008). Therefore, in the current study, researchers developed an animal model of IGUR by applying low protein diet (protein restriction diet) in pregnant rat and followed by giving high fat diet to infants after birth. In this model, researchers further explored the body weight increase of maternal rats, length and weight of the new born rats. To further explore whether the model causes diabetes, glycosylated serum protein levels and serum insulin levels were measured.

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MATERIALS AND METHODS

Animal husbandry: This study was approved by the Animal Research Committee of Sichuan University and was performed in accordance with the guidelines for the use of experimental animals, Experimental Animal Center of Sichuan University Huaxi Medical Center. Programmed mated female Sprague-Dawley (SD) rats were purchased from Sichuan University. Virgin rats (225-250 g) were single coupled for 12 h and those with expelled vaginal plugs and four non-gestating rats were selected and sent to the research center. After arrival, the animals were caged individually in a room at 23°C with 55% relative humidity and a 12:12 h light-dark cycle. Animals had free access to water.

Diets: The low-protein diet contained 9% protein by weight was isocaloric and was formulated to match low-protein diets published earlier (Snoeck *et al*., 1990). In the low-protein diet, fat content was 4.4% and carbohydrates were 77% by weight. The control protein diet (standard chow diet) was used to feed control rats. The control protein diet contained 19% protein, 6.2% fat and 75% carbohydrates by weight and contributed 18% kcal from fat. The high fat diet contained 42% kcal calories from fat and by weight as follows: 21% fat, 17% protein, 49% carbohydrates and 0.2% cholesterol.

Animal groups and study design: On postcoupling day 10, animals were randomly assigned to one of the three study groups: Food Restriction control, Normal Diet control and High Fat diet (FR, ND and HF, respectively, n = 7 or 8) so that body weights and body fat contents were similar. Rats in ND group were given normal diet during the gestation period. The food intake of animals in group FR and HF was restricted to 50% intake of non-gestating rats of similar body weight during last 11 days of gestation. After the gestation period, maternal rats in HF group were given high fat diet while maternal rats in FR group were given normal diet as ND control rats. All the new born rats in each group were given the diet as their maternal rats.

Measurement of length and body weight of maternal rats: The length of all maternal rats (including tail) was measured manually every 3 days and accurate to 0.1 cm. The body weight of all maternal rats was assessed by electronic balanced every 3 days.

Measurement of length and body weights of new born rats: The length of all new born rats (including tail) was measured manually every 4 days and accurate to 0.1 cm. The body weight of all new born rats was assessed by electronic balance every 4 days.

Biochemical assays: Glycosylated Serum Protein (GSP) concentrations were determined in duplicate using GSP ELISA kit purchased from Yuanye Biotechnology Company, Shanghai, China. Plasma insulin concentrations were measured in duplicate by radioimmunoassay using rat insulin as the standard. The within and between-assay coefficients of variation for the insulin assay were 4 and 10%, respectively.

Statistical analysis: Data were expressed as mean±SEM. Statistical analyses were performed using one-way ANOVA with Bonferroni correction and Student's unpaired t-test. The $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Body weights of food-restricted rat model, high fat diet rat model and control rats were analyzed: In utero, growth restriction has been associated with malnourishment during pregnancy, leading to the adult-onset metabolic disorders. To develop a mouse model of IUGR, researchers randomly divided rats in three groups and for the FR group, researchers fed SD females half dose of food diet, named food restriction, at the beginning on day 0 of gestation. Meanwhile, rats in HF group, researchers also fed those pregnant maternal rats half dose of food diet from day 0-6 of gestation. Then, after 6 days of gestation, rats in HF group were given high fat diet. Researchers measured body weights of all rats every 3 days. Figure 1 shows body weight after gestation. Rats fed with normal diet showed significant increased body weight during the gestation period. However, for the maternal-intervention groups (food restriction group and high fat diet group) led to significantly lower body weights than those in the normal diet group. Especially during the late stage of pregnancy (from day 12-18), there was no significant increase of body weights of rats in food restriction group and their body weights were significantly lowered than those of high fat diet group and those of normal diet group ($p<0.05$). Meanwhile, maternal rats when given high fat diet food, showed significant increase of body weight (day 7-18) however their body weights were significant lowered than those of rats in normal diet group ($p<0.05$). Therefore, food restriction could successfully induce decreased body weight during gestation. Although, high fat diet could induce the

