Effect of a high amino acid diet on antioxidant barrier parameters of rat skin. Part 1

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ABSTRACT

Introduction: Oxidative stress is largely responsible for numerous skin complications that occur in the course of various diseases as well as accelerated skin aging. A high amino acid diet, supplemented with whey protein concentrate (WPC), is well-balanced and has well-absorbing proteins, which are an ideal source of essential amino acids.

Purpose: To assess what changes will occur in the antioxidant barrier of unharmed skin of rats on a high amino acid diet.

Materials and methods: The study was conducted on sexually mature male Wistar rats (160-180g): 1. control (standard feed), 2. high amino acid diet (WPC-80 80% whey protein) administered for 7 days at a dose of 0.3g/kg of body weight, 3. WPC-80 for 7 days at a dose of 0.5g/kg of body weight, 4. WPC-80 for 14 days at a dose of 0.3g/kg of body weight, 5. WPC-80 for 14 days at a dose of 0.5g/kg of body weight. Total antioxidant capacity, total oxidative status and oxidative stress index were determined.

Results: Enrichment of a standard diet with WPC-80 did not affect the total oxidative status of undamaged healthy rat skin. This study shows that a diet rich in amino acids in rats caused an increase in total antioxidant capacity, but statistically significant values were obtained after 14 days of administering WPC at a dose of 0.5mg/kg of body weight.

Conclusions: Enrichment of a standard diet with WPC-80 strengthens the antioxidant barrier in unwounded healthy rat skin.

Keywords: Whey protein concentrate, oxidative stress, skin, rat

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INTRODUCTION

Whey Protein Concentrate (WPC), a protein isolate of animal origin, contains high concentrations of a full spectrum of essential (exogenous) amino acids [1]. Just 14 grams of whey protein covers the daily requirement of an adult for essential amino acids [2]. In this article, a high amino acid diet describes a properly balanced diet, in which additional supplementation with whey protein concentrate (WPC-80) consisting of highly available and compatible with the body essential amino acids, both exogenous and endogenous.

Oxidative stress is a process that results in an increased amount of reactive oxygen species (ROS) in the body that can lead to cellular structure damage. ROS are constantly generated, but a reason for their increase is a disproportion between ROS formation and the ability of enzymatic systems to neutralize them. ROS creation processes require the occurrence of mechanisms that protect the cell from damage as a result of oxidative stress. These mechanisms form an antioxidant barrier in the body as well as in the skin exposed to exogenous agents [3]. Antioxidants are compounds that are constantly present in the cells at low concentrations. The oxidation inhibition mechanism is one- or two-stage. The molecules of reactive oxygen species are converted into radicals with weaker activity. It is not fully possible to measure single antioxidant molecules, and the antioxidant activity of these molecules is additive, which is why measurements of total antioxidant capacity (TAS) and total oxidative status (TOS) are performed [4]. Total antioxidant capacity (TAS) is the body's overall possible antioxidant activity, without dividing them by their origin or mechanism of action. TAS is a key parameter that enables estimating the capacity of the body, organ or tissue to prevent oxidative stress [5]. Total oxidative status (TOS) is the body's overall possible oxidant activity, also without dividing them by origin or mechanism of action [6]. Due to the fact that a cell's oxidant and antioxidant potential can undergo constant changes, the concept of oxidative stress index (OSI) was introduced, which is an indicator showing changes in the dependence of these potentials and defined as the ratio of TOS to TAS [7].

Skin exposure to free oxygen radicals, internal and external, causes the activation of many mechanisms aimed at their elimination and prevention, resulting in oxidative stress development. Oxidative stress is largely responsible for numerous skin complications that occur in the course of various diseases as well as accelerated skin aging. Numerous studies have described the behavior of biochemical parameters in developed skin lesions or wound healing processes in various disease models, after using a diet with WPC as a diet to support treatment. However, little attention has been paid to changes occurring in skin subjected to a high amino acid diet and no clinically visible changes. Therefore, the aim of this study was to answer the question of how a high amino acid diet affects total oxidative status and total antioxidant capacity of unwounded healthy rat skin.

MATERIALS AND METHODS

The Local Ethics Committee for Experiments on Animals in Bialystok approved the study (No. 12/2011). The study was conducted on sexually mature 14-month-old, male, Wistar strain rats, with an initial body weight of 490-530 grams, from the Department of Experimental Pharmacology Medical University of Bialystok.

