



Oxidative stress and DNA damage in peripheral blood mononuclear cells from normal, obese, prediabetic and diabetic persons exposed to adrenaline in vitro



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ABSTRACT

Diabetes represents one of the major health concerns, especially in developed countries. Some hormones such as the stress hormone adrenaline can induce reactive oxygen species (ROS) and may worsen the diabetes. Therefore, the main aim of the investigation was to find out whether peripheral blood mononuclear cells (PBMCs) from normal persons have less DNA damage induced by adrenaline (0.1, 1 and 10 μM) in comparison to PBMCs from obese, prediabetic and diabetic patients. Also, the biochemical parameters of oxidative stress (TBARS, catalase) and lactate dehydrogenase were monitored. It was observed that higher concentrations of adrenaline (1 and 10 μM) induced DNA damage in the obese, prediabetic and diabetic groups. In healthy individuals only the highest concentration of adrenaline caused significant increase in the DNA damage. In summary, total comet score (TCS) comparison has shown significant differences between groups, and DNA damaging effects of adrenaline were most evident in diabetic patients. The results of the biochemical analysis also demonstrate that adrenaline exerts most obvious effects in diabetic individuals which is manifested as significant change of parameters of oxidative stress. In summary, the obtained results demonstrated that diabetics are more sensitive to genotoxic effects of adrenaline and this effect probably resulted from decreased antioxidative defence mechanisms in various stages of progression through diabetes. Therefore, these results could contribute to a better understanding of a role of endocrine factors to damage of cellular biomolecules which could be useful in finding novel therapeutic approaches and lifestyle changes with an aim to lower the possibility of diabetes complications.

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterised by hyperglycemia due to a defect in insulin secretion or/and due to a

defect in its effects. International Diabetes Federation (IDF) has described diabetes as global epidemic. The most prevalent form of diabetes is type 2 diabetes mellitus (T2DM) and it is expected to increase to 439 million by 2030 [1]. One of the important factors which contribute

Abbreviations: ROS, reactive oxygen species; DNA, deoxyribonucleic acid; TCS, total comet score; IDF, International Diabetes Federation; T2DM, type 2 diabetes mellitus; CAT, catalase; MDA, malondialdehyde; LDH, lactate dehydrogenase; BMI, body mass index; PBS, phosphate buffered saline; FPG, fasting plasma glucose; EDTA, ethylenediaminetetraacetic acid; LMPA, low melting point agarose; DMSO, dimethyl sulfoxide; TBARS, thiobarbituric acid reactive substances

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to an increased development of T2DM is obesity [2].

Free radicals are formed during normal metabolic processes of biosynthesis, biodegradation and biotransformation and they have numerous physiological roles, such as: taking part in cell signaling, defense against infective agents, regulation of gene expression [3]. If their production is uncontrolled and overruns antioxidative capacity of the cell, a pathological state arises, followed by oxidation of membrane lipids, cell proteins and DNA. This state may result in production of new cytotoxic ROS and even the cell death [4]. In diabetic cells, the amount of glucose oxidized in the Krebs tricarboxylic acid cycle is increased, leading to an increase in generation of reactive oxygen species (ROS) and resulting oxidative stress plays a key role in the pathogenesis and progression of diabetes and diabetic complications [5].

Hyperglycemia induces excessive production of superoxide anion in electron transport chain, leading to a numerous metabolic abnormalities and activation of pathways involved in pathogenesis of diabetes complications [6].

Relatively high concentrations of H_2O_2 are released at the site of any kind of inflammation, but also in uninfamed cells (fibroblasts, endothelial cells, chondrocytes) under the influence of cytokines such as interleukin-1 and TNF- α . Cells are protected from hydrogen peroxide by powerful enzyme systems such as catalase in peroxisomes and glutathione peroxidase in mitochondrion's [7].

Catalase is an enzyme with high expression in all tissues. Early and profound oxidative stress in any organ, especially in pancreas, leads to, probably compensatory, increase of the activity of antioxidative enzymes including catalase [8].

If the hydrogen peroxide is not removed by catalase it can react with ferric ions (the Fenton reaction) making the more potent hydroxyl radical. This radical causes mitochondrial membrane damage in situ leading to loss of ATP production, damage to DNA, cell membrane by modification of membrane proteins, lipid peroxidation and possibly irreversible cell destruction. [9].

Accumulating evidence clearly indicates an association between oxidative stress and T2DM [10]. It has been reported that T2DM patients show higher oxidative stress which is reflected in significant changes in parameters of oxidative stress compared to healthy individuals [11]. Also significant raises in DNA damage of PBMCs in diabetic patient have been observed [11]. It is interesting that oxidative stress already exist in prediabetic patients [12]. Therefore, oxidative stress is thought to play an important role in the progression of diabetes and its complications such as foot ulcers, myocardial infarction or cerebrovascular accidents [13].

