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Chronic high-protein diet induces oxidative stress and alters the salivary gland function in rats

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ABSTRACT

Objective: Chronic high protein intake leads to an increase in reactive oxygen species (ROS) generation. However, there is no data on the impact of high-protein diet on the antioxidant barrier, oxidative stress and secretory function in the salivary glands of healthy individuals.

Design: 16 male Wistar rats were randomly divided into 2 groups (n = 8): normal protein (C) and high-protein diet (HP) for 8 weeks. Salivary antioxidants: peroxidase (Px), catalase (CAT), superoxide dismutase 1 (SOD 1), uric acid (UA), total antioxidant status (TAS), total oxidant status (TOS) and the oxidative stress index (OSI), as well as protein carbonyls (PC), 4-hydroxynonenal protein adduct (4-HNE protein adduct), 8-isoprostanes (8-isoP), 8-hydroxy-2′-deoxyguanosine (8-OHdG) and protein content were determined in the salivary glands and plasma. Salivary unstimulated and stimulated flow rates were examined.

Results: Parotid Px, TAS, UA, TOS, OSI, PC were significantly higher, the total protein content was statistically lower in the HP group as compared to the control. Submandibular UA, TOS, OSI, 8-isoP, 4-HNE-protein adduct, 8-OHdG were statistically elevated, SOD 1 and Px were significantly lower in the HP group as compared to the control rats. The unstimulated salivary flow rate was significantly depressed in the HP group as compared to the controls.

Conclusions: Higher antioxidant capacity in the parotid glands of HP rats *vs.* control rats seems to be a response to a higher ROS formation. In the submandibular glands severe oxidative modification of almost all cellular components was observed. Administration of HP resulted in the weakening of the salivary gland function.

1. Introduction

Evidence shows that protein intake increases in an industrialized population and reaches a level more than twice the WHO recommended intake (WHO, 1985). There is almost no data on the benefits or side effects of high protein intake for healthy adults. It was documented that undesirable metabolic changes occur when the protein intake is 1.6 or more times higher than the recommended values (Metges & Barth, 2000). However, physiological and functional consequences of a chronic high protein intake have not been fully explained.

In contrast to fatty acids and glucose, proteins, when present in excess of the current demand, cannot be stored and they are metabolically processed immediately. Evidence shows that an adaptation to HP is associated with an increase in the activity of enzymes involved in protein digestion and increased amino acid metabolism, including amino acid transport, transamination, oxidative deamination, as well as, the capacities mainly for gluconeogenesis less ureagenesis, to maintain amino acid homeostasis in the body (Jean et al., 2001; Peters & Harper, 1985). It should be underlined that oxidative deamination of amino acids is related to the production of reducing equivalents, that must be reoxidized in the mitochondrial respiratory chain. This may contribute to an increased electron flow along the respiratory

Abbreviations: 4-HNE protein adduct, 4-hydroxynonenal protein adduct; 8-isoP, 8-isoprostanes; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CAT, catalase; OSI, oxidative stress index; PC, protein carbonyls; Px, peroxidase; ROS, reactive oxygen species; SOD 1, superoxide dismutase 1; TAS, total antioxidant status; TOS, total oxidant status; UA, uric acid

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chain and increased ROS formation (Petzke & Proll, 1994). This point of view is supported by data, which shows that chronic high protein intake results in an increased thermogenic response, which e.g. decreases nutritional efficiency of energy utilization, increases oxygen consumption and impairs oxidative phosphorylation capacities (Klein & Hoffmann, 1993; Petzke & Proll,1994; Porrata-Maury, Aust, Noack, & Eschrich, 1987).

An increased production of ROS, in the case of failure of the antioxidant systems to combat extra free radical generation, may lead to oxidative stress (OS) (Pamplona & Barja, 2006). One of its consequences is oxidative modification of polypeptide chains, as well as, single amino acid residues. This results in the formation of, for example, of reactive protein carbonyl derivatives (PC), the generation of peroxides due to a damage in polyunsaturated fatty acids (e.g.) 4-hydoxynonenal protein adduct (4-HNE protein adduct) or 8-isoprostanes (8-isoP) or the oxidative modification of DNA, leading to the formation of 8-hydroxy-D-guanosine (Lushchak, 2014b).

The question of whether higher protein intake induces physiologic OS remains controversial. It has been shown that high protein intake increases plasma oxidative modified protein bound amino acids of adult rats fed 60% casein but does not increase plasma protein carbonyl concentration (Petzke, Proll, Bruckner, & Metges, 1999). Camiletti-Moirón et al. (2015) showed that brain thiobarbituric acid reactive substances and protein carbonyls were higher in the high protein group compared to the normal protein group, which is contrary to the results of Petzke, Elsner, Proll, Thielecke, and Metges (2000).

Saliva produced by the salivary glands plays an important role in oxidant/antioxidant redox homeostasis. Saliva may be therefore considered as a major component of the oral host defenses, which constitute a first line of defense against ROS-induced agents in tobacco smoke, alcohol, drugs, as well as other xenobiotics in the diet (Nagler, Klein, Zarzhevsky, Drigues, & Reznick, 2002). The moisturizing properties of saliva facilitate articulation, swallowing and digestion. Saliva protects the surfaces of the teeth and the mucous membranes of the oral cavity against biological, chemical and mechanical insults (Sonesson, Wickström, Kinnby, Ericson, & Matsson, 2008). Thus, factors which compromise the salivary glands' function, change the amount and composition of the saliva secreted in the oral cavity and have adverse effects on oral health and the quality of life.

