

# The effect of protein deficiency on the small intestinal carbobidrase system after the transition to the definitive nutrition in rats

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**Abstract.** The effect of protein deficiency on enteral carbohydrases during post-lactotrophic feeding of rat pups was studied. During this period, low protein intake (8% of the energy value of the diet versus 27% in the norm) negatively affects the growth of body weight and pancreas of rats, leads to the disruption of the activity of intestinal enzymes without changing the morphological development of the small intestine.

## 1 Introduction

Protein-calorie malnutrition remains a challenging medical problem worldwide. It is of great importance for a person's life and performance, as it is either a prerequisite for diseases or their cause. Of particular interest is the influence of this factor on the child's body, which is extremely vulnerable to the effects of food deficiency in late prenatal and early postnatal ontogenesis, since the need for nutrients to maintain growth rate is high, and the ability to accumulate energy sources and direct their use to compensate for nutritional deficiency minimal. This pattern, well known in developmental biology, was established primarily on the basis of ontogenesis studies of growth rates, metabolic levels, activity of neuroendocrine regulatory systems, etc. The function of the gastrointestinal tract in this aspect has not been sufficiently studied. At the same time, significant advances achieved recently in the field of age-related physiology of digestion have demonstrated the direct participation of the gastrointestinal tract in the reaction of a growing organism to nutritional imbalance. It turned out that the transition of mammalian offspring from lactotrophic nutrition to definitive nutrition is characterized by a number of structural and functional changes at the molecular-subcellular, cellular and organ-system levels of organization, and that thanks to these structural and functional rearrangements, the growing organism acquires the ability to adapt to changing nutritional conditions during critical periods of life [11, 19-20].

It is known that the physiological mechanisms responsible for the implementation of adaptive changes in gastrointestinal function during critical periods of ontogenesis are extremely sensitive to the influence of various exogenous and endogenous factors, among which the dominant factor is nutrition. In animals that have suffered malnutrition during milk feeding, the gastrointestinal tract remains at the time of weaning from the mother not

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quite ready to assimilate definitive food, which in turn steadily retards the rate of growth and development of animals, increases the risk of disease and early death [11, 19].

Every year it becomes more and more difficult to provide food for all people on Earth in accordance with their physiological requirements. This issue is large-scale and covers not only economic, but also biological, medical, and environmental areas. For all known reasons, the overall yield in agriculture is decreasing. This situation is due to the fact that it also prevents an increase in the growth of ungulate cattle. This is clearly manifested in relation to proteins from the main food substances, which leads to a decrease in its content in existing food products, an increase in the cost of protein dishes [1-4]. At the same time, the number of different commercial dishes is growing in all countries, and among the population there is not a need for them, but an increase in desires. This condition is due to the fact that delicious food is consumed with love by many (especially young) people whose physiological need for carbohydrates is higher than the established norm. The result is a violation of the normative ratio of proteins, fats and carbohydrates in the daily diet [5-6]. The protein content in food, which is below the level of physiological need, causes a number of biological and medical problems [7-8]. Including the fact that there are not enough of them in consumer food products, causes many problems related to reproduction, growth-maturation, various diseases, tolerance to adverse stress factors, and problems with mental and physical activity. There is also the fact that protein deficiency in the diet most strongly affects the functional formation of a growing organism (from the embryo, which has already begun to develop in the womb, to the level of its maturity after birth). The deficiency of protein in the diet, which continues at this time, leads to the appearance of long-lasting and even lifelong defects in the emerging body [9-14].

## 2 Materials and methods

To study the consequences of protein deprivation during the transition of otogenesis to a definitive nutrition, we conducted studies on white Wistar rats born in a laboratory vivarium. The animals were kept in a well-ventilated room with a natural light-dark regime in polyethylene cages measuring 50x25x40 cm, with 6-8 rats in each, at an air temperature of 22-26 °C and a relative humidity of 40-60% and had free access to food and water. Among a large number of rats, females of approximately the same weight (180-250 g) were selected to mate with males of similar weight. When obvious signs of pregnancy appeared, the females were placed in individual cages measuring 35x20x30. During pregnancy and lactation, these rats received normal 27% protein diet. On the first day after birth, the rat pups were divided as 8 pups for each lactating female. At the end of the milk-feeding period, the rat pups were transferred to a protein-deficient diet for three weeks (from the 21st to the 42nd day of life). As a control, we used a group of rat pups born and raised by normally fed females and transferred after weaning to a balanced diet.

