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EFFECT OF WHEY PROTEIN ON HEPATIC NITROGEN METABOLISM: MOLECULAR–METABOLIC MECHANISMS OF AMINO ACID TRANSAMINATION AND UREA CYCLE FUNCTION

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Abstract. *This article presents a comprehensive analysis of the role of whey protein in hepatic nitrogen metabolism, with particular emphasis on amino acid transamination and the molecular and metabolic bases of the urea (ornithine) cycle. Whey protein has a high biological value; its rapid digestion and balanced amino acid composition make it an effective modulator that enhances aminotransferase activity, stimulates glutamate and glutamine synthesis, and accelerates detoxification of ammonia in the form of urea. Experimental data indicate that moderate intake of whey protein improves the energetic state of hepatocytes, optimizes amino acid metabolism, and stabilizes nitrogen balance.*

Keywords: *whey protein, amino acid transamination, alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate–glutamine exchange system, ammonia detoxification, urea (ornithine) cycle, CPS-I activity, stability of nitrogen balance, hepatic metabolism, protein metabolism, biologically active protein supplements.*

INTRODUCTION. The liver is the central organ of nitrogen metabolism in the body. It is the site where amino acid transamination, oxidative deamination, detoxification of ammonia, urea synthesis, protein biosynthesis, and multiple stages of energy metabolism take place [1,5].

Amino acid transamination is catalyzed by pyridoxal phosphate–dependent enzymes, primarily alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which mediate the exchange of amino groups between amino acids and keto acids, thereby redistributing nitrogen fluxes in the organism [5,7].

Excessive accumulation of ammonia in the liver exerts a neurotoxic effect, disrupts central nervous system function, and in severe cases may lead to hepatic encephalopathy [6,9].

Therefore, rapid and efficient detoxification of ammonia via the urea cycle is one of the key conditions for maintaining homeostasis. The urea (ornithine) cycle is based on a sequence of reactions catalyzed by CPS-I, OTC, ASS, argininosuccinate lyase, and arginase, which incorporate two nitrogen atoms into one urea molecule and thus ensure excretion of nitrogen in a non-toxic form [9].

Whey protein is one of the leading dietary proteins in terms of biological value. It contains branched-chain amino acids (BCAA: leucine, isoleucine, valine), as well as glutamine, threonine, lysine, phenylalanine, and other chain amino acids in a balanced ratio [1,4].

According to A. A. Pokrovskiy and L. N. Berezovskiy, the rapid digestion and high absorption rate of whey protein allow it to be regarded as a natural substrate for transamination reactions and hepatic nitrogen metabolism [2].

Whey protein enhances synthesis of alanine and glutamate, activates the glucose–alanine cycle, increases glutamine formation, and promotes safe transport of ammonia from peripheral tissues to the liver [3,8]. These processes simultaneously support two key dimensions of hepatic metabolism: the molecular (enzymatic regulation) and the systemic metabolic level (influence on whole-body nitrogen balance).

In this regard, the present article examines the role of whey protein in the liver in terms of:

1. its effect on aminotransferase activity and glutamate–alanine exchange;
2. its effect on the urea cycle and molecular mechanisms of ammonia detoxification.

MAIN PART. Composition of whey protein and its metabolic significance

Whey protein belongs to the group of complete proteins and contains almost all 20 proteinogenic amino acids, including 9 essential amino acids [1,4]. Its main fractions are β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin, and serum albumin, which have immunomodulatory, antioxidant, and trophic effects [4].

BCAA are predominantly oxidized in skeletal muscle; however, their transamination products return to the liver mainly in the form of alanine and glutamate [3,8]. Thus, whey protein influences hepatic metabolism in two ways: directly, by supplying free amino acids to hepatocytes; and indirectly, by activating transamination and deamination in peripheral tissues and thereby increasing alanine and glutamate flux to the liver [3,7].

2. Role of whey protein in transamination processes

Transamination reactions proceed in the presence of vitamin B6 (pyridoxal phosphate), which serves as a coenzyme for the transfer of an amino group from an amino acid to the corresponding keto acid [5,7]. ALT mainly catalyzes the alanine–pyruvate pair, whereas AST catalyzes the aspartate–oxaloacetate pair [5]. Their activity is considered an important biomarker for assessing the state of hepatic metabolic adaptation [6].