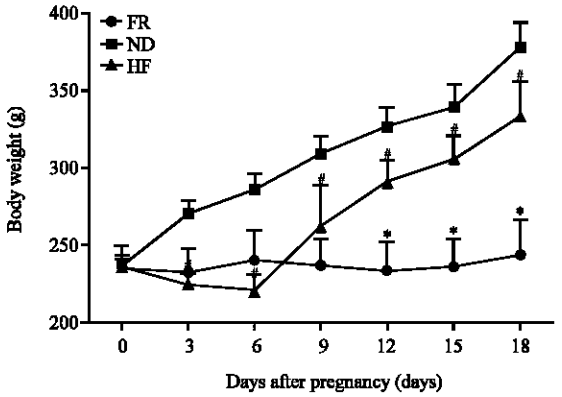

Fig. 1: Body weights of maternal rats after pregnancy were measured every 3 days. Rats in FR group were given restriction food as food control. Rats in ND were given normal diet after pregnancy as normal control and rats in HF group were given restriction food from day 0-6 and then given high fat diet during last 11 days of gestation (from day 7-18). Data are presented as mean value±SEM; n = 10-12 for each group (*$p<0.05$ for comparison of all other groups; #$p<0.05$ for comparison of ND group)

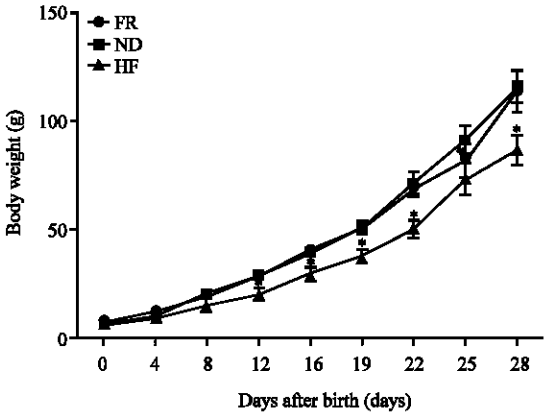

Fig. 2: Body weights of new born rats were measured at birth and during the suckling period. Body weights were measured every 4 days in first 2 weeks and every 3 days in the last 2 weeks of suckling period. Maternal rats in FR group and ND group were given normal diet after delivering new born rats while maternal rats in HF group were still given high fat diet after giving birth of new rats. Data are presented as mean value±SEM; n = 10-12 for each group (*$p<0.05$ for comparison of all other groups)

elevation of body weights during middle and late stages of pregnancy, the increased body weights were still significantly lower than the normal diet controls ($p<0.05$).

Body weights at birth and during the suckling period were analyzed: To further analysis whether new born rats were IUGR Models, researchers measured body weights of new born rats every 4 days in first 2 weeks and every 3 days in the last 2 weeks of suckling period. Figure 2 shows body weight at birth. New born rats in all three groups of rats possess similar body weights ($p>0.05$ at birth) and all groups of rats showed increased body weights with growth time. However, only one of the maternal intervention groups which is high fat diet group, led to significantly lower body weight than those in the normal diet control group after 12 days of birth. Therefore, high fat diet female offspring showed significant growth restriction compared with controls until they were killed at 4 weeks of age. Interestingly, relative to the control group, rats in food restriction group possess similar body weight, indicating these new born rats were accustomed to food restriction environment.

