Effect of a high amino acid diet on antioxidant barrier parameters of rat skin. Part 2

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ABSTRACT

Purpose: The imbalance between the formation of reactive oxygen species and antioxidant capacity of the body is known as oxidative stress. Exposition of the skin to free radicals, the origin of the internal and external causes activation of multiple mechanisms to eliminate them and prevent in this way the development of oxidative stress. The aim of this experiment was examining what changes are taking place in the antioxidant barrier of unwounded healthy skin of rats, who are on a high amino acids diet for 7 and 14 days at administered doses of 0.3 and 0.5 g/kg body weight.

Materials and Methods: The study was performed on male Wistar rats divided into 5 groups: 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. The concentration of superoxide dismutase 2 and 3, the concentration of catalase specific activity of glutathione peroxidase, the concentration of glutathione and total protein content were determined.

Results: The supplementation of the standard diet by the preparation of WPC-80 administered in a dose 0.5 g/kg body weight for 14 days containing methionine and cysteine (essential amino acids involved in the formation of glutathione), significantly increases the concentration of reduced glutathione.

Conclusions: Enrichment of a standard diet with WPC-80 caused by the significant increases of non-enzymatic antioxidant.

Keywords: WPC-80, skin, rat

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INTRODUCTION

Introducing targeted diet modification may provide or limit the amount of those components that are needed or unnecessary to the body. Such individualization of the diet may consist of changing the methods of preparing products for consumption or introducing modifications in the proportions of ingredients supplied. This may be necessary to achieve tangible benefits in the form of increasing the efficiency of anabolic or catabolic processes. Such a way of feeding is called an alternative diet [1].

WPC is a very efficient source of bodybuilding substrates [2,3].

For 100 g dry weight of WPC-80, the protein is 77.7 g (essential amino acids, exogenous and endogenous), 6.4 g for lipids and 8.5 g for carbohydrates. The caloric value per 100 g of the preparation is 402 kcal (1702 kJ) [4].

Powdered forms of whey are used as an ingredient of high-protein nutrients for infants, convalescents and athletes [5,6], functional foods [7] and anticancer preparations [8,9], used in the prevention and treatment of osteoporosis [10], lowering blood pressure and decreasing the heart rate [11].

The mitochondrial electron transporting system involved in cellular respiration provides free oxygen radicals formed from molecular oxygen in physiological processes. As red-ox reaction products, they are necessary to transmit information to and from cells to regulate their internal metabolism. The intracellular hydrogen peroxide (H_2O_2), not being a radical, is a very reactive molecule that during the oxidation of ferrous or cuprous ions transforms into the hydroxyl radical $^{\bullet}OH$ [12].

H_2O_2 is one of the substrates in peroxidase-catalyzed reactions (GSH-Px) or catalase (CAT) [13]. In contrast, mitochondrial superoxide dismutase (SOD), in the process of spontaneous or enzymatic dismutation, converts the superoxide anion radical ($O_2^{\bullet}$) into hydrogen peroxide [14].

Various types of antioxidants are involved in building an antioxidant barrier:

1. preventive, which inhibit the production of reactive oxygen species, including superoxide dismutases, catalase or peroxidase,
2. scavengers of free radicals whose task is to break the oxidative chain reaction including albumin, uric acid and vitamins,
3. repair enzymes that remove the effects of oxidation of proteins, genetic material and lipids, including proteases, lipases, DNA repair enzymes and transferases [15].

Another division of antioxidants divides them into enzymatic and non-enzymatic ones [16].

The aim of this work is to find an answer to the question of whether the high-amino acid diet affects the activity of enzymatic and nonenzymatic antioxidants of the skin?

MATERIALS AND METHODS

This study was approved by the Local Ethics Committee for Experiments on Animals in Bialystok (No. 12/2011). The study was conducted on sexually mature 14-month-old, Wistar male obtained from the Department of Experimental Pharmacology Medical University of Bialystok. The animals were housed individually in cages in animal rooms (20-21°C, 12 hours light/12 hours dark cycle, humidity depending on external environmental conditions, balanced granulated feed (Harsteller, Germany) (36% proteins, 54% carbohydrates, 10% fats)) at the Center for Experimental Medicine Medical University of Bialystok. After 5 days, 50 rats were divided into five groups:

1) control (C) (standard feed),
2) 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),
3) 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),
4) 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),
5) 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. The 0.5 mL solution of WPC-80 (with drinking water) was administered (0.3 g/kg of body weight or 0.5 g/kg of body weight) into the stomach [17].

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.

At the end of the experiment, under anaesthesia of ketamine (80 mg/kg of body weight) with xylazine (5 mg/kg of body weight), fragments of shaved back full skin section were collected (2 cm^2). 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. The concentration of superoxide dismutase 2 (SOD2) and 3 (SOD3), the concentration of catalase (CAT) specific activity of glutathione peroxidase (GSH-Px), the concentration of glutathione (GSH) and total protein content (tP) were determined in the supernatant fluid. All determinations were performed in duplicates.