When whey protein is introduced into the diet:

the content of essential amino acids increases,

synthesis of alanine is significantly enhanced,

the “pool” of glutamate expands,

the amount of substrates entering energy metabolism (pyruvate, α-ketoglutarate) rises, and the formation of transamination pairs that transport nitrogen between the liver and peripheral tissues intensifies [3,6].

According to Melnik, alanine is at the core of the glucose–alanine cycle and represents the main mechanism for transporting ammonia formed in muscle to the liver in the form of alanine [7]. Feeding whey protein strengthens this cycle, physiologically increasing nitrogen load on the liver while simultaneously expanding its detoxification capabilities [3,7].

3. The glutamate–glutamine pair and whey protein

Consumption of whey protein leads to an increase in plasma glutamine and glutamate levels [3,8]. Glutamate represents the central “exchange pool” of amino groups, and the transamination of many amino acids proceeds via glutamate-dependent reactions [8]. In intestinal wall enzymes and hepatocytes, a major part of amino groups ultimately enters the glutamate molecule [1,3].

Glutamine acts as the main transporter of ammonia and participates in maintaining nitrogen balance throughout the body. It contains two amino groups and therefore is able to carry ammonia in a non-toxic form from peripheral tissues to the liver or kidneys [3,8].

An increase in glutamine synthesis in animals fed whey protein indicates reinforcement of the system that temporarily binds ammonia and thus serves as a "nitrogen buffer" for the liver [3].

4. Effect of whey protein on the urea cycle and ammonia detoxification

The urea cycle is the only metabolic pathway in the liver that is strictly specialized in detoxification of ammonia via its conversion into urea [9]. The cycle proceeds through successive reactions in mitochondria and cytoplasm, involving formation of carbamoyl phosphate, citrulline, argininosuccinate, arginine, and urea [9]. CPS-I activity is the initial and rate-limiting stage of this cycle.

Arginine, aspartate, and glutamate contained in whey protein expand the substrate base of the ornithine cycle [1,2,8]. Experimental results show that a decrease in blood ammonia concentration and an increase in urea levels reflect activation of the urea cycle under the influence of whey protein [8,9]. This process is characterized by:

an increase in CPS-I activity, resulting in enhanced carbamoyl phosphate formation;
a rise in ornithine levels, which accelerates cycle turnover;
intensified aspartate flux, which stimulates argininosuccinate synthesis and breakdown;
and elevated arginase activity, which promotes urea production [9].

5. Whey protein, hepatic protein synthesis, and detoxification reserve

An increase in plasma albumin and total protein indicates enhanced protein biosynthesis in the hepatic parenchyma [10]. Albumin not only maintains oncotic pressure but also participates in the transport of numerous endogenous and exogenous substances, binds free radicals, and contributes to antioxidant defense [5,10]. Whey protein delivers high-quality amino acids to hepatocytes, supports their anabolic state, and accelerates reparative and regenerative processes [2,10].

Histological observations show that administration of whey protein in moderate doses improves the nucleus–cytoplasm ratio of hepatocytes, increases the number of mitochondria, and reduces degenerative changes, which indicates structural and energetic adaptation of liver cells [6,10].

METHODOLOGY. This study was aimed at determining the effect of whey protein on hepatic metabolic activity, amino acid transamination, and the enzymatic response of the urea cycle. The research design was based on an experimental model with a comprehensive analysis of biochemical parameters, enzyme activities, and the dynamics of nitrogen metabolism products in the liver.

The experiment lasted 30 days and involved four groups of animals (n = 40):

1. control group – no whey protein supplementation;
2. S1 group (0.5 g/kg) – low-dose whey protein;
3. S2 group (1.0 g/kg) – moderate physiological dose;
4. S3 group (1.5 g/kg) – high dose of whey protein.

Whey powder was administered orally once daily together with feed. The whey protein used in the study consisted of β-lactoglobulin, α-lactalbumin, immunoglobulins, lactoferrin, and serum albumin, with ratios corresponding to modern technological standards [1,4].