Adrenaline is a hormone and neurotransmitter with primary roles in mobilization of resources in response to emotional and physical stress. It is also an important regulator of insulin secretion [14]. Adrenaline is used as an emergency treatment for cardiac arrest, in the treatment of asthma, allergic reactions and glaucoma [15]. There are reports which suggest that administration of adrenaline exerts genotoxic effect via ROS [16]. Moreover, short term (less than 30 min) exposure of murine 3T3 cells to stress hormones (including the adrenaline) causes 5 fold increase in the DNA damage revealed by the Comet assay [17]. One of the possible mechanisms of elevated ROS is achieved through stimulation of β_2 adrenergic receptors [18]. In addition, adrenaline may undergo autooxidation and form quinone and semiquinone that enter in redox cycling which results in excess level of ROS and lead to oxidative stress [19].

Based on the above, we hypothesize that PBMCs of prediabetic and diabetic individuals may be more sensitive to the DNA damaging effect of adrenaline and oxidative stress which may result in worsening diabetes control. In order to investigate our hypothesis, we determined the genotoxic effects and as parameters of oxidative stress catalase (CAT), thiobarbituric acid reactive substances (TBARS) and lactate dehydrogenase (LDH) after administration of adrenaline in cells from healthy, obese, prediabetic and diabetic persons. To our knowledge, this type of study has not been conducted yet and the observed results

may contribute to better understanding the significance of adrenaline in progression of T2DM.

2. Material and methods

2.1. Study subjects

The present study was conducted on 28 females from 46 to 63 years old (7 persons in each group with the following mean \pm SEM age: healthy 53.0 ± 3.6 ; obese 61.0 ± 1.4 ; prediabetes 53.8 ± 2.3 ; T2DM 57.0 ± 2.9), who attended the Department of Endocrinology, Diabetes and Metabolic Diseases in Belgrade. Neither of the subjects had been ever diagnosed with malignancy, myocardial infarction, cerebrovascular insult or any other serious complication of diabetes. All of them were non-smokers, under no medication (except for drugs for controlling blood sugar level), and without use of any food supplements. All participants were informed about the procedure of the study and signed a written consent which was approved by the Ethical committee at the Faculty of Pharmacy (University of Belgrade).

Participants were divided into four groups (7 females in each group): female individuals with normal blood glucose levels (healthy group); group with normal blood glucose levels and body mass index (BMI) ≥ 30.0 kg/m² (obese group); group with impaired fasting plasma glucose (5.5 mmol/L $<$ FPG $<$ 7 mmol/L) (prediabetic group) - according to the recommendations of the World Health Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications [20] and group selected on the basis of having been diagnosed with T2DM previously using an oral glucose tolerance test and/or being on antihyperglycaemic medication (diabetic group). All subjects were completely physically examined in the morning after an overnight fast. Some antropometric parameters (body height, body weight, blood pressure) were measured the same morning, and body mass index was calculated as weight in kg per square meters of height (m²). Fasting plasma glucose (FPG) was determined by PAP enzymatic spectrophotometry at 505 nm, whereas glycosylated hemoglobin (HbA1c) was determined by capillary electrophoresis on Capillarys 3 Tera device.

2.2. Sampling and cell preparation

Blood samples were withdrawn by venipuncture from female donors. All samples within one group were taken on the same day, and all tested parameters were done on fresh samples. The blood was diluted (1:1) with RPMI medium, underlaid with Histopaque 1077 and centrifuged at 1900 g for 15 min. The PBMCs layer (buffy coat) was washed twice in RPMI 1640 medium, each wash was followed by centrifugation for 10 min at 1800g.

2.3. Comet assay

The alkaline version of Comet assay was performed according to Singh et al. [21] and Tice et al. [22] with minor modifications. Briefly, a suspension of isolated PBMCs in PBS was treated with adrenaline (0.1, 1, and 10 μ M, CAS No. 51-43-4) for 30 min at 37 °C. Immediately after the incubation period, the cell viability was checked by Trypan blue exclusion [23]. Each experiment included a positive control (100 μ M H_2O_2) and negative control (untreated cells). After incubation, 100 μ L of cell suspension was mixed with 100 μ L of 1% low melting point agarose (LMPA). 90 μ L of suspension was casted on a microscope slide precoated with 1% normal melting agarose and put in the fridge to solidify. Then the slides were immersed in cold lysis solution at pH 10 (2.5 M NaCl, 100 mM EDTA, 10 mM Tris pH 10, 1% Triton X-100, 10% DMSO) overnight at 4 °C. After lysis, slides were placed in a horizontal gel electrophoresis tank to allow DNA unwinding in cold electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH $>$ 13) for 30 min. Electrophoresis was carried out at 4 °C with electric current of 25 V (1.1 V/cm) for 30 min. The slides were then removed from the tray and washed

