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Maternal protein restriction affects gene expression and enzyme activity of intestinal disaccharidases in adult rat offspring

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Abstract

This study investigated the consequences of intrauterine protein restriction on the gastrointestinal tract and particularly on the gene expression and activity of intestinal disaccharidases in the adult offspring. Wistar rat dams were fed isocaloric diets containing 6% protein (restricted, n = 8) or 17% protein (control, n = 8) throughout gestation. Male offspring (n = 5-8 in each group) were evaluated at 3 or 16 weeks of age. Maternal protein restriction during pregnancy produced offspring with growth restriction from birth (5.7 ± 0.1 *vs* 6.3 ± 0.1 g; mean ± SE) to weaning (42.4 ± 1.3 *vs* 49.1 ± 1.6 g), although at 16 weeks of age their body weight was similar to control (421.7 ± 8.9 and 428.5 ± 8.5 g). Maternal protein restriction also increased lactase activity in the proximal (0.23 ± 0.02 *vs* 0.15 ± 0.02), medial (0.30 ± 0.06 *vs* 0.14 ± 0.01) and distal (0.43 ± 0.07 *vs* 0.07 ± 0.02 $U \cdot g^{-1} \cdot min^{-1}$) small intestine, and mRNA lactase abundance in the proximal intestine (7.96 ± 1.11 *vs* 2.38 ± 0.47 relative units) of 3-week-old offspring rats. In addition, maternal protein restriction increased sucrase activity (1.20 ± 0.02 *vs* 0.91 ± 0.02 $U \cdot g^{-1} \cdot min^{-1}$) and sucrase mRNA abundance (4.48 ± 0.51 *vs* 1.95 ± 0.17 relative units) in the duodenum of 16-week-old rats. In conclusion, the present study shows for the first time that intrauterine protein restriction affects gene expression of intestinal enzymes in offspring.

Key words: mRNA enzyme abundance; Maternal protein restriction; Disaccharidase activity; Fetal programming

Introduction

Recent studies have shown that nutritional changes during critical periods of prenatal development can lead to permanent alterations in the metabolism of the offspring, which can become more susceptible to chronic diseases (1). Disorders such as arterial hypertension, diabetes mellitus and obesity can be programmed during fetal life as a consequence of the maternal dietary regime during pregnancy (2-4). According to Hales and Barker (5), fetuses receiving insufficient nutrient levels in the intrauterine environment develop adaptive mechanisms to ensure survival, optimizing nutrient use and exhibiting a ‘‘thrifty’’ phenotype. After birth, however, individuals may develop physiological modifications if their nutrient offer is proportionally higher than that received during intrauterine life (6-8).

The effects of fetal programming on offspring metabolism and obesity are well known (9-11). However, the consequences regarding nutrient digestion, particularly at the molecular level, have yet to be studied. This information is required to understand food processing, the first step in nutrient metabolism (7).

Intestinal enzymes are partially responsible for food processing, and their activity can drastically change because of the high intestinal phenotypic flexibility (12). Disaccharidases are intestinal brush border enzymes that vary as a function of stimulus such as diet and animal age (13) and particularly protein malnutrition (14). These enzymes are directly involved in supplying energy to the organism and some of them, like sucrase, can be increased in obese individuals (15,16).

The short-term consequences of maternal protein restriction for the disaccharidases of weaned offspring include increased lactase activity (17,18). However, studies about the long-term effects of maternal dietary restriction during pregnancy on the intestinal enzymes of offspring are scarce, and results are contradictory. For instance, in these cases sucrase and maltase activity is decreased in offspring aged 2 and 4 months and markedly increased in 6-month-old rats (18).

The mechanisms underlying the control of enzyme activity remain to be elucidated. Molecular studies that

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assess the responses to maternal dietary restriction are not available, although the determination of mRNA levels of enzymes can provide information on enzyme synthesis. Enzyme synthesis and activity are crucial parameters from a developmental standpoint, but one does not necessarily reflect the other (19).

The present study investigated the effects of fetal programming, determined by gestational protein restriction, on gene expression and activity of the intestinal enzymes sucrose and maltase in the duodenum, jejunum and ileum of 3- and 16-week-old offspring rats and lactase in 3-week-old rats. Gastrointestinal organ weight was also determined.

Material and Methods

Animals

The male and female rats used to produce the offspring were provided by the central animal facility of Universidade Estadual Paulista, Botucatu, SP, Brazil. The procedures were previously approved by the Research Ethics Committee of Instituto de Biociências of the same institution.

