Delayed Consequences of Protein Deficiency during Lactation for the Development of Enzyme Systems of Digestive and Nondigestive Organs in Offspring

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Suboptimal protein nutrition during lactation has a negative impact on the digestive function of the small intestine and trophic barrier functions of the large intestine, liver, and kidneys due to significant enzyme deficiency (disaccharidase, peptidase, alkaline phosphatase) in 6-month-old offspring. Changes in enzyme activity in digestive and nondigestive organs play an important role in metabolic disorders promoting the development of "risk diseases" and reducing lifespan.

Key Words: *protein deficiency; lactation; digestive enzymes; intestine; liver; kidneys*

Insufficient nutrition during pregnancy and lactation can have negative consequences for structural and functional development of various systems and organs in the offspring during the early ontogeny and in the adulthood [6,13,14]. Maternal nutrition affects not only the growth and functional development of growing organism, but also on the health of offspring during adulthood and can cause diseases reducing their lifespan [10,11]. Despite abundant epidemiological data, the molecular mechanisms of maternal metabolic alimentary regulation of various systems, especially, the enzyme systems in digestive and nondigestive organs performing nutritional, trophic, and barrier functions are not studied. We previously found that protein deficiency during pregnancy and lactation significantly decreased activity of digestive enzymes in the small intestine in rat offspring both during early ontogeny and 2-4 months after birth [1,2]. However, the role of protein deficit during each critical periods (pregnancy and lactation) in the formation of the enzyme systems of digestive and nondigestive organs was not elucidated.

The purpose of the present study was to examine delayed effects of protein deficiency during lactation on activity of digestive enzymes participating in membrane and intracellular hydrolysis of nutrients and hydrolases of the large intestine, liver, and kidneys performing trophic and barrier functions and participating in intermediary metabolism.

MATERIALS AND METHODS

The experiments were carried out on 6-months-old Wistar male rats from the mothers kept on a protein-deficient diet (2.5-fold lower protein content compared to standard diet [2]) for 21 day during lactation. Starting from day 21 after birth rat pups and lactating females received normal balanced ration. Age-matched rats from mothers kept on complete ration were used as controls. Experimental and control rats were weaned on day 30 and kept on a standard diet.

Activities of membrane enzymes, sucrase (EC 3.2. 1.48), maltase (EC 3.2.1.20), alkaline phosphatase (EC 3.1.3.1), aminopeptidase M (EC 3.4.11.2), and cytosolic glycyl-L-leucine dipeptidase (EC 3.4.13.2) were determined in mucosa homogenates from the duodenum, jejunum, ileum, and large intestine. Activities of these enzymes were also measured in liver and kidney homogenates. Maltase activity was measured by glucose oxidase method [5], sucrase and aminopeptidase

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activities — by the method described elsewhere [3,7], dipeptidase activity was determined by glycine method [4], protein content was measured by the method of Lowry [4]. Alkaline phosphatase activity was measured using 0.6 mM n-nitrophenylphosphate as the substrate.

The results were processed statistically using Student's *t* test.

RESULTS

Dietary protein deficiency in lactating females significantly altered the enzyme activity in digestive and nondigestive organs of the offspring during their adulthood. However, the body weight, the weight and length of small and large intestine, and the weight of duodenum, jejunum, ileum, liver, and kidneys did not differ from control values.

Sucrase activity was maximum in the jejunum, low in the liver, and was absent in the kidneys in both control and experimental animals. In experimental animals, sucrase activity in the duodenum and liver was 2.3- and 1.2-fold below the control, respectively (Table 1).

Maltase activity was maximum in the jejunum; it was high in the duodenum, ileum, and kidneys, and low in the liver. Protein deficiency in lactating females decreased maltase activity in the duodenum, jejunum, and liver of their offspring by 1.5-, 1.2-, and 3.2 times, respectively (Table 1).

In control rats, alkaline phosphatase activity was maximum in the duodenum and minimum in the ileum, which pointed to a pronounced proximodistal gradient of phosphatase activity along the intestine. This gradient was less pronounced in experimental rats; alkaline phosphatase activity in the duodenum of these rats was 1.8-fold below the control. In the ileum and large intestine, activity of this enzyme was low in both rat groups, while in the kidneys it was 1.7-fold lower in experimental rats compared to control animals. Alkaline phosphatase activity was very low in the liver, and in experimental rats it was 2-fold lower than in controls (Table 1).

In both rat groups, aminopeptidase M activity was 2-fold lower in the duodenum compared to jejunum and ileum, and slightly higher than in the large intestine. The highest activity was found in the kidneys, in experimental rats it was 1.4-fold lower than in controls. In the liver, this enzyme activity was low being twice lower in experimental animals compared to the control (Table 1).

Glycyl-L-leucine dipeptidase activity was high in all examined digestive and nondigestive organs in both rat groups. The highest activity was found in kidneys, where it surpassed maximum activity in ileum. In experimental rats, this activity was significantly (1.7-fold) decreased in the duodenum, ileum, and liver, as well as in the kidneys (1.3-fold) compared to the control.

Thus, protein deficit in lactating females had a negative impact on digestive functions of the small intestine and trophic barrier functions of the large

TABLE 1. Enzyme Activity (μmol/min/g protein) in Small and Large Intestine, Liver, and Kidneys in 6-Month-Old Male Wistar Rats Fed by Mothers Maintained on Standard (Control) or Protein-Deficient (Experiment) Diet during Lactation (*M±m*)

Enzyme	Duodenum	Jejunum	Ileum	Large intestine	Kidney	Liver
Sucrase						
control	118±25	139±16	71±6	34±5	0	12±5
experiment	52±13*	102±11	48±14	20±3*	0.3	10±3
Maltase						
control	210±13	424±17	313±36	44±5	167±11	19±3
experiment	138±1*	355±6*	343±41	49±1	96±7*	6±1*
Alkaline phosphatase						
control	101±15	57±3	12±2	9±1	15±2	2.0±0.2
experiment	55±5*	60±9	18±3	12±1	9.0±0.3*	1.0±0.3*
Aminopeptidase M						
control	30±5	57±8	51±7	14±2	118±5	12±2
experiment	20±7	48±3	45±6	15±2	83±6*	6±0.2*
Glycyl-L-leucine dipeptidase						
control	108±16	270±16	273±38	139±12	609±50	211±32
experiment	62±11*	157±16*	393±40*	164±13	470±30*	122±11*

Note. *$p<0.05$ compared to the control.

intestine, liver, and kidneys in adult offspring. Function of these organs in adult offspring reared by protein-deficient mothers differs from that in controls reared by mothers maintained on normal ration. This manifested in reduced activity of digestive enzymes. Our experiments showed that such a stress factor as protein deprivation of lactating females is retained in biochemical memory of their offspring despite early (from day 21 after birth) transition to complete ration. It can be hypothesized that the negative effect of protein deficiency of lactating mothers is critical for the formation of metabolism in adult offspring [2]. It cannot be excluded that these changes in enzyme activity in digestive and nondigestive organs play a key role in metabolic disorders and promote the development of "risk diseases" in the first and subsequent generations. It was shown that nutrient substrates inhibit or activate various enzymes via common metabolites of the lipid, carbohydrate, and protein catabolism pathways modulating translational and posttranslational modifications of enzyme molecules [8,12]. These processes can inhibit synthesis of the enzymes or accelerate their degradation and are most likely genetically determined. These problems require further investigations.

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