Inadequate nutrition is thought to be a key factor in the development of pathological changes of oral homeostasis and it also alters the salivary glands' function (Elverdin et al., 2006; Fathy El-Maghraby, 2012; Huumonen & Larmas, 2005; Johnson, Lopez, & Navia, 1995; Kołodziej et al., 2017; Zalewska et al., 2014). However, still little is known about the impact of a high-protein diet on the secretory function and antioxidant barrier as well as OS markers in the salivary glands. Therefore, it is important to explain whether the chronic intake of a high level of protein contributes to salivary gland dysfunction and results in disturbed redox balance in the salivary glands.

2. Materials and methods

The study was conducted in accordance with the Guidelines laid down by the European Communities Council Directive of 24 November 1986 (86/609/EEC) and was approved by the Committee for Ethics Use of Animals in the Medical University in Bialystok, Poland (protocol number 89/2015, 2015/109).

2.1. Animals

16 male Wistar rats, weighing between 67–72 g, were housed individually in steel cages, maintained at 22–24 °C, under standard lighting conditions from 8.00 a.m.–8.00 p.m. The animals remained in constant eye contact with each other. Before the feeding experiment, all rats consumed commercially available rodent chow (Agropol Motycz Poland, 13.5 mg% of fat, 24 mg% of protein, 62.5 mg% of carbohydrates, energy value 0.011 mJ/g). After 5 days of adaptation, the rats were randomly (based on a computer generated sequence of numbers) divided into two dietary groups: control (C) and experimental (HP).

2.2. Diet

During the 8 weeks of the experiment, animals assigned to the control group were fed a pelleted feed (Agropol Motycz Poland, 13.5 mg% of fat, 24 mg% of protein, 62.5 mg% carbohydrates, energy value 0.011 mJ/g). Rats assigned to the experimental group were fed a high protein diet (Research Diets, Inc.; D03012801, containing 14 mg% fat, 44 mg% proteins, 33 mg% carbohydrates, energy value 0.0158 mJ/g).

During the adaptation period and the 8 weeks of the experiment, rats from both groups had unlimited access to drinking water and food.

2.3. Salivary flow measurement

The food intake and body weight were monitored weekly. After 8 weeks, following an overnight fasting, rats were anesthetized with phenobarbital (80 mg/kg body weight, i.p.). Next, the stimulated and unstimulated saliva secretion rates were measured. In order to make measurements of saliva secretion, rats were placed on a couch preheated to 37 °C, placed at an angle of 30°. Non stimulated salivary secretion was measured for 15 min, using a pre-weighted cotton ball inserted underneath the tongue (Knaś, Maciejczyk, Daniszewska et al., 2016; Knaś, Maciejczyk, Sawicka et al., 2016). After 5 min, the rats were peritoneally injected with 5 mg/kg BW pilocarpine nitrate (Sigma Chemical Co, St. Louis, MO, USA). A five minutes after the pilocarpine administration, pre weighted cotton ball was inserted into the oral cavity and the whole stimulated saliva was collected for 5 min (Picco et al., 2012). The volume of saliva collected was measured by subtracting the initial weight from the final weight of the cotton ball. One milligram of whole saliva corresponds to 1.0 μL.

2.4. Blood and salivary glands collection

Finally, whole blood was drawn from the abdominal aorta into chilled heparinized tubes. The blood was centrifuged at 4 ° C, 5 min, 3000*g* (MPW 351, MPW Med. Instruments, Warsaw, Poland) and thus obtained plasma was precooled in liquid nitrogen and stored at −80 °C. Afterwards, the salivary glands from one side were removed on one side, freeze-clamped with aluminum tongs, precooled in liquid nitrogen and stored at −80 °C. The glands on the other side were fixed in 10% formalin and then processed for paraffin embedding.

2.5. Preparation of the blood and salivary glands for biochemical and histological assays

The salivary glands and plasma were slowly thawed (4 °C) the day on which the biochemical determinations were performed. The salivary glands were weighed (laboratory balance KERN PLI 510–3 M) and placed in glass tubes, containing ice cold PBS (1:10). The salivary pieces intended for the determination of carbonyl groups were diluted (1:10) in a 50 mM phosphate buffer. The protease inhibitor (1 tablet/10 mL of the buffer) (Complete Mini Roche, France) and the antioxidant butylhydroxytoluene (10 μL 0.5 M BHT in acetonitrile per 1 mL of the buffer) (Sigma-Aldrich, Germany) were added to all samples (plasma and salivary glands). Next, the glands were homogenized on crushed ice (Omni TH, Omni International, Kennesaw, GA, USA), and sonificated (1800 J/sample, 20 s three times, on ice; ultrasonic cell disrupter, UP 400S, Hielscher, Teltow, Germany). Finally, homogenates were centrifuged for 20 min, 4 °C, 5000 × *g* (MPW Med Instruments, Warsaw, Poland) and supernatants were taken for biochemical analysis.