Sexually mature Wistar rats were housed in maternity cages (33 × 45 × 17 cm) with 2 females to 1 male to allow mating. On the morning of the following day, females were inspected for the presence of sperm in their vaginal smear, which indicated the first gestational day (day 0). This protocol was repeated until the number of pregnant females in each group was adequate.

From day 0 to the day they gave birth, female rats were fed an isocaloric diet [400 kcal/100 g, AIN-93G (20), manufactured by Pragsoluções Biociências, Brazil; Table 1] with normal protein content (17% protein, control group, n = 8) or low-protein content (6% protein, protein-restricted group, n = 8). After giving birth, females were fed a standard rodent chow (Purina, Brazil).

Table 1. Composition of the diet (400 kcal/100 g) supplied to the pregnant rats (AIN-93G).

Ingredient	Normal protein (17% protein)	Low protein (6% protein)
Casein (84% protein)*	202.00	71.50
Cornstarch	397.00	480.00
Dextrin	130.50	159.00
Sucrose	100.00	121.00
Soybean oil	70.00	70.00
Fiber (microcellulose)	50.00	50.00
Mineral mixture**	35.00	35.00
Vitamin mixture**	10.00	10.00
L-cystine	3.00	1.00
Choline chloride	2.50	2.50

*Corrected according to protein content of casein. **According to AIN-93G (20).

Offspring groups

After birth, litters were reduced to 4 male and 4 female rats to ensure the same litter size per dam. Rats born to dams fed the low-protein (6% protein) and control diet (17% protein) were studied at 3 weeks (PR3 and C3 groups, respectively) or 16 weeks of age (PR16 and C16 groups, respectively). Each group comprised 8 male rats born to different dams. Female offpring rats were not included in this study, because we did not intend to investigate the differences between genders. After weaning (3 weeks), the animals were transferred to individual cages and fed the standard diet *ad libitum*. All rats were weighed at birth and at 3 and 16 weeks of age. Epididymal and visceral white adipose tissues were dissected and weighed at the end of the study and the adiposity index was calculated (the sum of fat depots divided by body weight x 100). All offspring were culled at the same time of day to prevent the influence of circadian rhythm on enzyme activity. During the experiments, dams and offspring rats were housed in a temperature-controlled environment (22°C) on a 12-h light/dark cycle with free access to food and water.

Gastrointestinal organs

At the age of 3 and 16 weeks, rats from each group were sacrified by decapitation. The abdominal cavity was then opened and the gastrointestinal organs removed, washed with saline solution and weighed.

Intestinal enzymes

After median laparotomy, the entire small intestine, from the pyloric sphincter to the ileocecal junction, was removed and washed in ice-cold saline solution. Segments roughly corresponding to the duodenum (0-12 cm distal to the pyloric valve), jejunum (0-12 cm in the middle intestine), and ileum (0-12 cm proximal to the cecum) were excised. The mucosa of each segment was scraped and frozen in liquid nitrogen. The digestive enzymes lactase (EC 3.2.1.23), sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20) were determined by the method of Dahlquist (21). Briefly, this procedure consists of measuring glucose levels of homogenized intestinal mucosa aliquots and water incubated with the specific substrates (lactose, sucrose or maltose). The glucose content released during the reaction is determined by the glucose oxidase method, which allows the calculation of specific enzyme activity, expressed as units per gram of mucosa.

Gene expression analysis: real-time PCR

Gene expression in the duodenum, jejunum and ileum was determined by RNA extraction with Trizol reagent (Invitrogen Life Technologies, USA) according to manufacturer recommendations. Complementary DNA (cDNA) synthesis was performed from total RNA using the SuperScript First kit - Synthesis Strand System for RT-PCR (Invitrogen Life Technologies).

The amplification protocol of the Power SYBR® Green Master Mix kit (Applied Biosystems, USA) was performed to evaluate the gene expression of target genes, lactase (sense: S5′-TACATCAACGAGGTGCTCAAGGCT-3′, antisense: A5′-TAGCCGTCAATGAGGGAACGAACA-3′); sucrase (sense: S5′-AACAATCAAAGTCCTTGGGCT-GCG-3′, antisense: A5′-AAGCTTCCGGCAAGGTTGA-AGTTG-3′) and maltase (sense: S5′-TGACA-ACCAAATGGCACAAGGGAC-3′, antisense: A5′-ATGCTGCCAAGTCTCATCTCCTGT-3′) [GenBank accession Nos.: lactase NM_053841.1; sucrase X15546.1; maltase XM_002726326.3]. The mRNA expression of the constitutive gene α-actin was used to normalize the gene expression of lactase, sucrase and maltase.