The animals were housed individually in cages in animal rooms at the Center for Experimental Medicine Medical University of Bialystok. Throughout the experiment, the animals were provided with standard breeding conditions (20-21°C, 12 hours light/12 hours dark cycle, humidity depending on external environmental conditions) and had unrestricted access to drinking water. For 5 days, the animals acclimatized to the new conditions. During this time, they were fed a balanced granulated feed (Harsteller, Germany) (36% proteins, 54% carbohydrates, 10% fats). After 5 days, the rats were divided into five groups of ten animals each:

- control (C) (standard feed),
- 0.3/7d WPC-80 group (WPC-80 intragastrically, once a day, administered for 7 days at a dose of 0.3 g/kg of body weight),
- 0.5/7d WPC-80 group (WPC-80 intragastrically, once a day, administered for 7 days at a dose of 0.5 g/kg of body weight),
- 0.3/14d WPC-80 (WPC-80 intragastrically, once a day, administered for 14 days at a dose of 0.3 g/kg of body weight),
- 0.5/14d WPC-80 (WPC-80 intragastrically, once a day, administered for 14 days at a dose of 0.5 g/kg of body weight).

WPC-80 was obtained free of charge from the Dairy Cooperative in Mońki, in 2010. WPC-80 was weighed every day and its amount was calculated in accordance with the animal weight. Just before administration, it was mixed with drinking water using a mechanical stirrer. After short-term immobilization of the rats in a specially constructed device, a solution of WPC-80 in a volume of 0.5 mL in two doses of 0.3 g/kg of body weight or 0.5 g/kg of body weight was administered within approximately 30 seconds using a cannula inserted into the stomach [8].

WPC-80 was administered once a day between 8 and 10 a.m. for 7 or 14 days. Rats in the control group received drinking water (0.5 mL) intragastrically using the same time regiments.

After the appropriate experiment time passed, under ketamine anesthesia (80 mg/kg of body weight) with xylazine (5 mg/kg of body weight) given intraperitoneally, fragments of shaved back skin were collected (approximately 2 cm^2, full skin section) from all the examined animals. Tissues were collected by one experienced operator. Samples were weighed, deep frozen in liquid nitrogen, and stored at -80° C. The tissues were homogenized and sonicated, and the obtained homogenates were centrifuged Total antioxidant capacity (TAS) (Randox, Crumlin, UK) and total oxidative status (TOS) (PerOx, TOS/TOC, Immune Diagnostic, Bensheim, Germany) were determined in the supernatant fluid. Oxidative Stress Index (OSI) was calculated by TOS/TAS x 100. All determinations were performed in duplicates.

Lack of a normal distribution of the tested parameters was the basis for using nonparametric methods. An assessment of differences in the distribution of quantitative variables between the groups was performed using the ANOVA Kruskal-Wallis and the median test. The distribution of values of individual quantitative parameters was described using the median (minimum-maximum). Nonparametric Spearman's correlation coefficients were used to assess the relationship between quantitative variables [9]. Statistical calculations were done using Statistica (StatSoft, Krakow, Poland). A level of $p < 0.05$ was considered as statistically significant.

RESULTS

Median total antioxidant capacity was significantly higher in the 0.5/14d group compared with the remaining groups (C p=0.001; 0.3/7d p=0.001; 0.5/7d- p=0.001; 0.3/14d p=0.001, respectively) (Table 1).

Table 1. Total antioxidant capacity (TAS), total oxidative status (TOS), and oxidative stress index (OSI) of the skin of rats from the studied groups

Group	TAS (mol/mg total protein) M (min.-max.)	TOS (mol/mg total protein) M (min.-max.)	OSI M (min.-max.)
C	1.83 (1.65-2.01)	0.019 (0.01-0.021)	10.01 (8.11-14.14)
0.3/7d	1.85 (1.57-1.99)	0.02 (0.01-0.028)	9.88 (6.21-11.55)
0.5/7d	1.86 (1.63-1.95)	0.19 (0.01-0.025)	9.03 (6.09-12.21)
0.3/14d	1.89 (1.66-1.95)	0.18 (0.011-0.019)	8.59 (6.99-10.47)
0.5/14d	**2.16 (1.91-2.43)** ♦■●▲	0.02 (0.009-0.017)	**7.04 (6.31-8.83)** ♦■●▲

Abbreviations: **C**- control group, **0.3/7d**- group with a high amino acid diet with WPC-80 administered for 7 days at a dose of 0.3g/kg of body weight, **0.5/7d**- group with a high amino acid diet with WPC-80 administered for 7 days at a dose of 0.5g/kg of body weight, **0.3/14d**- group with a high amino acid diet with WPC-80 administered for 14 days at a dose of 0.3g/kg of body weight, **0.5/14d**- group with a high amino acid diet with WPC-80 administered for 14 days at a dose of 0.5g/kg of body weight, **M**- median, **min.**- minimum, **max.**- maximum, ♦- statistical significance *vs.* C ($p<0.05$), ■- statistical significance *vs.* 0.3/7d ($p<0.05$), ●- statistical significance *vs.* 0.5/7d ($p<0.05$), ▲- statistical significance *vs.* 0.3/14d ($p<0.05$).

