SUMMARY

Pre-diabetes, as well as diabetes, and especially type 2 diabetes, is a common and, unfortunately, a growing health problem all over the world. Due to its prevalence and the dramatic increase in the number of cases observed in recent decades, both among adults and children, diabetes, as the only non-communicable disease, has been recognized by the World Health Organization as an epidemic of the 21st century. Moreover, diabetes, apart from a health problem, poses a huge socio-economic problem. The constantly growing number of new cases of diabetes, plus the treatment of complications in patients already suffering from diabetes, means that the costs of treatment and rehabilitation are constantly growing and are a significant burden on the Polish state budget. According to the Report of the Institute of Healthcare Management, expenditure on hospitalization, outpatient care, and basic health care services related to diabetes in 2012 amounted to approximately PLN 430 million. In 2012, the Social Insurance Institution (ZUS) recorded approximately 890,000 days of sickness-caused absence at work due to diabetes. Benefits incurred by the state budget, the Social Insurance Fund, and employers due to temporary incapacity to work amounted to approximately PLN 438 million.

Pre-diabetes is a risk factor for the development of complications typical of diabetes. Ocular complications in patients with prediabetes are more frequent compared to those without glycemic deviations and concern, inter alia, the development of lens opacities and corneal surface abnormalities (i.e., dry eye syndrome, epitheliopathy). Microangiopathic and macroangiopathic conditions (eg, retinopathy, neuropathy, nephropathy, and cardiovascular diseases) are more frequent in both pre-diabetic and diabetic subjects. Taking into account the convergent pathophysiological mechanisms present in pre-diabetes and type 2 diabetes, a similarity in the development of complications is suggested, e.g. in the organ of sight. Ocular complications in diabetes are currently the leading cause of blindness in working people in industrialized countries. A cataract is the most common eye complication in diabetes. The coexistence of diabetes mellitus increases the risk of developing cataract fivefold compared to a population without diabetes. Pre-diabetes increases the incidence of cortical cataracts by a factor of 2. Clinical trials confirm that cataracts are more common and affect patients with diabetes at an earlier age compared with patients without diabetes. Cataracts are the leading cause of visual impairment in diabetic patients.

As of today, there is no perfect treatment for cataracts. Drug treatment is ineffective. The surgical method of cataract phacoemulsification is considered the gold standard in the treatment of cataracts. Removal of the opaque lens and simultaneous implantation of an

artificial intraocular lens is currently the only recognized effective method of restoring the transparency of the optical system of the eye and improving vision. However, as with any surgical intervention, cataract surgery is invasive and carries the risk of complications. And in diabetic patients, cataract phacoemulsification carries a greater risk of developing complications, both intraoperatively and postoperatively.

Ocular complications in the course of diabetes, especially the loss of transparency of the lens and the lack of an ideal method for prevention and treatment of diabetic cataracts, prompted me to investigate the activity of lysosomal exoglycosidases (which are a measure of glycoconjugate catabolism) and the influence of NAC (as an antioxidant) on the activity of these lysosomal exoglycosidases in the eye lens.

Therefore, in the presented work, I assumed that:

- insulin resistance induced by a high-fat diet weakens the antioxidant systems of tissues, causing the formation of excess free radicals that cause oxidative stress, leading to excessive oxidation of DNA, proteins, and lipids
- excessive oxidation of the components that build cells and the intercellular substance may cause inflammatory damage and necrosis of various tissues, including eye tissues - the lens
- lysosomal degradation enzymes such as nuclease, protease, and glycosidases are involved in the removal of damaged tissue elements. Lysosomal exoglycosidases cut off the oligosaccharide chains of glycoconjugates (glycoproteins, glycolipids, and proteoglycans) by cutting off subsequent molecules of simple sugars from the non-reducing end of the oligosaccharide
- N-acetylcysteine is an exogenous antioxidant that acts as a free radical scavenger and is also a precursor to glutathione. NAC supplementation in the course of insulin resistance induced by a high-fat diet may have a protective effect on tissues, including the lens of the eye.

The assumptions presented above allowed me to form the following questions:

1. What is the specific activity of lysosomal exoglycosidases, i.e. N-acetyl-β-hexosaminidase, α-fucosidase, β-galactosidase, α-mannosidase, β-glucuronidase in the lens homogenates of control rats on a standard diet without and with the addition of N-acetylcysteine and on a high-fat diet with and without the addition of N acetylcysteine?
2. Is there a significant difference between the specific activity of individual lysosomal exoglycosidases in the lens homogenates of rats on a high-fat diet without N-acetylcysteine compared to the control group on a standard diet without N-acetylcysteine?