Amplification reactions of the target genes were performed on 96-well plates containing Power SYBR® Green Master Mix (Applied Biosystems), cDNA and the specific oligonucleotide primers. Differences in gene expression rates were normalized by the expression frequency of α-actin, which proved to be the best endogenous control. The relative expression values for each gene were calculated using the ΔΔCt method with efficiency correction and using one control sample as calibrator (22). Mean efficiency values for each gene were calculated from the amplification profile of individual samples with the LinRegPCR software (23).

Statistical analysis

Data are reported as means ± SE. Differences between control and experimental animals at each age were analyzed by the Student *t*-test. Statistical significance was set at $P < 0.05$.

Results

Body measures

Animals born to protein-restricted dams were lighter ($P < 0.05$) than those born to control dams at birth (5.7 ± 0.1 *vs* 6.3 ± 0.1 g, respectively) and weaning (42.4 ± 1.3 *vs* 49.1 ± 1.6 g, respectively). At 16 weeks of age, however, offspring from protein-restricted and control groups had similar weight (421.7 ± 8.9 and 428.5 ± 8.5 g, respectively). The adiposity index at 16 weeks of age was higher ($P < 0.05$, Student *t*-test) in animals born to protein-restricted dams (2.2 ± 0.2 *vs* 1.7 ± 0.3%). The organs of rats born to protein-restricted dams were also lighter at weaning, but at 16 weeks of age there was no difference between groups (Table 2). The relationship between organ and body weight was similar for the protein-deprived and control groups.

Intestinal enzyme activity. Lactase activity in the duodenum, jejunum and ileum was higher in PR3 than in C3 rats (Figure 1). There was no statistical difference between PR3 and C3 rats in maltase activity in proximal, middle, and distal segments. Similar results were obtained for sucrase activity in proximal, middle and

Table 2. Organ weight and organ weight/body weight ratio of rats born to dams fed a protein-restricted (R) or a control diet (C).

	Organ weight (g)				Organ weight/body weight ratio			
	3-week-old		16-week-old		3-week-old		16-week-old	
	R	C	R	C	R	C	R	C
Stomach	0.35 ± 0.06[b]	0.42 ± 0.06[a]	2.00 ± 0.20	1.89 ± 0.20	0.82 ± 0.12	0.86 ± 0.11	0.48 ± 0.03[a]	0.44 ± 0.03[b]
Pancreas	0.14 ± 0.04[b]	0.19 ± 0.06[a]	1.09 ± 0.15	1.02 ± 0.19	0.33 ± 0.10	0.39 ± 0.10	0.26 ± 0.03	0.24 ± 0.05
Liver	1.73 ± 0.27[b]	2.00 ± 0.05[a]	15.24 ± 1.52	15.59 ± 1.20	3.87 ± 0.27	3.92 ± 0.29	3.63 ± 0.19	3.65 ± 0.35
SI	0.52 ± 0.12[b]	0.65 ± 0.08[a]	9.85 ± 0.78[a]	8.98 ± 0.57[b]	1.14 ± 0.23	1.29 ± 0.17	2.32 ± 0.35	2.26 ± 0.16
LI	2.19 ± 0.30[b]	2.53 ± 0.30[a]	4.06 ± 0.46[a]	3.86 ± 0.47[b]	5.04 ± 0.53	5.10 ± 1.16	0.90 ± 0.06	0.94 ± 0.11

Data are reported as means ± SE with n = 6-8 per group. SI = small intestine; LI = large intestine. R = 6% protein; C = 17% protein. Means with different superscript are statistically different between diets within ages ($P < 0.05$; Student *t*-test).