Supplementation of the standard diet with WPC-80 administered in various doses and for various periods of time did not affect the medians of total oxidative status of the skin of rats from the studied groups (Table 1).

Median oxidative stress index of unwounded rat skin was significantly higher in the 0.5/14d group compared with the remaining groups (C p=0.001; 0.3/7d p=0.001; 0.5/7d- p=0.001; 0.3/14d p=0.001, respectively) (Table 1).

The total antioxidant capacity (TAS) of rat skin positively correlated with the time of WPC-80 administration (very high correlation, p=0.019, r=0.721). Oxidative stress index correlated negatively with the time of examination (very high correlation, p=0.015, r=-0.735). The total antioxidant capacity (TAS) of rat skin positively correlated with WPC-80 dose (very high correlation, p=0.025, r=0.696). Oxidative stress index correlated negatively with the dose of dietary supplementation (high correlation, p=0.043, r=-0.648). Oxidative stress index correlated negatively with the total antioxidant capacity level (very high correlation, p=0.011, r=-0.878).

DISCUSSION

Human skin is an organ fulfilling a number of different functions, and unlike animal skin, it is also a source of aesthetic experiences for the environment. Skin cell disorders affect the preservation of the right barrier protecting from the

external environment and ensuring homeostasis of the whole organism. For the proper functioning of the whole skin, it is very important to provide substrates for the production and reproduction of protein structures. These substrates are endogenous and exogenous amino acids. The coenzymes of these processes are vitamins, especially A, E, and C, which are also free radical scavengers [10].

In the available literature, a number of positive effects of WPC supplementation has been documented. These include, among others, antioxidant and anticancer activity, resulting from high cysteine and methionine content, which are substrates used for intracellular glutathione (GSH) synthesis, a compound with antioxidant and free radical eliminating activity, stimulating leukocyte activity and modulating immune system function [11,12]. In addition, tumor necrosis factor α (TNF-α), present in whey at concentrations below 10ng/L, stimulates the release of inflammatory response mediators (INF-γ, IL-6, IL-8), inhibits tumor cell proliferation, and induces the apoptosis process [13].

The literature data indicate that WPC supplementation positively affects circulation through the hypotensive activity of α-lactorphine and β-lactorphine. Both α-lactorphine and β-lactorphine block the angiotensin-converting enzyme, thereby limiting blood vessel contraction. According to some researchers, nitric oxide (NO) plays a key role in the antihypertensive activity of α- and β-lactorphines, since their use together with NO synthase inhibitors removes their antihypertensive effect [14]. In people diagnosed with prehypertension or hypertension, it has been shown that WPC normalized blood pressure and did not cause hypotension [15]. Zhang et al. proved in their research that WPC has a positive effect on the blood lipid metabolism, which is important in people with atherosclerosis [16]. Improved circulation promotes improved tissue metabolism, and this can improve ROS elimination. The effect of reducing the amount of reactive oxygen species can also be the result of the antimicrobial and antiviral activity of WPC. It is a product rich in proteins from the immunoglobulin superfamily. The vast majority are immunoglobulin G, immunoglobulin A, and immunoglobulin M. They are responsible for the elimination of pathogens. Immunoglobulins block enzyme systems, inhibiting bacterial cell metabolism. They counteract the adhesion of pathogenic microorganisms to endothelial cells. They neutralize bacterial and viral toxins and mainly initiate a specific immune response [17].

Antibacterial and antiviral activity is also demonstrated by lactoferrin, a glycoprotein from the transferrin superfamily. It has binding properties of metal ions (Fe^{2+}, Al^{3+}, Co^{3+}, Cu^{2+}, Zn^{2+}), reducing their availability for microorganisms. It also demonstrates the ability to release lipopolysaccharides from the external cell walls of Gram-negative bacteria, inhibiting microbial cell growth [18]. WPC proteins stimulate anti-inflammatory cytokines (IL-4 and IL-10) and lower the concentration of proinflammatory cytokines (IL-1 and TNF-α) in an induced colitis animal model [19]. β-lactoglobulin is also a substrate for the production of antibacterial peptides. Changes modifying its structure lead to an increased immune response to viral infections. Whereas, α-lactalbumin successfully inhibits the activity of *Escherichia coli*. Modifications to its structure in the gastrointestinal tract activate the inhibition of HIV-1 replication [14, 15,20,21]. Elimination of early stages of infection significantly affects maintaining a cell's oxidative potential.

