Protein-Deficient Diet in Pregnant Females Impairs Functional Activity of Enzyme Systems in Digestive and Non-Digestive Organs in the Offspring

N. M. Timofeeva, L. A. Gordova, V. V. Egorova, and A. A. Nikitina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 2, pp. 136-139, February, 2003
Original article submitted September 12, 2002

Activity of disaccharidases in various portions of the small intestine markedly increased in 6-month-old offspring of pregnant females feeding a protein-deficient diet. Activities of amino- and dipeptidases decreased in the kidneys, while alkaline phosphatase activity remained practically unchanged. Probably, information about protein restriction in nutrition of pregnant females is stored in the biochemical memory of adult offspring and promotes the development of metabolic disturbances.

Key Words: *protein restriction; pregnancy; digestive enzymes; intestine; liver; kidneys*

The problem of early metabolic/nutritional programming is related to the effect of food manipulations in the maternal organism during critical periods (pregnancy and lactation) on the state of health not only in the offspring at early and late ontogeny, but also in next generations. The main structural and definitive characteristics of organs and systems are formed before birth. In humans, 9 months spent in the maternal organism determine the risk of heart attacks, diabetes mellitus, and hypertension tens years later [7,12].

Epidemiological studies indicate that early metabolic/nutritional programming determines metabolic characteristics in the offspring. However, the molecular mechanisms of this process remain unclear. Protein deficiency is highly prevalent among people. Therefore, it is necessary to evaluate consequences of feeding a low-protein diet during pregnancy. Chronic diseases spoiling appetite and impairing food assimilation, toxemia of pregnancy, and malabsorption contribute to protein deficiency.

Our previous studies showed that functional activity of enzymes in digestive and non-digestive organs changes in adult offspring of females feeding a low-protein diet during lactation [2,3]. Changes in enzyme systems in adult offspring of females receiving a protein-deficient diet during pregnancy remain unclear.

Here we studied the delayed effect of protein restriction in nutrition of pregnant females on activity of intestinal digestive enzymes and hydrolases in the liver and kidneys performing trophic and barrier functions in adult offspring.

MATERIALS AND METHODS

Experiments were performed on 5 male Wistar rats aging 6 months whose mothers fed isocaloric diets during pregnancy. Protein content in these diets was 2.5 times lower than in the control. The control group included 5 rats of the same age whose mothers received a balanced diet during pregnancy [1]. Experimental and control rats were reared by mothers for 30 days and then transferred to a standard diet.

Activities of membrane-bound enzymes invertase (EC 3.2.1.48), maltase (3.2.1.20), alkaline phosphatase (EC 3.1.3.1), and aminopeptidase M (EC 3.4.11.2) and predominantly intracellular glycyl-L-leucine dipeptidase (EC 3.4.13.2) were measured in homogenized duodenal, jejunal, and ileal mucosa and liver and kid-

I. P. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg. ***Address for correspondence:*** nmtimof@pavlov.infran.ru. Timofeeva N. M.

0007-4888/03/1352-0117$25.00

ney homogenates. Maltase activity was estimated by the glucose oxidase method [8]. Invertase activity was determined as described elsewhere [5]. Aminopeptidase M was measured by the method described in [10]. Dipeptidase activity was estimated by the glycine method [6]. Alkaline phosphatase activity was determined using 0.6 mM p-nitrophenyl phosphate as the substrate. Protein content was measured as described elsewhere [11].

The results were analyzed by Student's *t* test.

RESULTS

Protein restriction in nutrition of pregnant females was accompanied by a decrease in body weight of 6-month-old offspring by 28% (323±28 *vs.* 447±17 g, $p<0.05$). The weight of the jejunal and colonic mucosa, liver, and kidneys decreased by 26, 43, 37, and 25%, respectively (Table 1).

Enzyme activities in these organs underwent considerable changes (Table 1). In experimental rats invertase activity in the duodenum, jejunum, ileum, and liver increased by 1.8, 2.5, 3.2, and 3 times, respectively. In the large intestine enzyme activity did not differ from the control. Invertase activity was not detected in the kidneys.

