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• *BRIEF REPORTS* •

Effect of early nutrition on intestine development of intrauterine growth retardation in rats and its correlation to leptin

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Abstract

AIM: To investigate the intestine and body development of intrauterine growth retardation (IUGR) rats under early different protein diet and to analyze the correlation between leptin and intestine and body development.

METHODS: An IUGR rat model was established by food restriction of pregnant female rats. Fifty-six neonatal IUGR rats and 24 neonatal normal rats were randomly divided into normal control group (C group), IUGR model group (SC group), low protein diet IUGR group (SL group), and high protein diet IUGR group (SH group). Eight rats were killed per group at wk 0, 4, and 12. Serum leptin, body weight (BW), body length (BL), intestinal weight (IW), intestinal length (IL), and intestinal disaccharidase (including lactase, maltase, and saccharase) were detected.

RESULTS: BW (4.50±0.41 g), BL (5.96±0.40 cm), IW (0.05±0.01 g), and IL (15.9±2.8 cm) in neonatal IUGR rats were much lower than those in C group (6.01±0.55 g, 6.26±0.44 cm, 0.10±0.02 g, 21.8±2.7 cm, $P<0.05$), while intestinal lactase and maltase activities were higher than those in C group. SH group showed the fastest catch up growth and their BW, BL, IW, and IL reached the C group level at wk 4. SC group showed relatively slower catch up growth than SH group, and their BW, BL, IW did not reach the C group level at wk 4. SL group did not show intestine and body catch up growth. Intestinal maltase [344±33 μmol/(min·g)] and saccharase activities [138±32 μmol/(min·g)] in SL group were both markedly lower than those in C group [751±102, 258±27 μmol/(min·g), $P<0.05$]. There were no significant differences in lactase activities at wk 4 and disaccharidase activities at wk 12 among all groups ($P>0.05$). The leptin level in SL group (0.58±0.12 ng/mL) was the highest in all groups, and much lower in SH group (0.21±0.03 ng/mL) than that in any other IUGR groups at wk 4 ($P<0.05$). Leptin was negatively related to BW ($r = -0.556$, $P = 0.001$), IW ($r = -0.692$, $P = 0.001$) and IL ($r = -0.738$, $P = 0.000$) at wk 4, while no correlation was found at wk 12.

CONCLUSION: High protein diet is a reasonable early nutritional mode to IUGR rats in promoting intestine and body catch up growth.

Key words: Intrauterine growth retardation; Rat; Intestine development; Disaccharidase; Leptin; Nutritional intervention

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INTRODUCTION

The incidence rate of intrauterine growth retardation (IUGR) is about 7.5-8.7% in our country. Development of the stomach and intestine is closely related to the body growth, it affects and is also reversely affected by nutrition. As a protein coded by ob gene (obesity gene), leptin is a neuroendocrine regulatory factor secreted from mature adipocytes into blood. Leptin can pass through blood-brain barrier and has a central effect on feeding behavior. It was reported[1] that IUGR rats have temporarily high leptin level to regulate growth hormone secretion and growth during catch up growth. Since the infant growth is mainly regulated by nutrition, and nutritional intake is controlled by the development of stomach and intestine and leptin level, our study aimed to investigate the intestine, intestinal disaccharidase and body development and their relation to serum leptin at wk 4 (childhood) and 12 (adulthood) in order to offer some animal research data for early nutritional intervention of IUGR.

MATERIALS AND METHODS

Materials and animals

Leptin kit was purchased from Diagnostic Systems Laboratories (USA). Glucose and disaccharidase enzyme kits were purchased from Great Wall Company (Baoding, Hebei Province, China).

The second class SD female rats were bought from Animal Center of Sun Yat-Sen University and mated with

male rats. IUGR model was established by food restriction of pregnant female rats[2]. The standard of IUGR was that the birth weight -2SD lower than control normal group (5.1 g). Fifty-six newborn IUGR rats and 24 newborn normal rats were randomly divided into normal control group (C group), IUGR model group (SC group), low protein diet IUGR group (SL group), and high protein diet IUGR group (SH group). The former two groups were fed with 22.5% normal protein diet, while the latter two groups with 11.3% low-protein diet and 28.6% high-protein diet respectively. All the rats were weaned when they were 3 wk old and fed with original diet till the 4th wk and then with normal protein diet till the end of the experiment. Components of the diets are shown in Table 1.

Table 1 Content ratio of different components (g) in 100 g food

Components	Protein	Fat	Carbohydrate	Total energy (kJ/100 g)
Normal diet	22.5	3.9	57.8	1 583.4
High protein diet	28.6	3.9	56.8	1 625.4
Low protein diet	11.3	8.5	58.7	1 558.2
χ^2	10.09	3.04	0.09	–
P	0.006[a]	0.22	0.96	–

[a]*P*<0.05 *vs* other.