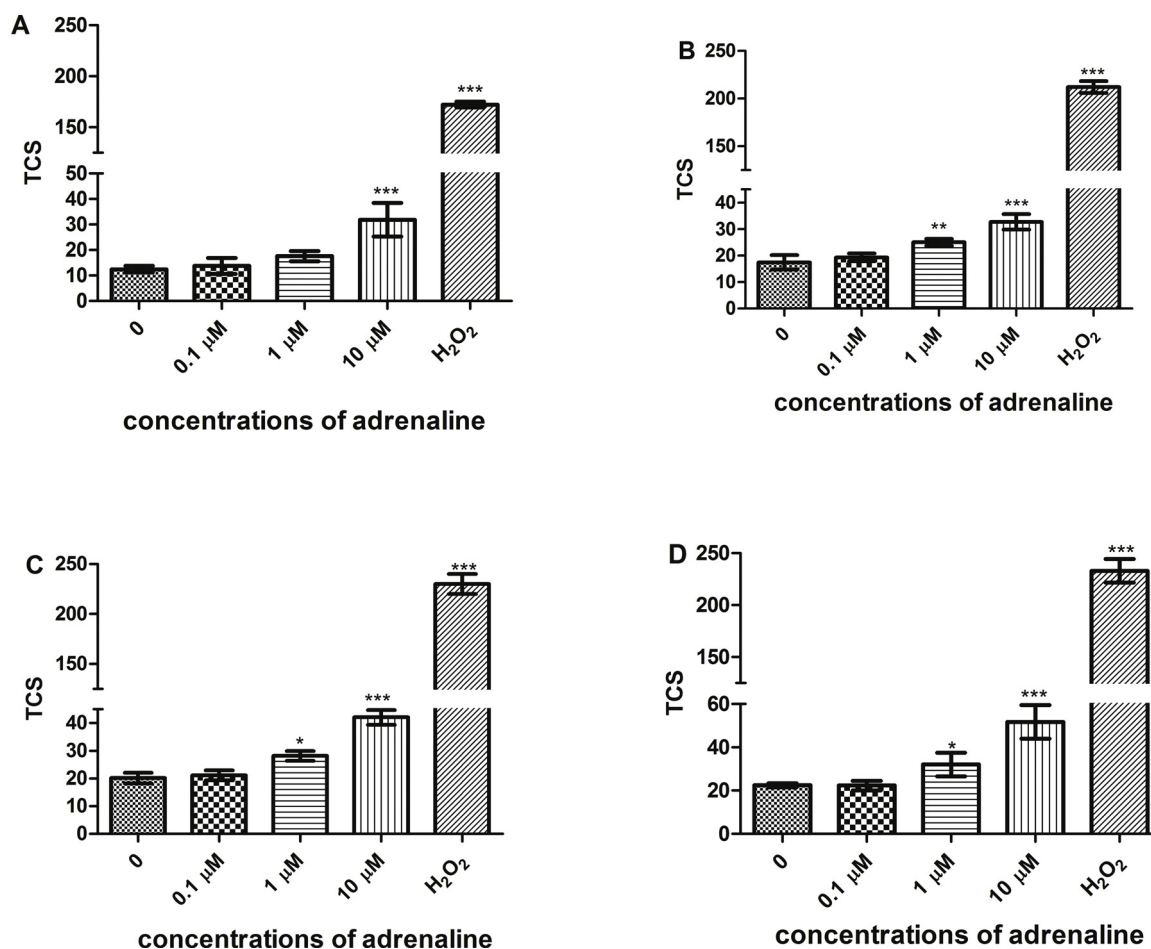


Fig. 1. DNA damage in various stages of progression through diabetes mellitus type 2: A – normal persons; B – obese with normal glycoregulation; C – patients with prediabetes; D – patients with type 2 diabetes mellitus (T2DM). TCS – total comet score. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Bonferroni test).

with neutralizing buffer (0.4 M Tris HCl, pH 7.5) for 5 min. The neutralisation was repeated three times. Finally, the slides were fixed with ice cold methanol, dried and stored. Before analysis, the slides were rehydrated with ice cold distilled water and stained with ethidium bromide (20 μg/mL). The slides were examined under a fluorescence microscope (AxioImager Z1, Carl Zeiss; excitation filter, 515–560 nm; emission filter, 590 nm). Comet were scored visually as described by Anderson et al. [24] and expressed as the total comet score (TCS) according to Collins [25]. In visual scoring the estimated level of DNA damage results in five classes of comets: (A) no damage, < 5%; (B) low level damage, 5–20%; (C) medium level damage, 20–40%; (D) high level damage, 40–95%; (E) total damage, > 95% („hedgehogs comets“). (Fig.1). TCS is calculated according to the formula:

$$\text{TCS} = 1 \times B + 2 \times C + 3 \times D + 4 \times E$$

where B to E represents percentage of cells with various level of DNA damage.