Changes in maltase activity were similar. Enzyme activity in the duodenum, jejunum, ileum, and large intestine increased by 1.8, 2, 1.9, and 1.3 times, respectively, compared to the control. Maltase activity in the kidneys and liver did not differ between experimental and control animals.

Our previous studies showed that invertase and maltase activities markedly decrease in the duodenum, jejunum, ileum, large intestine, liver, and kidneys of 2- and 4-month-old rats whose mothers fed a protein-deficient diet during pregnancy [4]. Changes in disaccharidase activities are paradoxical. When pregnant females fed a low-protein diet enzyme activities in digestive and non-digestive organs decreased in the offspring aged 2 and 4 months (by 1.5-5 times), but markedly increased in 6-month-old rats.

As differentiated from disaccharidases, aminopeptidase M activity in 6-month-old experimental rats decreased only in the duodenum and kidneys (by 1.6 and 1.4 times, respectively). Enzyme activity in other portions of the small intestine, large intestine, and liver did not differ from the control. In 2- and 4-month-old offspring of experimental animals enzyme activity in these organs was 2-5 times lower than in the control [4].

TABLE 1. Weight (g) and Activities of Enzymes (μmol/mg protein/min) in Intestinal Mucosa, Liver, and Kidneys in 6-Month-Old Male Wistar Rats Whose Mothers Received Balanced (Control) or Protein-Deficient Diet (Experiment) during Pregnancy (*M*±*m*, *n*=5)

Parameter	Duodenum	Jejunum	Ileum	Large intestine	Liver	Kidneys
Weight						
control	0.60±0.05	1.90±0.12	1.40±0.12	0.70±0.09	17.00±1.55	3.20±0.17
experiment	0.50±0.04	1.44±0.11*	1.20±0.20	0.40±0.04*	10.70±1.36*	2.40±0.22*
Invertase						
control	62.4±8.8	57.5±5.9	19.8±3.9	7.2±1.1	6.7±1.3	0
experiment	111.0±8.0*	143.0±8.7*	64.4±10.5*	7.00±0.88	20.1±5.0*	0.40±0.24*
Maltase						
control	389.6±51.4	684.1±14.6	428.0±46.3	51.2±0.5	24.2±4.4	197.8±15.8
experiment	699.1±71.5*	1383.0±71.0*	813.5±73.5*	64.9±5.6*	21.8±3.3	184.3±13.6
Alkaline phosphatase						
control	183.6±26.4	9.2±20.2	23.7±6.0	5.7±0.4	1.4±0.16	24.9±0.6
experiment	135.2±15.9	110.0±11.6	15.0±2.1	3.9±0.6*	1.04±0.17	25.7±2.3
Aminopeptidase M						
control	48.2±4.4	75.6±7.0	79.6±9.4	13.7±3.7	14.6±2.8	164.2±14.5
experiment	29.1±4.2*	94.5±4.2*	90.4±7.0	7.8±1.7	7.0±1.4*	117.8±7.0*
Glycyl-L-leucine dipeptidase						
control	158.2±20.1	380.9±69.8	488.0±82.0	155.3±18.4	167.6±12.8	1091.9±62.0
experiment	178.3±30.9	453.5±33.7	864.8±80.0*	137.2±10.0	200.8±28.0	675.6±93.9*

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

In 6-month-old experimental rats activity of glycyl-L-leucine dipeptidase acting on the surface of brush-border membranes and inside enterocytes did not differ from the control. The only exception was the ileum, where enzyme activity 1.5-fold surpassed the normal. In 2-month-old experimental rats glycyl-L-leucine dipeptidase activity in test organs was 1.5 times higher than in control animals. However, in 4-month-old rats enzyme activity decreased by 2.5-5 times compared to the control [4].

These data indicate that activity of peptide hydrolases responsible for enzymatic treatment of nutrient proteins underwent considerable changes in the offspring of rats feeding a protein-deficient diet during pregnancy.