Methods

Body weight (BW) and body length (BL, from nose to tail) of each rat were measured at wk 0, 4, and 12. Eight newborn female rats of IUGR group and C group were killed right after birth. Another eight female rats were killed per group after being fasted for 10-12 h at wk 4 and 12 respectively. Blood sample was taken from the eyeballs and kept at -30 ℃. Serum leptin was monitored by ELISA.

Abdomen was opened right after the rats were killed. Intestinal weight (IW) and intestinal length (IL) were measured from the beginning of jejunum to the end of ileum, and then 10 cm proximal end of the jejunum (about 3 cm from the beginning of jejunum) was cut, weighed, and homogenized. The disaccharidase activities (including lactase, saccharase, and maltase) were measured.

Statistical analysis

Results were expressed as mean±SD. All data were analyzed by the SPSS 10.0 statistical package. ANOVA test was used to compare these four groups. Least significant difference was used to compare every two groups when variance was regular, while Dunnett T3 test was used when variance was irregular. Pearson's test was used in correlation analysis.

RESULTS

Development of body and intestine and disaccharide activity in newborn IUGR rats and normal rats

The BW, BL, IW, and IL of newborn IUGR rats were significantly lower than those of C group ($P<0.05$), while lactase and maltase activities were higher than those in C group. There was no significant difference in saccharase activity between the two groups (Table 2).

Development of intestine and body of rats

SH group showed the fastest catch up growth, and BW, BL, IW, and IL reached the C group level at wk 4 ($P>0.05$). BW, BL, IW, and IL in SL group at wk 4 and 12 were all markedly lower than those in normal control group ($P<0.05$). BW, BL, and IW in SC group at wk 4 were all significantly lower than those in C group ($P<0.05$). IL in SC group was lower than that in C group at wk 12. There were no significant differences in BW, BL, and IW between the two groups ($P>0.05$).

Intestinal maltase and saccharase activities in SL group were markedly lower than those in C group ($P<0.05$). There was no significant difference in lactase activity between the two groups at wk 4 ($P>0.05$). Maltase activity in SH group was lower than that in C group ($P<0.05$). There were no significant differences in disaccharidase activity between groups at wk 12 ($P>0.05$).

Leptin in SL group was significantly higher than that in SC and C groups, and lower in SH group than that in SC group at wk 4 ($P<0.05$). Leptin in SC group was higher than that in C group and lower in SH and SL group than that in SC group ($P<0.05$). There were no significant differences between these two groups and C group at wk 12 ($P>0.05$). Results are shown in Table 2.

Correlation analysis

The following results were found in correlation analysis of leptin and BW, BL, IW, and IL. Leptin and BW had a negative correlation at wk 4 ($r = -0.556$, $P = 0.001$), while

Table 2 Leptin, BW, BL, IW, IL, and disaccharidase activities in all groups (mean±SD) *n* = 8/group

Age (yr)	Group	Leptin (ng/mL)	BW (g)	BL (cm)	IW (g)	IL (cm)	Lactase	Saccharase	Maltase
0 wk	C	ND	6.01±0.55	6.26±0.44	0.10±0.02	21.8±2.7	315±19	13±6	58±20
	SC	ND	4.50±0.41[a]	5.96±0.40[a]	0.05±0.01[a]	15.9±2.8[a]	383±39[a]	15±8	269±17[a]
4th wk	C	0.26±0.08	60.8±9.5	23.1±1.4	2.2±0.2	74.8±9.1	54±15	258±27	751±102
	SC	0.36±0.20	52.0±10.9[a]	21.0±2.5[a]	1.4±0.3[a]	70.3±3.4	39±14	254±23	797±95
	SH	0.21±0.03[c]	70.0±4.5[c]	23.6±0.5[c]	1.9±0.2	80.7±9.5	62±8	230±21	368±26[a,c]
	SL	0.58±0.12[a,c]	21.4±3.5[a,c]	16.0±1.3[a,c]	0.8±0.4[a,c]	53.9±3.1[a,c]	66±22	138±32[a,c]	344±33[a,c]
12th wk	C	0.40±0.23	235.5±43.4	38.5±0.8	4.3±0.7	122.7±12.6	48±3	230±32	713±158
	SC	0.79±0.41[a]	208.6±21.6	36.9±1.7	3.9±0.9	101.2±5.8[a]	50±21	262±43	670±157
	SH	0.42±0.15[c]	254.8±23.5[a,c]	37.1±0.4	4.1±0.4	122.3±13.0	48±12	242±27	682±144
	SL	0.38±0.25[c]	169.3±6.7[a,c]	33.2±0.8[a,c]	3.3±0.6[a]	99.6±6.9[a]	53±12	239±35	568±52

Disaccharidase activity unit: μmol/(min·g) protein. [a]*P*<0.05, IUGR group *vs* C group; [c]*P*<0.05, SL, and SH groups *vs* SC group. ND: not determined.

had no correlation at wk 12; leptin and BL had no correlation at wk 4 and 12; leptin and IW had a negative correlation at wk 4 (r = -0.692, P = 0.001), while had no correlation at wk 12; leptin and IL had a negative correlation at wk 4 (r = -0.738, P = 0.001), while had no correlation at wk 12.