2.4. Determination of catalase (CAT)

Catalase activity was assayed in suspension of isolated PBMCs in PBS after treatment with adrenaline using H₂O₂ as substrate and activity was followed by measuring the decrease in absorbance at 240 nm [26]. The enzyme activity was expressed in U/mL. Each experiment included a positive control (100 μM H₂O₂) and negative control (untreated cells).

2.5. Determination of lipid peroxidation (TBARS)

Lipid peroxidation was assessed in suspension of isolated PBMCs in PBS after treatment with adrenaline, by measuring thiobarbituric acid reactive substances test (TBARS) according to the methods described by Gutteridge [27] and Traverso et al. [28]. The assay evaluated the formation of a colored adduct after the stoichiometric reaction between thiobarbituric acid (TBA) and several lipid derived aldehydes, including malondialdehyde (MDA). The TBARS level released in the samples was measured at 535 nm. The results were expressed in nmol/mL. Each experiment included a positive control (100 μM H₂O₂) and negative control (untreated cells).

2.6. Determination of lactate dehydrogenase (LDH)

After the incubation period with adrenaline, the suspension of PBMCs in PBS was centrifugated for 5 min at 2 000 rpm and extracellular LDH determined in the supernatant. Each experiment included a positive control (100 μM H₂O₂) and a negative control (untreated cells). Lactate dehydrogenase was determined by spectrophotometry. The reaction solution (2.0 ml) containing Tris HCl buffer (61.43 mM, pH 7.4) and NADH (0.18 mM) was supplemented with pyruvate (0.6 mM). The 100 μl of the supernatant was then added in reaction solution. The extinction was detected at 339 nm after 30 s, 1, 2 and 3 min at 25 °C [29]. The enzyme activity was expressed in U/mL.

Table 1
Basic characteristics of the study groups.

Parameters	Normal	Obese	Prediabetics	T2DM
Age (years)	53.00 ± 3.54	61.00 ± 1.41	53.83 ± 2.27	57.00 ± 2.94
FPG (mmol/L)	5.16 ± 0.30	5.30 ± 0.22	5.57 ± 0.17	5.45 ± 0.33
HbA1c (%)	5.68 ± 0.16	6.05 ± 0.29	5.70 ± 0.11	6.05 ± 0.19
BM (kg)	70.78 ± 9.72	84.65 ± 2.96	85.30 ± 8.77	78.75 ± 6.65
BH (cm)	165.20 ± 2.52	167.00 ± 2.74	165.67 ± 4.12	175.50 ± 3.84
BMI (kg/m ²)	26.16 ± 3.46	30.4 ± 0.27	30.77 ± 2.40	25.40 ± 1.27
Systolic BP (mmHg)	124.00 ± 4.00	132.50 ± 10.31	121.67 ± 9.10	122.50 ± 2.50
Dyastolic BP (mmHg)	82.00 ± 2.00	82.50 ± 4.79	76.67 ± 5.58	80.00 ± 0.00

The results are presented as mean ± SE; FPG- fasting plasma glucose; BM – body mass; BH- body height; BMI – body mass index; BP- blood pressure. There were no statistically significant differences among groups for any analysed parameter ($p > 0.05$ in one-way ANOVA and Tukey's test).