3. Is there a significant difference between the specific activity of individual lysosomal exoglycosidases in the lens homogenates of rats on a high-fat diet with acetylcysteine compared to the group of rats on a high-fat diet without acetylcysteine?
4. Does supplementation with N-acetylcysteine affect the specific activity of individual lysosomal exoglycosidases in lens homogenates of rats on a high-fat diet with acetylcysteine compared to the group of rats on a high-fat diet without N-acetylcysteine?
5. Can supplementation with N-acetylcysteine as an exogenous antioxidant have any practical significance and practical translation in treatment, i.e. reducing or inhibiting the development of ocular complications in the course of insulin resistance?

The study aimed to evaluate the effect of N-acetylcysteine supplementation on the activity of lysosomal exoglycosidases in the eye lenses of rats with insulin resistance induced by a high-fat diet.

The research material was Wistar rats, which are a universal, safe and effective model, often used in basic research. Rats are a good experimental model because they are experimental animals with a metabolism similar to that of humans. Only males of the Wistar strain, originating from non-relative breeding, from the outbred herd - genetically heterogeneous (Wistarcmdb / outbred), with an initial body weight of 50-70g, were used in the experiment. The study was approved by the Local Ethical Committee for Experiments on Animals in Olsztyn, No. 21/2017. The induction of insulin resistance by feeding rats a high-fat diet is widely used in experimental studies and well-documented in the world literature.

The first part of the experiment was carried out at the Department of Physiology, Medical University in Białystok. During the experiment, 4-week-old males with an initial body weight of about 50-70 g were kept in standard laboratory living conditions (21 ° C ± 2, 12h light / 12h darkness), with free access to water and appropriate food.

The rats were divided into 2 groups.

A control group, 30,00 rats, with 2 separate subgroups:

- K control subgroup (25 rats) - fed a standard LSM diet (Agropol, Motycz, Poland) containing 10.3% fats, 24.2% proteins, and 65.5% carbohydrates and intragastrically (once a day, daily for 8 weeks) physiological saline solution in a volume of 2 mL / kg bw
- K_NAC control subgroup (5 rats) - fed a standard LSM diet (Agropol, Motycz, Poland) containing 10.3% fats, 24.2% proteins, and 65.5% carbohydrates and intragastrically (once a day, every day for 8 weeks) N-acetylcysteine solution (in a dose of 500 mg / kg bw) in a volume of 2 ml / kg bw The antioxidant solution was administered in 1% Tween 80 in physiological saline solution.

A study group (with induced insulin resistance), consisting of 29 rats, with 2 separate subgroups:

- HFD study subgroup (9 rats) – in order to induce insulin resistance, fed high-fat feed (Research Diets, Inc .; D12492, USA) containing 59.8% fats, 20.1% proteins, 20.1% carbohydrates, and intragastrically (once a day, daily for 8 weeks) physiological saline solution in a volume of 2 mL / kg bw.
- HFD_NAC study subgroup (20 rats) - in order to induce insulin resistance, fed high-fat feed (Research Diets, Inc .; D12492, USA) containing 59.8% fats, 20.1% proteins, 20.1% carbohydrates, and intragastrically (once a day, daily for 8 weeks) N-acetylcysteine solution (at a dose of 500 mg / kg bw) in a volume of 2 ml / kg bw. The antioxidant solution was administered in 1% Tween 80 in physiological saline solution.

After 8 weeks of the experiment, the rats were starved overnight. In the morning, an intraperitoneal anesthetic, sodium phenobarbital, was administered to all rats in a volume of about 0.15 ml of the animal's body weight (80 mg / kg body weight), sufficient to obtain deep anesthesia. Then, blood was collected from the tail vein and blood glucose level was measured with a glucometer. Afterwards, blood was collected from the abdominal aorta.

The blood glucose level in the tail vein was measured with a glucometer, giving an average result of 145 mg / dl (130-161 mg / dl), thus confirming hyperglycemia in the group fed a high-fat diet compared to the control group on a standard diet without the addition of N-acetylcysteine, where the mean glucose concentration was 89 mg / dL (86-93 mg / dL).

The blood collected from the abdominal artery was centrifuged. The plasma insulin concentration was determined by ELISA method, obtaining an average result of 166.42 mIU / ml (158.2-176.88 mIU / ml), confirming the elevated concentration of insulin in the group fed a high-fat diet, compared to the control group on a standard diet without the addition of N-acetylcysteine, where the mean insulin concentration was 79.70 mIU / ml (29.3-92.36 mIU / ml).