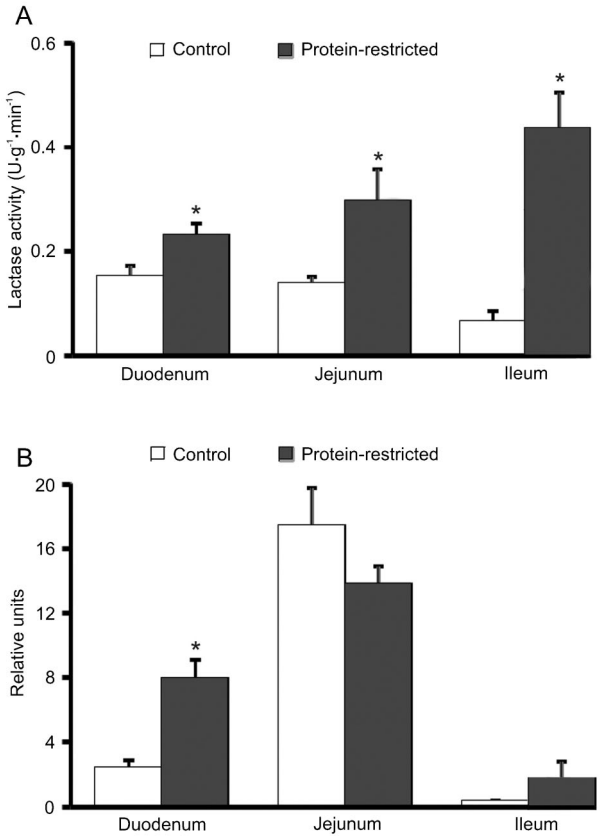

Figure 1. Lactase activity (*A*) and lactase mRNA abundance (*B*) in the intestine of 3-week-old rats (n = 5-8 animals) born to dams fed a protein-restricted (6% protein) or a control diet (17% protein). Data are reported as means ± SE. *P < 0.05 compared to control (Student *t*-test).

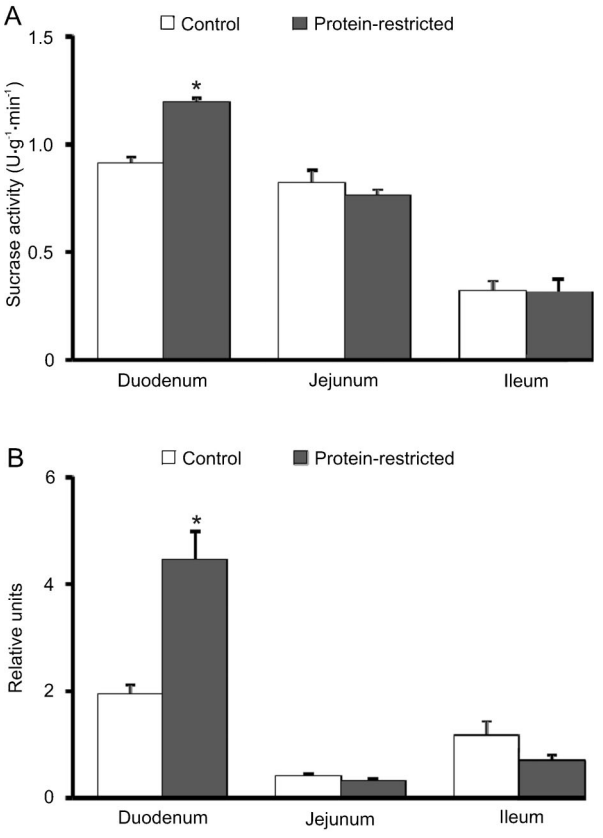

Figure 2. Sucrase activity (*A*) and sucrase mRNA abundance (*B*) in the intestine of 16-week-old rats (n = 5-8 animals) born to dams fed a protein-restricted (6% protein) or a control diet (17% protein). Data are reported as means ± SE. *P < 0.05 compared to control (Student *t*-test).

distal intestine. PR16 rats had higher sucrase activity in the duodenum compared to C16 (Figure 2). However, maltase activity did not differ between PR16 and C16 in the proximal, middle or distal intestinal segments.

Gene expression of intestinal enzymes

Lactase mRNA expression in the duodenum was higher ($P < 0.05$) in PR3 than in C3 animals (Figure 1). Dam protein restriction during pregnancy did not affect the abundance of maltase and sucrase mRNA in the proximal, middle or distal intestinal segments of 3-week-old rats.

In PR16, the expression of sucrase mRNA in the duodenum was higher ($P < 0.05$) than in C16 (Figure 2). However, there was no significant increase in maltase mRNA expression in proximal, middle or distal segments.

Discussion

The present study evaluated gene expression and intestinal disaccharidase activity as a consequence of intrauterine protein restriction. The responses varied as a function of the intestinal segment, enzyme type and animal age.

Rats born to protein-restricted dams showed higher lactase mRNA abundance at 3 weeks of age and higher sucrase mRNA abundance at 16 weeks. Furthermore, 3-week-old animals born to protein-restricted dams showed higher lactase activity in the three intestinal segments. Sixteen-week-old rats showed higher sucrase activity in the duodenum.