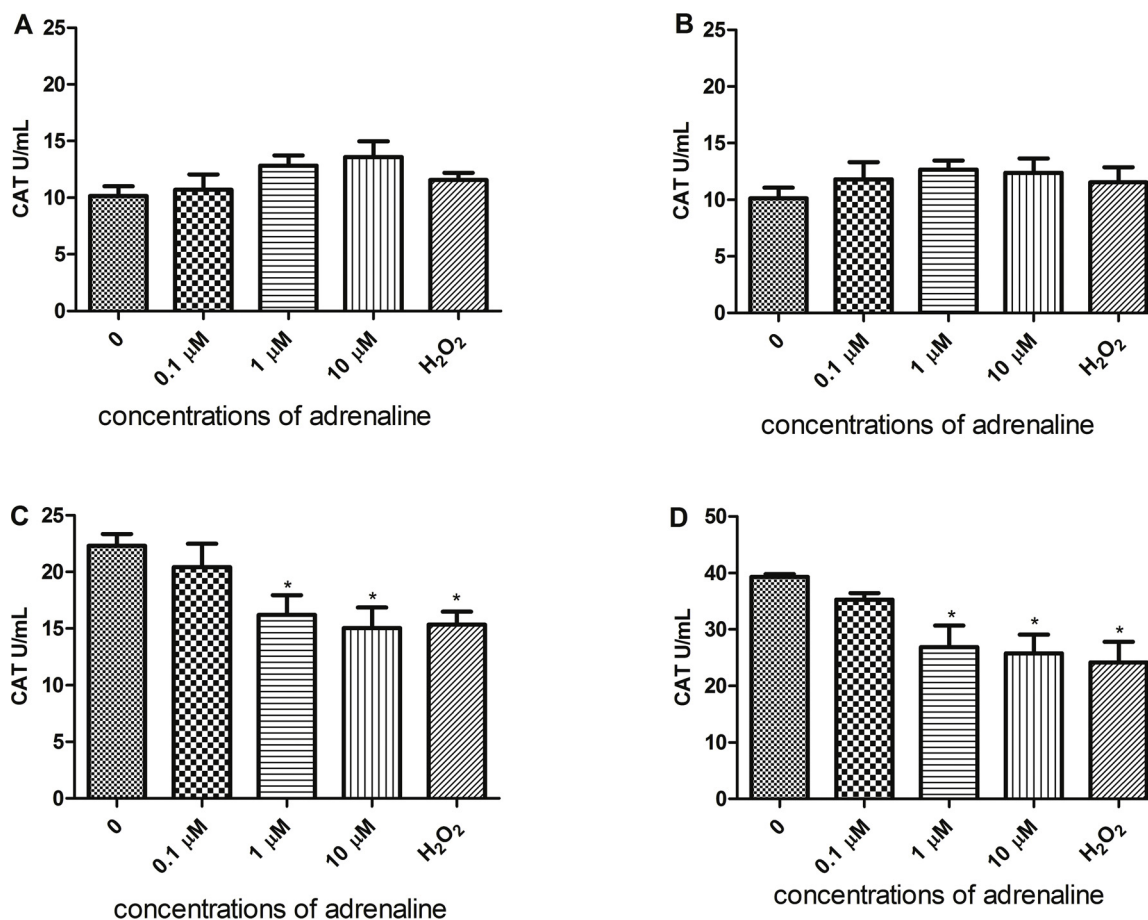


Fig. 2. Activity of catalase (CAT) in PBMCs of A) normal, B) obese C) prediabetics D) T2DM persons treated with viros concentrations of adrenaline and positive control (H₂O₂). The results are presented as means ± standard error of mean (SEM). * $p < 0.05$ in comparison to the negative control (zero point), by Dunnett test.

2.7. Statistical analysis

Due to the homogeneity of the data obtained in the Comet assay (coefficient of variation < 30%), one-way analysis of variance (ANOVA) was used, followed by Bonferroni test. Basic antropometric and biochemical analysis among groups were analysed by one-way ANOVA and Tukey's test. Statistical significance of the differences of catalase, TBARS and LDH was determined by means of the ANOVA test followed by Dunnett test. The area under the curve (AUC) was calculated with baseline adjusted for all groups and parameters, followed by Kruskal-Wallis and Dunn's test for heterogenous dataset, while for homogeneous dataset we used one-way ANOVA and post hoc Tukey's test. Statistical analysis was performed with GraphPad Prism version 6 (GraphPad, San Diego, CA, USA). All values are presented as the mean ± SE. A p value of less than 0.05 was considered to be statistically

significant.

3. Results

The basic clinical characteristics of study groups are shown in Table 1. The groups were homogenous for all parameters (age, FPG, HbA1c, blood pressure, BMI). Homogenous results for FPG and HbA1c demonstrate that all subjects had good glycaemic control, which was supported by medication in prediabetics and diabetics. Also, none of the subjects involved in this study had a history of some serious medical condition connected to diabetes complications (stroke, heart attack, cancer, absence of any systemic disease or infection in previous months).

