7. Summary

High fat diet induced insulin resistance disrupts the antioxidant systems of the parotid and submandibular glands, leading to peroxidation of DNA, protein and lipids. In consequence causing morphological changes of the glands, which is reflected in the changes to the quantity and quality of saliva produced. N-acetylcysteine is an exogenous antioxidant, which works as a free radical scavenger. It is also a glutathione precursor. It has been proven that N-acetylcysteine supplementation activates antioxidant enzymes, prevents oxidative stress development, improves sensitivity towards insulin and also lowers insulin concentration in the serum of the high fat diet induced insulin resistant rats. There are no scientific reports regarding the results of exogenous antioxidant supplementation, including N-acetylcysteine on antioxidant defense and function of salivary glands during high fat diet induced insulin resistance.

The aim of my studies were to evaluate the impact of NAC supplementation on the parameters of redox balance and the secretory function of the parotid and salivary glands of the rats after the 8 weeks of high fat diet. Rats were cared for in accordance with the principles of the Local Committee for Ethical Use of Animals in Olsztyn (consent number 21/2017).

The research was conducted on male Wistar rats with the initial weight of 50-72g. The animals were kept in standard laboratory conditions at a controlled temperature (21C), light (12h light/12h darkness), with free access to water and chow.

The rats were randomly assigned to 4 groups of 10 rats each:

- group 1 - control group, rats were fed standard type LSM chow (Agropol Motycz Poland), containing 10.3% fat, 24.2% protein and 65.5% carbohydrates and daily intragastrically saline 2mL/kg of body weight (once a day, every day for 8 weeks),
- group 2- rats were fed standard type LSM chow and intragastrically N-acetylcysteine solution (500mg/kg body weight) in 2mL/kg volume for 8 weeks
- group 3- rats were fed high fat chow (Research Diet, USA) containing 59.8% fat, 20.1% protein and 20.1% carbohydrates and intragastrically saline in 2mL/mg body weight for 8 weeks
- group 4 -rats were fed high fat chow and intragastrically N-acetylcysteine solution (500mg/kg body weight), once a day, everyday for 8 weeks n 2mL/kg body weight volume

8 weeks after being fed various diets, the rats were anaesthetised with pentobarbital (80mg/kg of body weight intraperitoneally). A tail blood analysis was done and stimulated and nonstimulated salivary flows were measured in all the groups of rats. Next the aorta blood was

collected and the salivary glands were dissected. The salivary glands were homogenised, sonificated and centrifuged (20 min, 4C, 5000 *x* g). The supernatants were used for further tests. In the supernatant and plasma we determined:

- using ELISA : the concentration of the glycation endproducts (AGEs), the concentration of lipid oxidation adducts 4-hydroxynonenal (4-HNE) and the concentration of the DNA oxidation products (8-hydroxy-2-deoxyguanosine 8-OHdG)
- calorimetrically - the concentration of advanced oxidation protein products (AOPP), reduced glutathione(GSH), total protein, total oxidation status (TOS), total antioxidation status (TAS), salivary peroxidase (Px),superoxide dismutase(SOD) and catalase (CAT) activities

The plasma insulin concentration was determined by ELISA.The insulin sensitivity was calculated using HOMA-IR index. The statistical analysis was carried out using Statistica 10.0 (Kruskal-Wallis ANOVA, the Spearman correlation coefficient $p<0.05$).

We proved that the NAC supplementation strengthens the enzymatic (SOD, CAT, Px) and nonenzymatic (GSH, TAS) antioxidant mechanisms of the parotid glands in the high fat fed rats. In the submandibular glands of HFD rats receiving NAC, we observed a significant increase of GSH and TAS levels. NAC prevents oxidative damage to the parotid glands of the high fat diet rats and prevents the reduction of stimulated saliva in this group of rats.

NAC supplementation did not prevent decrease of the protein content in the salivary glands of the high fat diet fed rats.