We applied nonparametric statistic methods (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 [18]. Statistical calculations were done using Statistica 13 (StatSoft, Cracov, Poland). A level of $p < 0.05$ was considered as statistically significant.

RESULTS

We have shown that supplementation of the standard diet with WPC-80 administered in different doses and for various periods of time does not affect medians of SOD2, SOD3, CAT and GSH-Px concentrations of rat skin from the studied groups (Table 1).

We showed that the median concentration of reduced glutathione was significantly higher in the group 0.5/14d in comparison to the other groups (respectively, ♦ - $p = 0.0001$, ■ - $p = 0.0001$, ● - $p = 0.0002$, ▲ - $p = 0.0002$) in the skin of rats (Table 1).

Supplementation of the standard diet with WPC-80 administered in a dose of 0.3g/kg body weight for 14 days (0.3/14d) significantly increases the total protein content in the skin of rats in comparison to the K group ($p=0.0006$) and group 0,3/7d ($p=0.0008$) and given in a dose of 0.5g/kg body weight for 14 days (0.5/14d) significantly increases the total protein content compared to the K group ($p = 0.00001$), groups 0.3/7d ($p = 0.00002$), groups 0.5/7d ($p = 0.007$) and groups 0.3/14d ($p = 0.001$) (Table 1).

In addition, we showed that the concentration of highly positive glutathione ($p = 0.029$, $r = 0.684$), and the total protein content almost full positive ($p = 0.001$, $r = 0.903$) correlates with the time of the experiment.

The concentration of reduced glutathione and total protein content also high positively ($p = 0.022$, $r = 0.709$, $p = 0.031$, $r = 0.501$) correlate with the dose of the given WPC-80 preparation.

Table 1. The concentration of superoxide dismutase 2 (SOD2) and 3 (SOD3), concentration of catalase (CAT) specific activity of gluthatione peroxidase (GSH-Px), the concentration of glutathione (GSH) and total protein content (tP) in unwounded healthy skin of rat

Group	SOD2 (µg/mg of protein) M (min.-max.)	SOD3 (µg/mg of protein) M (min.-max.)	CAT (µg/mg of protein) M (min.-max.)	GPx (µIU/mg of protein) M (min.-max.)	GSH (µg/mg of protein) M (min.-max.)	tP (mg/mL) M (min.-max.)
K	0.05 (0.02-0.065)	0.059 (0.029-0.079)	1.82 (1.32-1.99)	0.56 (0.31-0.87)	3.32 (1.78-3.55)	145.31 (122.51-167.66)
0,3/7d	0.05 (0.025-0.057)	0.055 (0.02-0.071)	1.79 (1.28-2.15)	0.59 (0.2-0.96)	3.41 (1.69-3.87)	146.26 (123.28-187.92)
0,5/7d	0.048 (0.029-0.062)	0.051 (0.02-0.062)	1.79 (1.26-2.21)	0.55 (0.41-0.83)	3.87 (1.85-4.01)	207.53 (173.2-240.1)
0,3/14d	0.049 (0.02-0.059)	0.05 (0.015-0.067)	1.8 (1.31-2.26)	0.56 (0.39-0.91)	3.85 (1.96-3.99)	**247.69 (225.11-288.32)** ♦■
0,5/14d	0.053 (0.028-0.062)	0.055 (0.009-0.073)	1.83 (1.04-2.31)	0.58 (0.39-0.88)	**5.66 (4.04-6.25)** ♦■●▲	**369.91 (308.3-411.82)** ♦■●▲

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

DISCUSSION

Free radical scavengers are unevenly distributed in the individual layers of the skin. Available publications show that the highest concentration of substances responsible for scavenging free radicals is present in the upper layers of the epidermis, which the authors associate with a higher partial pressure of oxygen. In the epidermis, compared to the dermis, higher concentrations of catalase, glutathione peroxidase, glutathione reductase and ascorbic acid have been demonstrated. The stratum corneum proteins also require protection against active forms of oxygen. In this layer, the protective role is played by vitamins C and E and uric acid [19]. 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 degeneration processes [14].