To carry out biochemical and morphological analyses, 6-8 rat pups were sacrificed by decapitation on the day the application ended and every other week for three weeks [15]. In all cases, analyzes of the structural and functional states of the digestive function were preceded by determinations of body weight, pancreas weight, and small intestine weight. During the experiment, food consumption and mortality of rat pups were recorded.

In the homogenate of the pancreas and the contents of the small intestine of these rat pups, the activity of alpha-amylase was determined; in the homogenate of the small intestinal mucosa, the activities of lactase, maltase and sucrase were determined.

Amylolytic activity was expressed in mg of substrate digested in 1 min and calculated per 1 g of pancreatic tissue (specific activity) and for the entire mass of organ tissue (total activity), in the case of small intestinal chyme - per 1 cm of length of the small intestine (specific activity) and along its entire length (total activity).

The activity of enteral enzymes was expressed in micromoles of the product formed per 1 min. Enzymatic activity was calculated per 1 g of mucosa (specific activity) and for the entire mass of the organ (total activity).

The structure of the small intestinal mucosa was also studied. For light optical examination, pieces of tissue measuring 2x2x5 mm were fixed in 4 % depolymerized paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 24 hours. After washing in phosphate- saline buffer and dehydration in increasing concentrations of ethanol solutions, the pieces were embedded in paraffin. 4 µm thick sections were stained with hematoxylin and eosin. Morphology was performed at 280x magnification.

Protein deficiency was created by reducing the amount of protein in the rats food to 8% instead of 27% of the norm and, thus, compensating the energy value of the food by appropriately increasing the amount of carbohydrates [17].

Statistical processing of the obtained data was carried out using the Student and Fisher test based on the calculation of the arithmetic mean (M), the mean error of the mean (m) and the reliability index (P). Differences were considered significant at P<0.05

### 3 Results

The rat pups grew up in conditions of a normal balanced feeding, while the body weight of 42-day-old rats decreased by 37.5% compared to the control group, and the pancreatic mass decreased by 57.6% when the milk was switched to protein deficiency in the final diet as soon as the absorption period ended (day 21). It should be noted that under such conditions, the mass of the small intestine of rats, its mucosa, the number of cells in the villi, the number of cells relative to the length of the villi, the height of the villi and the depth of the crypt did not undergo significant changes (Table 1).

Thus, the presence of a certain pattern is clearly evident here, namely that a diet with a lack of protein within three weeks after the transition of animals from milk to definitive nutrition selectively suppresses the growth of animals and the structural development of the pancreas, while the growth indicators of the small intestine and the morphometric characteristics of its mucosa, nutritional imbalance in this period of ontogenesis does not have such a sharp inhibitory effect.

**Table 1.** The effect of protein deficiency on the growth of animals and the structural characteristics of the digestive organs during the early transition to a definitive nutrition (42 day old rats).

Studied indicators	Groups of animals			
	control	experimental	%	P
body weight (g)	103.8±8.2	64.9±5.4	-37.5	<0.01
pancreas weight (mg)	583.3±64.1	247.5±24.7	-57.6	<0.001
small intestine weight (mg)	4430.5±487.3	3397.8±340.1	-23.3	>0.25
mucosa weight (mg)	3232.0±258.3	2517.9±226.6	-22.1	>0.1
number of cells in the villi	133.7±9.2	136.8±9.9	+2.3	>0.5
number of cells in 100 µm of villi	19.1±1.6	21.5±1.9	+12.6	>0.5
number of cells in the crypt	65.0±5.8	61.1±5.6	-6.0	>0.5
villi length (µm)	350.0±35.6	318±30.0	-9.1	>0.5
crypt depth (µm)	106.0±10.0	100.0±9.0	-5.7	>0.5

According to the functional characteristics of the pancreas and small intestine, a much more complex condition was observed in our experiments in this series. In this case, pancreatic homogenate and alpha-amylase activity in the small intestine fluid (both specific and general) decrease sharply due to a lack of protein. This condition indicates inhibition of the synthesis of this enzyme in the pancreas and its pumping into the intestinal cavity (Table 2).