It should be noted that WPC has anabolic and corrective activity. The most frequently occurring growth factors in whey and WPC (0.5% whey proteins) are insulin-like growth factors (IGF-1, IGF-2), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β) and fibroblast growth factor 2 (FGF-2) [22, 23]. IGF-1 stimulates protein synthesis and indirectly stimulates the production of growth hormone (GH), which results in increased body weight. IGF-2 also has anabolic activity [24]. Platelet-derived growth factor directly stimulates cells to grow and divide. It also increases the synthesis of collagen and glycosaminoglycans in the process of wound healing [25]. Transforming growth factor β increases cell proliferation and differentiation. It stimulates damaged tissue regeneration and the movement of epithelial cells, restoring epithelial barrier continuity [26,27]. Fibroblast growth factor 2 (FGF2), stimulating the synthesis of intracellular DNA, increases the proliferation of fibroblasts, melanocytes, chondrocytes, and smooth muscle cells [23,27].

The skin, as the body's barrier, contains a well-organized system of chemical and enzymatic antioxidants that are able to act in a synergistic manner. Complexes of skin antioxidants protect cells from damage by oxygen free radicals and prevent the production of oxidation products of proteins, cell membrane lipids and DNA. When oxidative stress significantly weakens the skin's antioxidant ability, subsequent modification of the REDOX processes taking place in the cell leads to the impairment of cellular homeostasis, which results in the generation of cell degenaration processes. In the skin, these processes are additionally intensified by external environment factors. Thus, the skin's antioxidant barrier plays a very important role in maintaining skin cell homeostasis [28].

Along with the increase in the human lifespan, problems related to various metabolic disorders arise, disrupting proper cell functioning, which occurs infrequently in young people. Due to the prolongation of life expectancy and the average age of the population, skin changes will become more and more common.

Another problem is the skin aging process and increased risk of developing precancerous lesions as well as cancer. Risk factors for more frequent incidence of this type of disease are immunosuppression resulting from the patient's underlying disease, iatrogenesis, age, and chronic exposure to ultraviolet radiation. It has long been known that excessive exposure to the sun is one of the significant factors responsible for skin cancer development. The effects of ultraviolet radiation are mutagenic as well as damaging to the protein structures responsible for the proper course of numerous cellular metabolic pathways. In younger skin with efficient repair mechanisms, these problems are unnoticeable. Also, photoprotection significantly limits the development of the tumorigenesis process.

Until now, skin protection from the action of reactive oxygen species has been based on the use of preparations applied topically on the skin. However, there are few scientific reports on the compensation of oxygen free radical activity through the use of dietary supplementation with suitable oral protein preparations. Whey and the protein concentrates obtained from it (WPC) are a great source of amino acids and other natural active ingredients necessary to create proteins in the body, as well as to ensure proper metabolic cell function. They are also a starting point for the reproduction and regeneration of endogenous enzyme systems that provide protection from the effects of excess reactive oxygen species [10].

The relationship between convalescence and proper diet has been emphasized many times in the literature, because a reduced protein intake limits many metabolic processes, including collagen production associated with the healing process, especially in the proliferation phase. Providing the right amount of proteins, micronutrients, and vitamins in this period can be important for particular stages of wound healing [29].

Recent research indicates that whey may increase the body's antioxidant activity, has anti-inflammatory effects by stimulating the immune system, and develops food tolerance by regulating the activity of regulatory T cells (Tregs). A diet rich in amino acids, with WPC, reduces the risk of bacterial infections due to the presence of lactoferrin, which inhibits bacterial proliferation [30].

This study shows that a diet rich in amino acids in rats caused an increase in TAS (total antioxidant capacity), but statistically significant values were obtained after 14 days of administering WPC at a dose of 0.5 mg/kg of body weight. The trend of TAS growth was already visible after 7 days, but the result was not statistically significant. The observed increase in TAS can be explained by the fact that the longer supplementation of amino acids contained in the WPC diet allowed to saturate the demand for amino acids and obtain a higher concentration of the non-enzymatic antioxidant, glutathione. An increase in TAS as a result of supplementation was also indicated by other authors, who were investigating the effect of supplements on the intensification of oxidative stress and wound healing [10].

The use of a whey diet also affected the oxidative stress index (OSI). A statistically significant decrease in OSI values was achieved in the same way as in the case of TAS only after 14 days of using a diet rich in amino acids at a dose of 0.5mg/kg of body weight. There were no significant changes in total oxidative status (TOS) in any of the studied groups, which indicates that a diet rich in amino acids using WPC-80 did not cause oxidative stress. Observation is a beneficial factor indicating the safety of this type of supplementation, allowing to improve the cell's antioxidant capacity and inhibit the development of some skin pathologies associated with the action of reactive oxygen species.

CONCLUSIONS

Enrichment of a standard diet with WPC-80 did not affect total oxidative status of unwounded healthy rat skin. Enrichment of a standard diet with WPC-80 administered at a dose of 0.5g/kg of body weight for 14 days increased total antioxidant capacity of unwounded healthy rat skin.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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