At all experimental points, the cell viability in Trypan blue exclusion test was acceptable (over 85%). The results of primary DNA

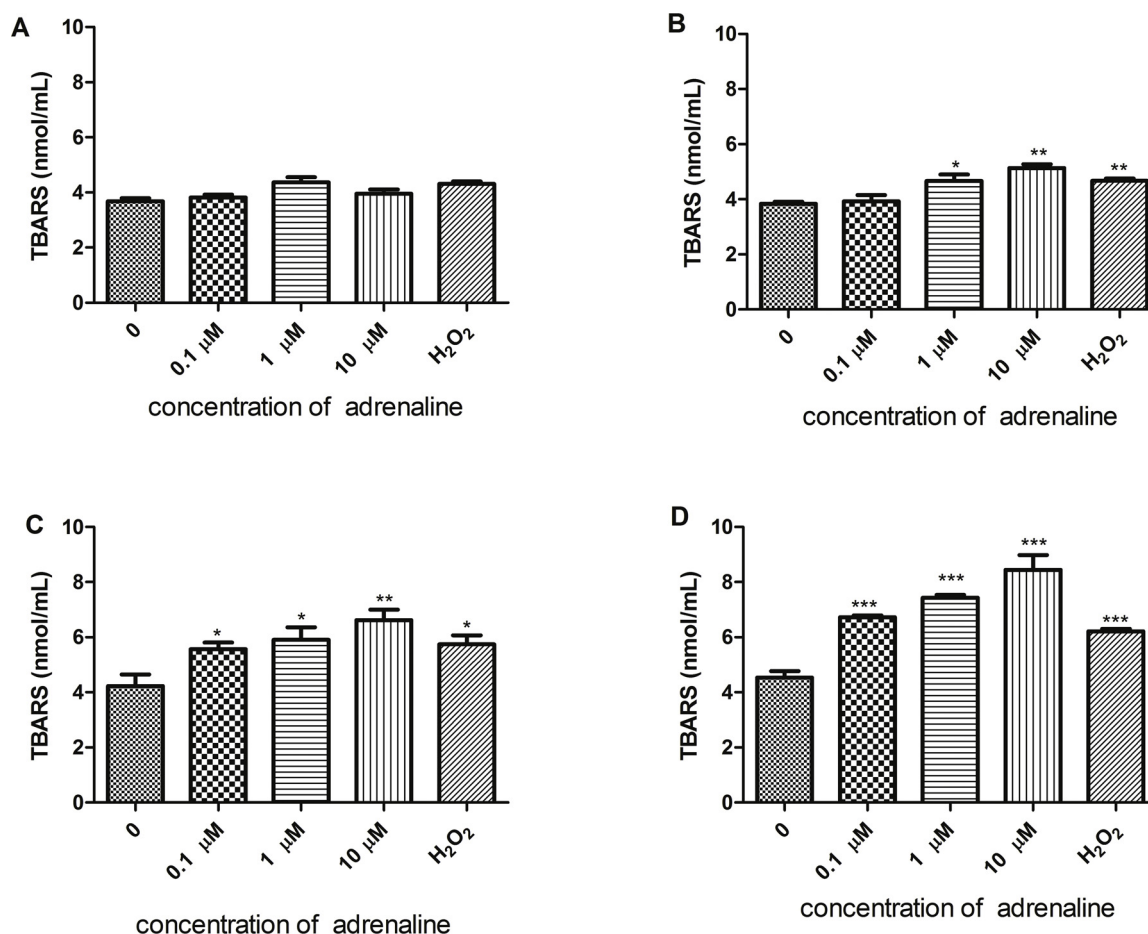


Fig. 3. Concentration of thiobarbituric acid reactive substances (TBARS) as an indicator of lipid peroxidation in PBMCs of A) normal, B) obese, C) prediabetics and D) T2DM patients treated with various concentrations of adrenaline and the positive control (H₂O₂). The results are presented as means \pm SEM. * p < 0.05, ** p < 0.01; *** p < 0.001 in comparison to the negative control, by Dunnett test.

damage in various groups (healthy, obese, prediabetics and T2DM patients) are presented in Fig. 1. In healthy individuals only the highest concentration of adrenaline (10 μM) exerted significant increase (p < 0.001) in DNA damage which is reflected in higher value of TCS score compared to untreated cells (A). However, in PBMCs of obese individuals significant increase in DNA damage was noted at 1 μM (p < 0.01) and 10 μM concentration of adrenaline (p < 0.001), (B). Similar results were observed in the prediabetic and T2DM groups where 1 μM of adrenaline induce significant rise (p < 0.05) in DNA migration, while the highest concentration of adrenaline (10 μM) caused an obvious DNA damaging effect (p < 0.001) compared to untreated cells (C,D). As expected, in all tested groups the positive control (100 μM H₂O₂) caused highly significant DNA damage (p < 0.001) in PBMCs. In summary, the basic difference in reactivity to adrenaline among the investigated groups is noticed at its concentration of 1 μM since PBMCs from normal persons did not have increased TCS score, whereas PBMCs from all other groups had at least slightly significant increase of TCS score. Interestingly, 10-fold higher concentration of adrenaline (10 μM) caused highly significant DNA damage in all groups of analysed persons.