Body lengths at birth and during the suckling period were analyzed: As body length will increase with the growth of new born rats, researchers measured body lengths of new born rats every 4 days in 1st 2 weeks and every 3 days in the last 2 weeks of suckling period. All groups of rats exhibited similar body length at their birth day ($p>0.05$) and all groups of rats showed increased body lengths with growth time. However, after 12 days of growth, new born rats in high fat diet group showed significant lower body lengths than those of normal diet group and those of food restriction group. Interestingly, relative to the normal diet control group, rats in food restriction group possess similar body length indicating these new born rats were accustomed to food restriction environment (Fig. 3).

Glucose homeostasis of new born rats was analyzed: To further explore whether malnourishment during pregnancy would lead to glucose homeostasis disorder in the offspring, researchers measured Glycosylated Serum Proteins (GSPs) concentrations. Beisswenger *et al.* (1993) suggested that glycosylated serum proteins may be more sensitive than HbA1c assay to the greater fluctuations in blood glucose levels generally associated with IDDM. Therefore, in this study, glycosylated serum proteins concentrations were measured every month. During the suckling period, the offspring of maternal rats who were fed normal diet showed comparatively lower glycosylated serum proteins concentrations than those whose maternal rats were fed high fat diet ($p<0.0001$) indicating that those new born rats whose female rats were fed high fat diet developed mild fasting hyperglycemia (Fig. 4). To further

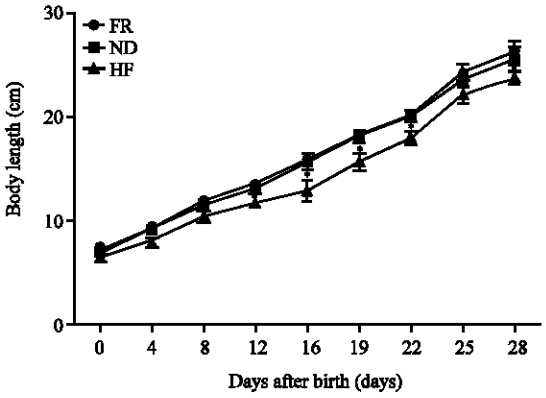

Fig. 3: Body lengths of new born rats were measured at birth and during the suckling period. Body length was measured every 4 days in first 2 weeks and every 3 days in the last 2 weeks of suckling period. Data are presented as mean value±SEM; n = 10-12 for each group (*p<0.05 for comparison of all other groups)

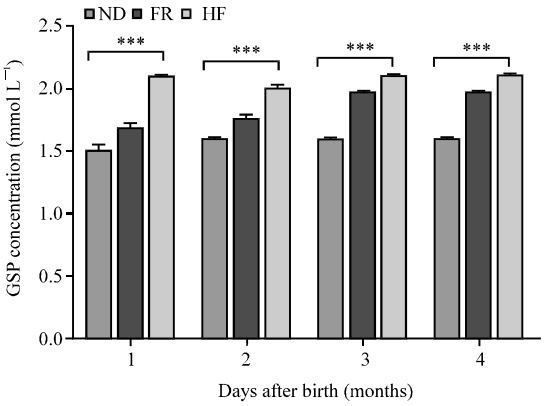

Fig. 4: Glycosylated serum proteins concentrations were measured every month until sacrificed at the 4th month. Data are presented as mean value±SEM; n = 10-12 for each group (***p<0.0001 for comparison of normal diet groups)

investigate whether high fat diet would induce the diabetic mellitus of new born rats and to study whether this hyperglycemia would be progressively worsened as the animals aged, researchers measured the glycosylated serum proteins concentration for the consecutive 4 months (including suckling period). After suckling period, new born rats in ND group were given normal diet, new born rats in FR group were also given food restriction diet and new born rats in HF group were given high fat diet too. In this study, researchers observed comparatively high glycosylated serum proteins levels of new born rats in HF group in the next 3 months after suckling period (2.09±0.03 mmol L^{-1} for suckling period, 1.99±0.04 mmol L^{-1} for the 2nd month, 2.0±0.03 mmol L^{-1} for the 3rd month and 2.09±0.03 mmol L^{-1} for the 4th month).