The up-regulation of lactase and sucrase mRNA can be considered to be a specific adaptive response to intrauterine protein restriction. The positive association between lactase and sucrase activity and gene expression suggests that transcriptional controls are involved in the fetal programming of these genes. Transcriptional regulation is considered to play a pivotal role in the carbohydrate-induced enhancement of sucrase and lactase expression (24). Moreover, transcriptional control is considered to be the major mechanism in the regulation of lactase-phlorizin hydrolase (25).

The increase in disaccharidase activity is consistent

with the prediction that animals suffering from intrauterine restriction optimize nutrient use to ensure survival (26). Similar results were observed for lactase in offspring rats born to dams fed only 50% of the adequate food supply (17) and for sucrase and maltase in offspring rats born to dams receiving only 60% of the adequate dietary protein content (18). However, they contradict the findings of Weaver et al. (17) for 42-day-old and 1-year-old rats subjected to similar intrauterine protein restriction. These different results can be attributed to the age of the animals used by Weaver et al. (17). Enzyme activity is changing at both ages. In fact, at 42 days of age the activity is increasing toward its highest level and at 1 year it is decreasing toward its lowest level (13). Moreover, an early study showed that the adaptive response of the intestine is reduced in old age (27).

The high enzyme activity in rats born to protein-restricted dams can increase individual short- and mid-term viability. However, increased disaccharidase activity may also contribute to the development of obesity by fetal programming since it can lead to increased carbohydrate absorption in the small intestine (15). In fact, leptin-deficient obese mice show increased sucrase activity (28) and obese rats fed a high-fat diet show an increase in maltase activity (29).

In the present study, rats born to protein-restricted dams showed a higher adiposity index at 16 weeks of age although their body weight was similar to that of the control group. Body fat has been considered to be a more sensitive criterion than body weight for assessing obesity (30). These investigators verified a 35-40% increase in body fat but a 10% increase in total body weight in rats fed a high-fat diet. Dissociation between body weight and adiposity index was also observed by Lopes et al. (31) in a study about the obesity caused by salt restriction during the perinatal phase. It was not an objective of the present study to determine the mechanisms of such dissociation, but further studies on this subject would be worth developing.

The effects investigated in the present study were primarily identified in the duodenum, corroborating earlier findings from our research group on the fetal programming of intestinal transporters (Pinheiro DF, Pinheiro PFF, Buratini Jr J, Castilho ACS, Lima PF, Trinca LA, et al., unpublished results) and other studies on the consequences of dietary changes. For instance, fructose absorption by animals subjected to food restriction for 24 and 270 days was higher in the proximal intestine portion (32), and the effects of a carbohydrate-rich diet on sucrase activity in mice was also higher in the duodenum (33). The activity of disaccharidases occurs along the longitudinal axes of the small intestine, with the highest activity occurring in the jejunum (13). An increase in enzyme activity could be expected in rats born to protein-restricted dams since it could improve the digestion and assimilation of nutrients. However, the adaptive response affecting only the duodenum observed here deserves further investigation. Enzymatic activity and mRNA abundance increased in the duodenum of 3-week-old rats from the protein-deprived groups, although they had a lighter small intestine. This specific increase in enzyme activity may therefore compensate for weight reduction, favoring better nutrient use. The weight of other body organs in 3-week-old animals was also affected by intrauterine protein restriction. The 16-week-old rats showed a heavier small and large intestine.

Alterations in enzymatic activity have been associated with morphofunctional changes in the mucosa. Montoya et al. (34) detected a decrease in enzyme activity in the intestine of rats with a smaller villus height due to the chronic consumption of a protein-free diet. In addition, Ferraris et al. (33) suggested that the proportion of immature and mature differentiated intestinal cells (the latter in higher amounts) in the atrophic mucosa of adult animals subjected to feed restriction accounts for the increase in enzymatic activity. In contrast, studies carried out in our laboratory have shown that intrauterine restriction increases enterocyte proliferation in 3- and 16-week-old rats (Pinheiro DF, Pinheiro PFF, Buratini Jr J, Castilho ACS, Lima PF, Trinca LA, et al., unpublished results). Therefore, we believe that the increased lactase activity in the jejunum and ileum, which was not associated with an increase in mRNA abundance, may be attributed at least in part to increased cell proliferation.

The present study shows for the first time that intrauterine protein restriction affects the gene expression of intestinal enzymes in the offspring. Moreover, it reinforces the idea that specific enzymatic activity can be programmed during early development.

Further studies are necessary to determine if other digestive enzymes, including pancreatic enzymes, can also be affected by a low-protein diet during gestation.

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