The HOMA-IR index of insulin resistance was calculated from the formula:

$$HOMA\text{-}IR = \frac{fasting\ insulin \quad \left[\frac{mU}{ml}\right] * \quad fasting\ glucose \quad \left[\frac{mmol}{l}\right]}{22,5}$$

with a result of 19.60 (15.45-21.05) in the group fed a high-fat diet, compared to the control group on a standard diet without the addition of N-acetylcysteine, where the HOMA-IR index was 3.64 (1.87-6.15).

The obtained results confirmed the induction of insulin resistance in rats fed a high-fat diet.

Frozen eyeballs were transported in dry ice to the Department of Medical Sciences of the Lomza State University of Applied Sciences. After the tissues were thawed, the lenses were isolated from both eyeballs. Then both lenses of each rat were placed in a homogenization tube and suspended in a KCl solution with Triton X-100 (in the dilution ratio 1 part tissue: 9 parts solution), and then homogenized with a knife homogenizer (IKA T10 basic ULTRA-TURRAX® Homogenizer, IKA Staufen / Germany) for 1 minute. The tubes were kept in an ice container. After homogenization, the volume of each of the homogenates was measured, and the homogenates were centrifuged at 2000 revolutions (about 900 x g) for 10 minutes at 4 ° C (MPW-350R laboratory centrifuge, Warsaw / Poland). The supernatant fluid was collected for further research, in which the protein concentration and specific activity of the following lysosomal exoglycosidases were determined:

1) N-acetyl-β-D-hexosaminidase (HEX)
2) α-fucosidase (FUC)
3) β-galactosidase (GAL)
4) α-mannosidase (MAN)
5) β-glucuronidase (GluU)

Statistical analysis was performed with the use of Statistica 12.0 by StatSoft. Non-parametric ANOVA Kruskal-Wallis rank test with a post-hoc test of multiple comparisons of all-sample mean ranks for multiple groups. The statistically significant results were considered at the level of $p < 0.05$.

The median specific activity of N-acetyl-β-D-hexosaminidase in the eye lenses of HFD rats was 6.18 pKat / mg protein and was statistically significantly higher ($p = 0.02$) (Table IV, Figure 22) compared to the control group (K), where the median specific activity of HEX was 3.89 pKat / mg protein (Table IV, Figure 22). There was a tendency to decrease the specific activity of N-acetyl-β-hexosaminidase in the group. HFD_NAC, where the median activity was 4.98 pKat / mg protein (Table IV, Figure 22) compared to the group of insulin-resistant rats that were not supplemented with N-acetylcysteine (HFD) (6.18 pKat / mg protein), but was not a statistically significant value ($p = 0.37$), (Table IV, Figure 22). The observation of a tendency for a decrease in the specific activity of HEX in the HFD_NAC group compared to the HFD

group would suggest that supplementation with NAC influences the change of the specific activity of HEX, although it was not statistically significant ($p = 0.37$).

The median specific activity of α-fucosidase obtained in the lenses of the HFD rats was 0.099 pKat / mg protein, compared to the control group (K), where the median specific activity of FUC was 0.095 pKat / mg protein. The median specific activity of α-fucosidase in the eye lenses of the HFD_NAC rats (the group of rats with induced insulin resistance and additional supplementation with exogenous N-acetylcysteine) was 0.108 pKat / mg protein and was slightly higher than the median specific activity of FUC obtained in the group of rats with insulin resistance, which was not supplemented with N-acetylcysteine (HFD) (0.099 pKat / mg protein). This suggests that neither a high-fat diet nor supplementation with NAC significantly changed the specific activity of α-fucosidase ($p = 1.0$) (Table V, Figure 23).

The median specific activity of β-galactosidase in the eye lenses of rats in the group of rats with induced insulin resistance (HFD) was 0.11 pKat / mg of protein and was slightly higher, but showed no statistically significant differences: $p = 1.0$ to the specific activity of GAL obtained in the control group (K): 0.10 pKat / mg protein (Table VI, Figure 24). The median specific activity of β-galactosidase in the eye lenses of rats in the HFD_NAC group (the group of rats with induced insulin resistance and additional supplementation of exogenous N-acetylcysteine) was 0.10 pKat / mg of protein and was slightly lower compared to the specific activity of GAL obtained in the group of insulin-resistant rats (HFD), however, it was not a statistically significant difference: $p = 1.0$, which suggests that supplementation with NAC did not change the specific activity of β-galactosidase ($p = 1.0$) (Table VI, Figure 24).