The results of catalase activity are presented on Fig. 2. There was a statistically significant decrease of catalase activity in PBMCs from prediabetics and T2DM patients after the treatment with high concentrations of adrenaline (1 and 10 μM) in comparison to the negative control. Namely, the activity of the enzyme was lowered by 27.40% and 32.56% (p < 0.05) in prediabetics, while 31.68% and 34.50% (p < 0.05) in diabetic patients. Roughly the same percentage of catalase inhibition was caused by hydrogen peroxide in both groups of

subjects (p < 0.05).

In order to examine the influence of increased ROS on the level of cell membrane damage in the PBMCs of the examined subjects (normal, obese, prediabetics and T2DM), under the influence of various concentrations of adrenaline in vitro, we determined the concentrations of TBARS, as well as activity of LDH as a specific marker of cell membrane damage in PBMCs [30].

The results of the effects of various concentrations of adrenaline on cell membrane damage are presented in the Fig. 3.

In healthy person PBMCs there was a weak increase at all tested concentrations, but it did not reach statistical significance in comparison to the negative control level. In PBMCs of obese subjects there was a significant increase of membrane lipids damage at higher concentrations of adrenaline: 1 and 10 μM (p < 0.05 and p < 0.01). The examined concentrations of adrenaline caused an increase of lipid peroxidation in prediabetics with the level of significance of p < 0.05 (0.1 and 1 μM adrenaline) and p < 0.01 (10 μM adrenaline). However, in T2DM subjects all concentrations of adrenaline caused an obvious damage of membrane lipids at the level of statistical significance of p < 0.001. The concentration of TBARS was increased in comparison to the untreated PBMCs for 55.7%, 70.90% and 95.15% (confidence interval, CI 95%) for increasing concentrations of adrenaline of 0.1, 1 and 10 μM, respectively. In summary, significant differences between the investigated groups with respect to harmful effects of adrenaline on the cell membrane were most obvious in diabetic patients. PBMCs treated with hydrogen peroxide had also damage of cell membrane, and the level of statistical significance was different: p < 0.01 in obese, p < 0.05 in prediabetics and p < 0.001 in T2DM patients.