As increased fasting insulin concentration would lead to insulin resistance which is a hallmark of type 2 diabetes, researchers next assessed fasting insulin levels of 3 groups of rats in the consecutive 4 months after birth. Insulin levels of all groups of rats showed increase tendency with the growth time. However, during and after the suckling period, rats in HF group showed significantly increased serum insulin levels compared with those of rats in ND group ($p<0.0001$) indicating that rats in HF group possess great potential of insulin resistance and tendency of forming type 2 diabetes. Interesting, although rats in FR group did not show significantly increased serum insulin levels during the suckling period ($p>0.05$), their serum insulin levels were significantly higher than those of normal controls ($p<0.01$ for the 2nd month and $p<0.05$ for the 3rd and 4th month). Taken together, these data indicate that high fat diet induces increased glycosylated serum proteins levels and increased fasting insulin levels in the new born rats (Fig. 5).

Nutrient insufficiency limits the availability of substrates to the fetus and retards growth during gestation. Several lines of evidence suggest that it may also have permanent consequences after birth. Epidemiological studies have revealed strong statistical links between poor fetal growth and the subsequent development of type 2 diabetes in adulthood (Barker and Osmond, 1986; Barker *et al*., 1993; Ravelli *et al*., 1976; Valdez *et al*., 1994; Hales *et al*., 1991; Rich-Edwards *et al*., 1999). In this study, researchers observed that in malnourishment environment, maternal rats showed significantly lowered body weight compared with the controls and their body weight even could not showed increase tendency during their pregnancy period indicating that malnourishment obviously retard the growth of those female rats during gestation.

Although, the new born offspring of those mal-nutrient female rats did not show significant growth inhibition from their body weight and body length perspective, their glycosylated serum proteins levels were significantly increased which showed that these rats were hyperglycemia. Meanwhile, assessment of fasting insulin levels also indicates that the offspring grown up in malnourishment environment possess the potential of developing type 2 diabetes. Therefore, these data from food restriction animal models of intrauterine growth retardation induced by malnutrition support the concept that poor fetal growth has permanent consequences in adulthood. These observations are in accordance with

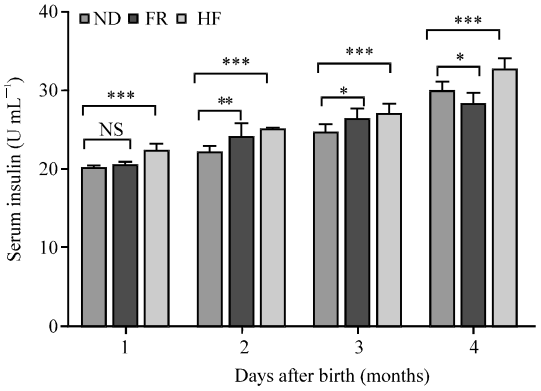

Fig. 5: Fasting insulin concentrations were measured every month until sacrificed at the 4th month. Data are presented as mean value±SEM; n = 10-12 for each group (*p<0.05 for comparison of ND group; **p<0.01 for comparison of ND group; ***p<0.0001 for comparison of ND group; NS: No Significant difference)

Barker's hypothesis. And the observation of malnourishment induced type 2 diabetes has been made in a large number of populations worldwide. Barker, Osmund and Hales conducted some of the earliest studies in England that explored the association between neonatal anthropometrics and later development of glucose intolerance and diabetes in individuals aged 50-64 years (Barker and Osmond, 1986; Barker *et al*., 1993). They described a significant correlation of decreased birth weight with impaired glucose tolerance, hypertension and diabetes independent of adult body mass (Valdez *et al*., 1994).