**Table 2.** The effect of protein deficiency on the activity of pancreatic and small intestine enzymes in the initial period of transition to a definitive diet (42 day old rats).

Studied indicators		Groups of animals			
		control	experimental	%	P
pancreatic $\alpha$ -amylase	in 1 g of the tissue (g/min)	8.6±1.3	2.4±0.3	-72.1	<0.01
	in the total mass of the tissue (g/min)	5.0±0.7	0.6±0.1	-88.0	<0.001
intestinal cavity $\alpha$ -amylase	in 1cm length (mg/min)	2.72±0.25	0.5±0.06	-81.5	<0.001
	in full length (mg/min)	194.8±20.1	35.1±3.7	-82.0	<0.001
maltase	in 1 g of the mucosa (mkmol/min)	124.3±6.5	68.3±5.0	-45.0	<0.001
	in the total mass of the mucosa (mkmol/min)	401.7±26.3	172.0±12.4	-57.2	<0.001
sucrase	in 1 g of the mucosa (mkmol/min)	6.9±0.5	5.7±0.3	-17.4	>0.1
	in the total mass of the mucosa (mkmol/min)	22.3±1.7	14.4±1.2	-35.2	<0.01
lactase	in 1 g of the mucosa (mkmol/min)	0.91±0.08	0.65±0.05	-28.6	<0.02
	in the total mass of the mucosa (mkmol/min)	2.9±0.3	1.6±0.2	-44.8	<0.01

About enzymes associated with the intestinal membrane, it can be noted that both specific and general maltase activity is sharply reduced from them. And the specific activity of sucrose varies slightly, and the overall activity decreases at a strong level (Table 2). The specific activity of lactase decreases slightly (28.6%), the overall activity decreases significantly (44.8%). It is obvious that in rat pups transferred to definitive food with protein deficiency after the end of the lactotrophic diet, the indicators of pancreatic growth and its functional activity are sharply suppressed. In this case, the small intestine is not structurally damaged much, but its enzymatic activity is reduced to varying degrees for different enzymes.

Thus, insufficiency of protein in the diet of rat pups in the early stages after the transition from milk feeding to definitive nutrition (the first three weeks) reduces the activity of enzymes involved in the intestinal hydrolysis of polysaccharides and oligosaccharides with relative constancy of the mucous mass and structural characteristics of the small intestine against the background of a sharp slowdown in the growth rate of animals and pancreatic mass.

## 4 Discussion

Based on the results obtained, it should be noted that with the end of the lactotrophic period, the growth of the pancreas and its functional properties sharply decrease in rats whose protein is transferred to a less pronounced diet than usual. At the same time, in the small intestine, various changes in the activity of enzymes are manifested in it, without significant structural changes.

Many clinical and experimental data will be formed from the small intestine of all mammals before birth, and at this time the mother will have the ability to hydrolyze all the nutrients contained in milk in the membrane (18, 19). As noted by A.M. Ugolev, there is a non-synchronicity of the ontogenesis of the digestive system conveyor, which at the time of birth prevails over the cavity of digestion in the membrane. The mechanism of development of the digestive process in the intestinal cavity directly occurs during the period of milk feeding, the end of which is accompanied by a transition to definitive nutrition (20, 21). In rats, this time corresponds to the end of the 3rd week of life after birth. Alpha-amylase activity in them was detected on the 16th day of gestation (22). This indicator is 15-20% (23) compared to that of adult rats by the last day of development in the womb. After that, during the 2nd week of the child's life after birth, the synthesis of enzymes, the mass of the gland and the mass of ossinal cells increases. These changes lead to an increase in the total activity of pancreatic alpha-amylase by 1000 times compared to the level in the fetus.

## 5 Conclusion

Thus, the results obtained by us, together with the data presented in the literature, show that nutritional imbalance in the early stages after the transition of animals from milk feeding to definitive food reduces the rate of both structural and functional development of the pancreas while simultaneously selectively damaging individual components of the function of the small intestine against the background of the relative stability of its structural characteristics

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