Membrane-bound alkaline phosphatase cleaves phosphoric acid esters and is involved in transport of nutrients. In 6-month-old rats subjected to protein restriction during prenatal development enzyme activity decreased by 80% in the ileum, but increased by 40% in the kidneys. However, alkaline phosphatase activity in the test organs decreased in experimental rats aging 2 and 4 months [4].

Therefore, information about food restriction in pregnant females is stored in the biochemical memory and impairs nutritive, trophic, and barrier functions of digestive and non-digestive organs in adult offspring. These changes were observed although lactating females, pups, and rats isolated from mothers fed a balanced diet starting from the first hours after birth to examination at an age of 6 months.

It should be emphasized that enzyme activities in the offspring of females feeding a low-protein diet during pregnancy underwent opposite changes. In experimental rats activities of disaccharidases invertase and maltase were much higher than in the control. Aminopeptidase M activity in experimental rats was low in the duodenum and kidneys, but did not differ from normal in other organs. Changes in activities of dipeptidase and alkaline phosphatase were less significant. Previous studied showed that enzyme activities in digestive and non-digestive organs decrease more significantly in 6-month-old offspring of animals subjected to protein restriction during lactation (up to 21 days) [2]. Disaccharidase activity in various organs of these rats did not increase so significantly as in animals whose mothers fed a low-protein diet during pregnancy. However, enzyme activities in 2- and 4-month-old animals subjected to protein restriction during intrauterine and early postnatal development underwent similar changes. Enzyme activity in these rats was much lower than in animals developed under normal conditions of protein supply [3,4].

Information about the negative effect of protein restriction in maternal nutrition on the synthesis of enzymatic proteins in digestive and non-digestive organs of adult offspring is probably stored in the genetic code during prenatal and early postnatal development and produces various changes in enzyme activities. In the offspring of females feeding a low-protein diet during pregnancy and lactation, programming of metabolic functions is probably realized at the level of individual genes and results in the induction or repression of gene expression [9]. Each cell can express a lower or greater amount of the enzyme. Otherwise, the number of cells can decrease or increase. It is important that although experimental animals fed a balanced diet after birth their body weight was lower than in the control (Table 1).

It cannot be excluded that adverse early nutritional programming associated with protein restriction in pregnant females modulates enzyme activity in the small and large intestine, liver, and kidneys in adult offspring, which promotes metabolic disturbances, causes various diseases, and accelerates aging.

This work was supported by the Russian Foundation for Basic Research (grant No. 01-04-49617).

REFERENCES

1. N. M. Timofeeva, *Ros. Fiziol. Zh.*, **86**, 1531-1538 (2000).
2. N. M. Timofeeva, L. A. Gordova, V. V. Egorova, and A. A. Nikitina, *Byull. Eksp. Biol. Med.*, **133**, No. 4, 374-376 (2002).
3. N. M. Timofeeva, L. A. Gordova, V. V. Egorova, and A. A. Nikitina, *Ros. Zh. Gastroenterol. Gepatol. Koloproktol.*, **11**, No. 4, 9-15 (2001).
4. N. M. Timofeeva, L. A. Gordova, V. V. Egorova, *et al.*, *Ros. Fiziol. Zh.*, **88**, No. 8, 814-821 (2002).
5. A. M. Ugolev and N. N. Iezuitova, *Study of Human Digestive System* [in Russian], Leningrad (1969).
6. A. M. Ugolev and N. M. Timofeeva, *Study of Human Digestive System* [in Russian], Leningrad (1969).
7. D. J. P. Barker, *Lancet*, **341**, 938-941 (1993).
8. A. Dahlqvist, *Analyt. Biochem.*, **7**, 18-25 (1964).
9. M. Desai, N. J. Crowther, A. Lucas, and C. N. Hales, *Brit. J. Nutr.*, **76**, 591-603 (1997).
10. W. Farr, N. Rehfeld, D. Reichelt, and R. J. Haschen, *Z. Med. Labortech.*, **9**, No. 2, 78-86 (1968).
11. O. H. Lowry, N. Y. Rosenbrough, A. L. Farr, and R. Y. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
12. A. Lucas, R. Morley, and E. Isaacs, *Nutr. Rev.*, **59**, No. 8, S24-S33 (2001).