Although, maternal malnourishment could successfully induce IUGR Models, herein we proposed a new model of IUGR which are induced by high fat diet during the gestation period. To test the hypothesis that high cholesterol in high fat diet may participate in the development of IUGR and associated metabolic syndrome traits in adulthood, researchers developed and characterized two mouse models for IUGR: one of food restriction and one of high cholesterol by high fat diet. As discussed above, the food restriction model mimicked the well-established rat IUGR Model under malnourishment environment and complemented a previously described food-restricted IUGR rat model characterized for hyperglycemia by increased glycosylated serum proteins levels and insulin resistance by increased fasting insulin levels. The basis of type 2 diabetes in humans is incompletely understood. However, insulin resistance and hyperglycemia are the two predominant and characteristic features. In the study, the IUGR offspring of high fat diet group became glucose intolerant with increased glycosylated serum proteins levels and insulin resistance by increased fasting insulin levels compared with controls. Meanwhile, body weight and body length of those new born offspring in high fat diet group were greatly inhibited compared with controls indicating that new born rats in HF group did not catch up with controls in weight and length.

Earlier studies have observed a correlation between high maternal cholesterol concentrations and enhanced lesions of atherosclerosis in the offspring in rabbits (Napoli *et al*., 2000; Palinski and Napoli, 2002) and in $LDLR^{-/-}$ mice (Napoli *et al*., 2002). Studies on high maternal fat intake and increased tendency of type 2 diabetes were firstly reported. Herein, researchers firstly reported that a high fat diet fed to the pregnant animal throughout gestation, lactation and even suckling period of the new born rats induces severe growth retardation in the offspring. The growth retardation could be explained by both body weight and body length of the new born rats. This model of fetal growth retardation has many advantages over other animal models: severe growth inhibition from body weight and body length perspective; IUGR rats by high fat diet easy to develop diabetes with a phenotype remarkably similar to that observed in humans with type 2 diabetes as hyperglycemia and progressive insulin resistance; most importantly, high fat diet model is easy to develop IUGR and type 2 diabetes. Therefore, the high fat diet induced IUGR rat represents one of the best experimental tools for studying the impact of high cholesterol during gestation of female rats and growth period of new rats on the evolution of diabetes. It is becoming increasingly apparent that embryonic and fetal cells have a complex system integrating nutritional signals from their environment to maximize the potential for survival. The association of maternal malnutrition or maternal high cholesterol and IUGR leading to adult-onset metabolic disorders such as obesity, type 2 diabetes and atherosclerosis has been demonstrated in several epidemiological studies (Barker and Osmond, 1986; Bhargava *et al*., 2004; Mi *et al*., 2000; Poston, 2001). The results mimicked population studies in humans where maternal malnutrition/high cholesterol and the resultant low birth weight were identified as the common risk factors for adult-onset diseases. In addition, the studies demonstrated that maternal protein restriction and hypercholesterolemia were both associated with hyperglycemia and insulin resistance in offspring. Therefore, maternal protein restriction and hypercholesterolemia may be an important antecedent in both models of IUGR and may be an important link in the mechanisms that contribute to adult-onset glucose intolerance.

CONCLUSION

The results indicate that maternal protein restriction and hypercholesterolemia lead to the progressive development of a type 2 diabetic phenotype in IUGR rats. These animals exhibit mild hyperglycemia in enhanced serum insulin concentration very early in life (suckling period) and continued to maintain hyperglycemia and higher insulin levels for 4 months. The data presented here support the hypothesis that both maternal malnourishment and maternal hyper-cholesterol environment can induce permanent changes in glucose homeostasis after birth. Moreover, maternal high cholesterol food could induce more severe hyperglycemia and insulin resistance than maternal protein restriction. The fetus in malnourishment adapts to an altered environment in utero that may enhance its short-term survival probability at the suckling period but ultimately form changed glucose homeostasis and insulin adoption. It could be explained that the cells are reprogrammed to enable the fetus as a whole to survive under conditions of nutrient deprivation or hyper-cholesterol environment. However, this reprogramming persists throughout life, leading to the development of type 2 diabetes in adulthood. Although, the direct causality and subsequent physiologic consequences of the high cholesterol during gestation and suckling period induced IUGR and type 2 diabetes remain unclear, this new model of IUGR provides a Novel Model and means of gaining insights into the pathogenesis of type 2 diabetes.

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