DISCUSSION

Multiple lines of evidence show that about 20-50% IUGR cannot catch up the growth after birth, and remains small till they become adults. Growth is mainly regulated by heredity, nutrition, and endocrine system. The early postnatal growth (the first year after birth) is mainly regulated by nutrition. Mother malnutrition during pregnancy not only delays the fetal growth, but also impairs many organ and tissue structures and functions of the fetus[3]. Zhang *et al*.[2], showed that the weight, length and many morphologic structures of intestine in newborn IUGR rats are significantly lower than those of normal newborn rats. Only few reports about the development of stomach and intestine and disaccharidase activity of IUGR are available at present.

Due to the effect of early nutrition on growth, Lucas[4] have raised the "nutritional programming" hypothesis: nutritional conditions during the critical or sensitive stage of growth have long-term or life-long effects on organic structure or function. The mechanism is that early nutrition stimulates clone selection and differentiation of blast cells and causes irreversible changes in cell number of organism. Based on the hypothesis, we carried out the early nutritional intervention during the first 4 wk after birth -a sensitive and critical stage of growth. We investigated its effects on the development of intestine and jejunum mucosa disaccharidase activity and their correlations with serum leptin.

Our research showed that the impaired development of intestine recovered quickly in SH group after high protein diet was given after birth. Ziegler *et al*.[5], found that the development of intestine mainly depends on the proliferation of cells before weaning. Enough nutrition can not only promote intestinal neuron's activity and increase blood supply of viscera but also stimulate the secretion of growth factor. Intestinal epithelium can absorb more nutrition for catch up growth. Although SC group was fed with normal diet after birth, it failed to catch up the normal control group at wk 4. SL group even failed to catch up normal growth at wk 12 and remained small till they became adults, suggesting that early postnatal protein diet has great effects on the development of intestine. Intestine and body catch up growth of IUGR rats depends on sufficient nutrition after birth, especially enough protein.

Intestine is the main organ to absorb sugar and protein. Disaccharidase, gastrin, and intestinal hormones play an important role in this process. Lactase and maltase are often produced at the late stage of gestation, while saccharase activity increases quickly only after intake of solid food. Stomach and intestine develops very fast in neonatal stage. As the stomach and intestine of neonates become mature, their lactase activity decreases, while maltase and saccharase activities increase[6]. Therefore, it is beneficial to the understanding of the development of stomach and intestine by detecting disaccharidase activities. Our research suggested that the premature of lactase and maltase activity in newborn IUGR rats was an adaptive compensation to intrauterine malnutrition. Saccharase activity in newborn IUGR rats was not affected, suggesting that saccharase is not regulated by intrauterine nutrition. Our research also demonstrated that maltase and saccharase activities in SL groups were markedly lower than those in C and SC groups at wk 4, which is consistent with the study by Nichol *et al*.[7]. The relatively higher lactase activity in SL group may be an accommodation response to lack of nutritional supply. The nutrition of neonatal rats is mainly from milk and lactose is the main component of carbohydrate in milk. The high lactase activity is an advantage for neonatal rats to absorb more lactose to compensate for the shortage of protein. But the reason why maltase activity in SH group was markedly lower than that in C and SC groups at wk 4 is still unknown. Gomez *et al*.[8], gave high protein diet to the rats suffering from abdominal wound and found that its lactase activity is markedly higher than in other rats given normal level protein diet. Further research is needed to test whether the decrease of maltase activity in SH group is related to the increase of lactase activity. There was no significant difference in disaccharidase activity between groups at wk 12, suggesting that disaccharidase activity might change with different kinds of food after birth, such as milk, solid food, and different protein diet.

It was reported that IUGR rats are insensitive to leptin during catch up growth[9]. Jaquet *et al*.[10], found that IUGR children are relatively resistant to leptin in order to catch up growth and leptin is closely related to catch up growth and fat tissue development. We know that blood and umbilical leptin level of newborn baby is positively related to birth weight. Leptin reflects not only the body fat level but also nutritional status. Leptin is a medium between neuroendocrine system and fat tissue. It builds a negative loop between fat tissue and neuropeptide Y (NPY). When weight decreases to some extent, the inhibitory effect of leptin on the secretion of NPY from hypothalamus is also weakened[11]. NPY is a strong stimulator of appetite, high level of NPY is advantageous for IUGR children to take in more nutrition and energy to accumulate body fat.

Correlation analysis showed that leptin was negatively related to BW, IW, and length at wk 4. The BW in SL group was the lowest but leptin level was the highest in all groups at wk 4. We found that IUGR rats were relatively resistant to leptin during catch up growth, but this phenomenon disappeared at wk 12, suggesting that leptin resistance is only related to catch up growth in IUGR children.

In conclusion, high protein diet is a reasonable early nutritional mode for IUGR rats. High leptin level in IUGR rats is related to catch up growth and nutritional status.

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