The following parameters were measured during the experiment:

ALT (alanine aminotransferase) activity [5];
AST (aspartate aminotransferase) activity [5,6];
blood ammonia (NH_3) concentration [7,9];

urea concentration [8,9];

CPS-I (carbamoyl phosphate synthetase-1) activity [9];

total protein and albumin levels in blood [10];

concentrations of glutamate, glutamine, ornithine, and alanine [3,8];

histological structure and changes in liver tissue [6].

The data obtained were analyzed by ANOVA, and intergroup differences were evaluated using the Tukey HSD test. A significance level of $P < 0.05$ was accepted [17].

ANALYSIS. In the experimental groups, ALT and AST activity was significantly higher than in the control group, indicating that whey protein acts not as a simple metabolic burden but as a substrate that activates transamination processes [5,7]. An increase in ALT activity reflected enhanced alanine–pyruvate exchange and activation of the glucose–alanine cycle, which denotes a higher nitrogen flux from muscle to liver and, as a consequence, increased glutamate synthesis as a by-product [7,8]. Elevated AST activity indicated enhanced aspartate flux into the urea cycle, since aspartate is the second major nitrogen donor in urea formation [9].

In group S2 (1.0 g/kg), ALT and AST activities were the highest among all experimental groups, suggesting that this dose of whey protein is an optimal metabolic stimulator for the hepatic transamination apparatus. Increasing the dose to 1.5 g/kg in group S3 did not significantly augment the metabolic response, indicating that a physiological “plateau” had been reached [2,7].

A marked increase in glutamate and glutamine concentrations demonstrated that the central pools of amino acid metabolism were reinforced [3,8]. Glutamate occupies a pivotal position in most transamination reactions, whereas glutamine serves as the main nitrogen “reservoir” and carrier of ammonia in a non-toxic form. A 20–35 % rise in glutamine synthesis in animals receiving whey protein indicated strengthening of the system that temporarily binds ammonia in the organism [3].

A decrease in blood ammonia levels combined with a rise in urea concentration confirmed that whey protein activated the urea cycle at the enzymatic level [8,9]. The increase in CPS-I activity showed that carbamoyl phosphate formation was accelerated; higher ornithine levels suggested faster turnover of the cycle [9]. Furthermore, enhanced aspartate flux intensified argininosuccinate synthesis and cleavage, while increased arginase activity promoted more rapid urea formation [9].

Higher total protein and albumin levels in blood reflected activation of protein biosynthesis in the hepatic parenchyma [10]. Positive changes in albumin content directly affected oncotic pressure, transport functions, and the efficiency of the antioxidant system [5,10].

Histological analysis in group S2 showed improvement in the morpho-functional state of hepatocytes, an increase in the number and activity of mitochondria, and a reduction in degenerative changes [6].

RESULTS. Based on the findings:

transamination processes — ALT and AST activities increased significantly under the influence of whey protein, indicating intensified amino acid metabolism;

the glutamate–glutamine system — central pools of amino acid metabolism were strengthened, and nitrogen transport improved;

the urea cycle — detoxification of ammonia in the form of urea accelerated by approximately 30–45 %, with increased activities of CPS-I and other cycle enzymes;

protein synthesis — total protein and albumin levels increased, reflecting enhanced functional status of the hepatic parenchyma;

optimal dose — group S2 (1.0 g/kg) showed the best results for all biochemical and histological indicators;

hepatic energy reserves — mitochondrial activity increased, and detoxification capacity of the liver was elevated.

These results confirm that whey protein is a high biological value nutrient that supports hepatic metabolism [1–3,8–10].

CONCLUSION. Whey protein supports two key mechanisms of hepatic nitrogen metabolism: amino acid transamination and the urea cycle. It increases the activity of transamination enzymes, expands the pools of glutamate and glutamine, ensures rapid and efficient detoxification of ammonia in the form of urea, and improves the energetic and metabolic activity of hepatocytes [1–3,7–9].

A moderate dose of whey protein (1.0 g/kg) demonstrated the highest efficiency, providing a solid scientific basis for its use as a biologically active dietary supplement. The use of whey protein appears justified for supporting liver function, alleviating the consequences of intense physical exertion, in diet therapy regimens, as well as in veterinary practice to stimulate growth and productivity and to improve metabolic stability [11–16].

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