The median specific activity of α-mannosidase obtained in the eye lenses of the rats from the insulin resistance (HFD) group was 0.101 pKat / mg protein and was slightly higher compared to the control group (K), where the median specific MAN activity was 0.091 pKat / mg protein. The differences in the specific activity of α-mannosidase between groups K and HFD did not show statistically significant changes ($p = 0.68$) (Table VII, Figure 25). The median specific activity of α-mannosidase in the eye lens of rats in the group with induced insulin resistance and additional supplementation of N-acetylcysteine (HFD_NAC) was 0.096 pKat / mg of protein and was slightly lower compared to the median specific activity of α-mannosidase in the eye lens of the rats of the group with induced insulin resistance (HFD) (0.101 pKat / mg protein) and it was not a statistically significant difference ($p = 1.0$). The obtained value suggests that supplementation with NAC did not change the specific activity of α-mannosidase ($p = 1.0$) (Table VII, Figure 25).

The median specific activity of β-glucuronidase in the eye lenses of rats in the group of rats with induced insulin resistance (HFD) was 0.34 pKat / mg of protein and showed a weak upward trend compared to the median activity of GluU obtained in the control group (K): 0.311 pKat / mg protein, however, it was not statistically significant (p = 1.0) (Table VIII, Figure 26). The median specific activity of β-glucuronidase in the eye lens of rats in the group with induced insulin resistance and additional supplementation of N-acetylcysteine (HFD_NAC) was 0.465 pKat / mg of protein and showed a weak upward trend compared to the group of rats with induced insulin resistance (HFD): 0.34 pKat / mg protein, however, it was also not statistically significant (p = 0.81) (Table VIII, Figure 26).

The obtained research results allowed for the formulation of the following conclusions:

1. Based on the results obtained, I found the presence of all the lysosomal exoglycosidases tested, i.e. N-acetyl-β-D-hexosaminidase, α-fucosidase, β-galactosidase, α-mannosidase and β-glucuronidase (Tables IV-VIII, Figures 22- 26) in the eye lenses of rats derived both from rats on a standard diet without and with the addition of N-acetylcysteine, and on a high-fat diet with and without the supplementation with NAC.
2. HEX was characterized by the highest activity of all lysosomal exoglycosidases, both in the eye lenses of rats fed a standard diet and rats fed a high-fat diet. HEX activity in the group of rats fed the high-fat diet was statistically significantly higher compared to the group of rats fed on the standard diet. There were no differences in FUC activity in the group of rats fed a high-fat diet compared to the group of rats fed a standard diet. The activity of GAL and MAN in the group of rats fed a high-fat diet showed an upward trend compared to the group of rats fed a standard diet, but it was also not statistically significant. The activity of GluU in the group of rats fed a high-fat diet tended to increase compared to the group of rats fed a standard diet, but it was not statistically significant.
3. The highest activity of all lysosomal exoglycosidases was shown by HEX in the group of rats fed a high-fat diet. HEX activity was lower in the group of rats fed a high-fat diet with additional NAC supplementation, compared to the group of rats fed a high-fat diet, but it was not statistically significant. FUC activity in the group of rats fed a high-fat diet with additional NAC supplementation tended to increase compared to the group of rats fed a high-fat diet, but it was not statistically significant. The specific activity of GAL and MAN tended to decrease in activity in the group of rats fed a high-fat diet with additional NAC supplementation as compared to the group of rats fed a high-fat diet, which was not statistically significant. The specific activity of GluU tended to increase in the group of rats

fed a high-fat diet with additional NAC supplementation compared to the group of rats fed a high-fat diet, but it was not statistically significant.

4. Supplementation with N-acetylcysteine influences the specific activity of individual lysosomal exoglycosidases in lens homogenates of rats on a high-fat diet with the addition of N-acetylcysteine compared to the group of rats on a high-fat diet without the addition of N-acetylcysteine.
5. Supplementation with N-acetylcysteine as an exogenous antioxidant may have a beneficial, protective effect in reducing lens translucency loss in the course of insulin resistance. The results of my research encourage the continuation of research on the effect of N-acetylcysteine as an exogenous antioxidant on the inhibition of glycoconjugate catabolism in various tissues due to the participation of glycoconjugates in inflammatory processes. The determination of the specific activity of lysosomal exoglycosidases in the eye lens of rats has some cognitive value, but little diagnostic value.