The diet is an important protection factor, or it promotes the development of cancer. Many research models examine the effectiveness of using a diet based on whey proteins. In experimental studies on animal models, it was observed that whey proteins increase the level of glutathione in cancer cells. The result of this process is slowing the growth of mammary gland or colon tumours [20,21]. In rats where colorectal tumours were experimentally induced, the incidence of tumours decreased by as much as 40% after using the diet with the whey protein preparation [22]. Subsequent experiments showed that whey proteins significantly statistically reduced the incidence and growth of tumours formed under the influence of a carcinogenic substance - dimethylhydrazine. It was found that after the use of whey proteins, the area of the intestine occupied by the tumour significantly decreased. It was also shown that the highest level of intracellular glutathione, whose concentration was evaluated in hepatic cells, was in the group of rats receiving whey [23,24]. Other research results describe the effect of whey on the antioxidant potential, inhibiting the negative effects of androgens, which even at physiological concentrations are able to reduce the level of glutathione in prostate cells, enabling the development of prostate cancer [20]. Although the skin is quite often exposed to oxidative stress, there are not many reports on the effects of supplements of nutrients, especially glutathione (GSH) improving the oxidation-reducing potential of the skin [19,25].

The place where glutathione is synthesized in the body is the liver that is responsible for the body's detoxification processes. The glutathione synthesis process is limited by the number of substrates available; mainly cysteine, which in the free state is toxic and easily oxidized [20,26]. In the body, this amino acid is mainly present in the oxidized form called cystine (a stable form) resulting from the joining of two molecules of a cysteine amino acid through a disulphide bond. At the moment of transport, cystine is immediately reduced to the two cysteine molecules [26]. In these studies, we showed that an increase in glutathione levels reduced in the skin is possible, however statistically significant differences occurred only with the dose of 0.5 g kg / m WPC-80 used in the diet for 14 days. These results suggest that effective supplementation should take longer. In the work of other authors, glutathione has been shown to increase the oxidation-reduction potential of the skin through synergistic interaction with vitamin C and vitamin E. This ability applies only to live cells and only when glutathione is synthesized in the cell. The local application on the skin does not bring any benefits, because glutathione does not penetrate through the stratum corneum due to the too large molecular weight (307 kD) [25]. Perhaps the protective effect of this scavenger of free radicals secures only living cells, whereas cells that are terminally differentiated do not require this type of protection.

The high nutritional values of proteins isolated from whey are evidenced by the results of nutritional tests conducted mainly on rats. Indicators of protein growth rate and biological value (the amount of protein processed for anabolic purposes in relation to the protein taken from the diet, digested and absorbed) for whey proteins significantly exceed their reference values (the index was calculated as 100 for egg proteins) [7]. At the base of high biological value of whey protein preparations lies the content of the full spectrum of amino acids, including exogenous amino acids such as methionine and cysteine, necessary for the production of glutathione (GSH), which is a non-enzymatic factor that increases skin oxidation potential. The extremely rich composition of WPC creates the possibility of using this type of protein concentrate in patients requiring supplementation, among others after surgical procedures to accelerate convalescence, support wound healing, after severe infectious diseases, in the period of increased demand for building materials. Being a rich source of proteins, they can accelerate and at the same time have a positive influence on the tissue regeneration process in patients after injuries or after surgery [27].

It is known that not only GSH can contribute to reducing the number of free oxygen radicals in the cell. After evaluation, in this study, the activity of enzymatic systems responsible for the scavenging of free oxygen radicals showed that there is no statistically significant difference between the control group and the activity of CAT, SOD2 and SOD3 and GSH-Px. Other authors also point to the major role of glutathione as a diet-induced antioxidant than to the activity of other cell enzyme systems responsible for the removal of free oxygen

radicals. However, synergistic enzymatic, as well as redox systems [28].

Nowadays, many models of a healthy lifestyle are promoted and selecting them from the one that "does not hurt" can be very important for the development and prevention of many civilization diseases. It seems that such an alternative in the light of numerous studies gives the enrichment of the standard diet with whey proteins.

CONCLUSIONS

The supplementation of the standard diet by the preparation of WPC-80 administered in a dose 0.5 g/kg body weight for 14 days containing methionine and cysteine (essential amino acids involved in the formation of glutathione) cause by the significant increases of non-enzymatic antioxidant.

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This study was unfunded.

Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

1. Reguła J. Characteristics and evaluation of various alternative diets. Forum Zaburz Metabol. 2013;4:115-21 (Polish)
2. Król J, Litwińczuk A, Zarajczyk A, Litwińczuk Z. Alpha-lactoglobulin and beta-lactoglobulin as biologically active compounds of the milk protein fraction. Med Wet. 2008;64(12):1375-78 (Polish)
3. Leman J. Whey protein as a food allergy factor in humans. Prz Mlecz. 2001;2:82-5 (Polish)
4. Koprowicz T. Evaluation of the action of whey protein concentrate (WPC-80) and its interaction with metronidazole on selected biochemical and morphological parameters of blood in rats. Dissertation, Medical University of Bialystok:2013 (Polish)
5. Krissansen GW. Emerging health properties of whey proteins and their clinical implications. J Am Col Nutr. 2007 Dec;26(6):713S-23S
6. Czerwionka-Szaflarska M, Zawadzka-Gralec A. Food allergy in infants and children - symptoms of diagnostics, treatment. Pol Merkuriusz Lek. 2007;138:443-8 (Polish)
7. Szczurek W. Whey processing products and their use in fodder for broiler chickens - nutritional and physiological aspects. Wiad Zootech. 2008;4:41-52 (Polish)
8. Madureira AR, Pereira CI, Gomes AMP, Pintado ME, Malcata FX. Bovine whey proteins - overview on their main biological properties. Food Res Int. 2007 Dec;40(10):1197-211
9. Tsai WY, Chang WH, Chen CH, Lu FJ. Enchancing effect of patented whey protein isolate (Immunocal) on cytotoxicity of an anticancer drug. Nutr Cancer. 2000;38(2):200-8.
10. Kruger CL, Marano KM, Morita Y, Takada Y, Kawakami H, Kobayashi T, Sunaga M, Furukawa M, Kawamura K. Safety evaluation of a milk basic protein fraction. Food Chem Toxicol. 2007 Jul;45:1301-7.
11. Pins JJ, Keenan JM. Effects of whey peptides on cardiovascular disease risk factors. J Clin Hypertens (Greenwich). 2006;8(11):775-82.
12. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. FEBS Lett. 2000 Dec;486(1):10-3.
13. Nulton-Persson AC, Szweda LI. Modulation of mitochondrial function by hydrogen peroxide. J Biol Chem. 2001 Jun;276(26):23357-61.
14. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012 Jan;5(1):9-19.
15. Battino M, Ferreir MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. J Clin Periodontol. 2002 Mar;29(3):189-94.
16. Bartosz G. The second face of oxygen. Free radicals in nature. Warszawa: Wydawnictwo Naukowe PWN; 2003, p35-120 (Polish)
17. Pérez-Cano FJ, Marín-Gallén S, Castell M, Rodríguez-Palmero M, Rivero M, Castellote C, Franch A. Supplementing suckling rats with whey protein concentrate modulates the immune response and ameliorates rat rotavirus-induced diarrhea. J Nutr. 2008 Dec;138(12):2392-98.
18. Stanisz A. An affordable statistics course using STASTISTICA PL on examples. Kraków: StatSoft; 2006, p288-91. (Polish)
19. Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K. Oxidative stress in aging human skin. Biomolecules. 2015 Apr;5(2):545-89.
20. Bounous G. Whey Protein Concentrate (WPC) and Glutathione Modulation in Cancer Treatment. Anticancer Res. 2000 Nov-Dec;20(6C):4785-92
21. Ripple MO, Henry WF, Rago RP, Wilding G. Pro-oxidant–antioxidant shift inducted by androgen treatment of human prostate carcinoma cells. J Natl Cancer Inst. 1997 Jan;89(1):40-8.
22. Hakkak R, Korourian S, Shelnutt SR, Lensing S, Ronis MJ, Badger TM. Diets containing whey proteins or soy protein isolate protect against 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors in female rats.

Cancer Epidemiol Biomarkers Prev. 2000 Jan;9(1):113-7.
23. Chatterton DEW, Smithers G, Roupas P, Brodkorb A. Bioactivity of β-lactoglobulin and α-lactalbumin - Technological implications for processing. Int Dairy J. 2006 Nov; 16(11):1229-40
24. McIntosh GH, Regester GQ, Le Leu RK, Royle PJ, Smithers GW. Dairy proteins protect against dimethylhydrazine - induced intestinal cancers in rats. J Nutr. 1995 Apr;125(4):809-16
25. Tsai FJ, Wang YD, Chen CC, Hsieh C, Cheng ZJ, Wu YJ. Evaluation of the antioxidative capability of commonly used antioxidants in dermocosmetics by in vivo detection of protein carbonylation in human stratum corneum. J Photochem Photobiol B. 2012 Jul;112:7-15
26. Bukowska B. Glutathione: biosynthesis, inducing factors and concentration in selected disease entities. Med Pr. 2004;55:501-9 (Polish)
27. Żebrowska M, Maciejczyk M, Sadowska A, Prokopiuk S, Krzyżanowska-Grycel E, Jamiołkowska-Stawowska M, Car H. Whey proteins and oxidative stress. [in]: Health in the 21st century: challenges, problems, dilemmas... Tom. 1. (red.) Jankowiak B, Kowalewska B, Rolka H. Lomza: Lomza State University of Applied Science, 2015: 165-73p. (Polish)
28. Haraguchi FK, Silva ME, Neves LX, dos Santos RC, Pedrosa ML. Whey protein precludes lipid and protein oxidation and improves body weight gain in resistance-exercised rats. Eur J Nutr. 2011 Aug;50(5):331-9.