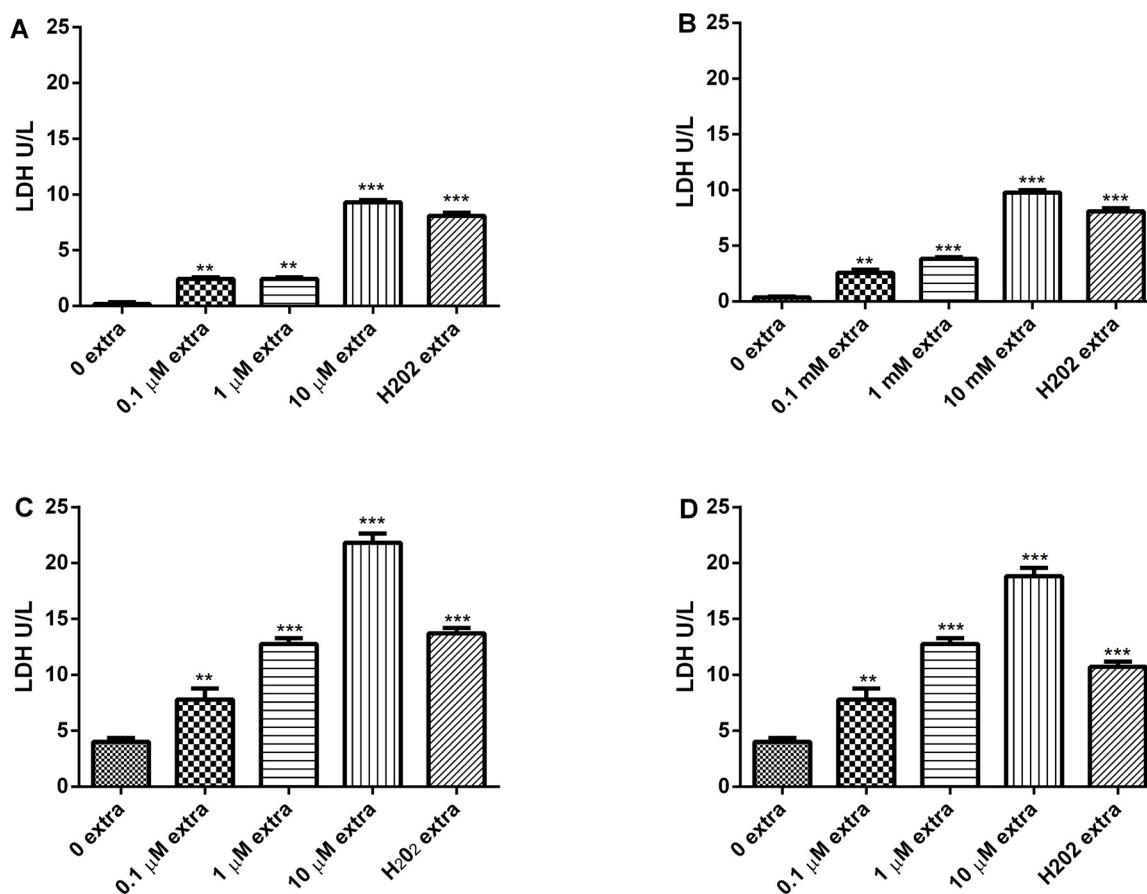


Fig. 4. Extracellular lactate dehydrogenase activity (LDH) as an indicator of cell cytotoxicity in PBMCs of A) normal, B) obese, C) prediabetics and D) T2DM, treated with various concentrations of adrenaline and positive control (H_2O_2). The results are presented as means \pm SEM. ** $p < 0.01$; *** $p < 0.001$ in comparison to the negative control group, by Dunnett test.

The results of the amount of released enzyme lactate dehydrogenase in all investigated groups are presented in Fig. 4.

On the basis of the amount of this enzyme we can conclude that there is a tendency of release of LDH in all groups treated with various doses of adrenaline with a high level of statistical significance. The most intensive changes are obtained in prediabetics and T2DM groups treated with concentrations of 0.1 μM ($p < 0.01$) and 1 μM of adrenaline ($p < 0.001$), with a maximal activity at 10 μM of adrenaline ($p < 0.001$). The same effect was observed with H_2O_2 ($p < 0.001$).

Results of the analysis of area under the curve (AUC) for each investigated parameter are presented at the Fig. 5. On the basis of the obtained results it is obvious that total comet score (TCS) is increased ($p < 0.0001$) in all investigated groups (obese, prediabetics and diabetics) when compared to normal subjects. Prediabetics and T2DM groups had increased level of cell membrane damage (AUC of TBARS, AUC of LDH) in comparison to normal and obese persons with various levels of statistical significance (Fig. 5). On the other hand, increase of AUC for CAT was observed in prediabetics ($p < 0.05$) and T2DM group ($p < 0.001$), compared to the control and obese subjects.

4. Discussion

It is well established that diabetes is associated with increased production of reactive oxygen species (ROS) which is one of the most important factors for the disease progression and development of chronic complications [11,31]. In addition to decreased level of oxidative DNA damage, diabetics have lower capacity of antioxidative defence mechanisms [32].

In this study, we treated isolated human PBMCs with three

concentrations of adrenaline (0.1, 1 and 10 μM). These concentrations are much higher than therapeutic doses of adrenaline for treatment of cardiac arrest. We chose these concentrations to build on previous in vitro results that we have published [33]. All concentrations of adrenaline in our investigation were much higher than blood adrenaline in some severe pathophysiological conditions such as pheochromocytoma. However, the highest concentration of 10 μM is much lower than highest possible concentration (10 mM) recommended for in vitro Comet assay of relatively un toxic compounds [22].

It should be mentioned that dose of adrenaline depends not only on its concentration but also on the treatment duration. In our previous investigation [33] of genotoxic effects of adrenaline on PBMCs even the concentration of 0.001 μM was high enough to produce significant DNA damage after 15 min of treatment, while after 1 h the concentration of 5 μM was high enough to increase DNA migration in the Comet assay. In the present investigation, an intermediate time of 30 min was good enough to make 1 μM of adrenaline to cause a significant level of DNA damage. Therefore, at shorter time of treatment DNA damage is not repaired enough giving the increased level of DNA damage in the Comet assay.

The increased DNA damage under the influence of adrenaline was evident at a concentration of 1 μM in obese, prediabetic and diabetic persons, showing that their PBMCs are more sensitive to genotoxic effects of adrenaline, despite the fact that all our subjects had good glycaemic control. Namely, impaired glucose regulation causes higher level of DNA damage, especially in patients diagnosed with T2DM [34,35]. Since only normal subjects in our investigation did not respond to intermediate dose (1 μM) of adrenaline in the Comet assay, even the obesity is a risk factor for increased sensitivity of PBMCs to DNA

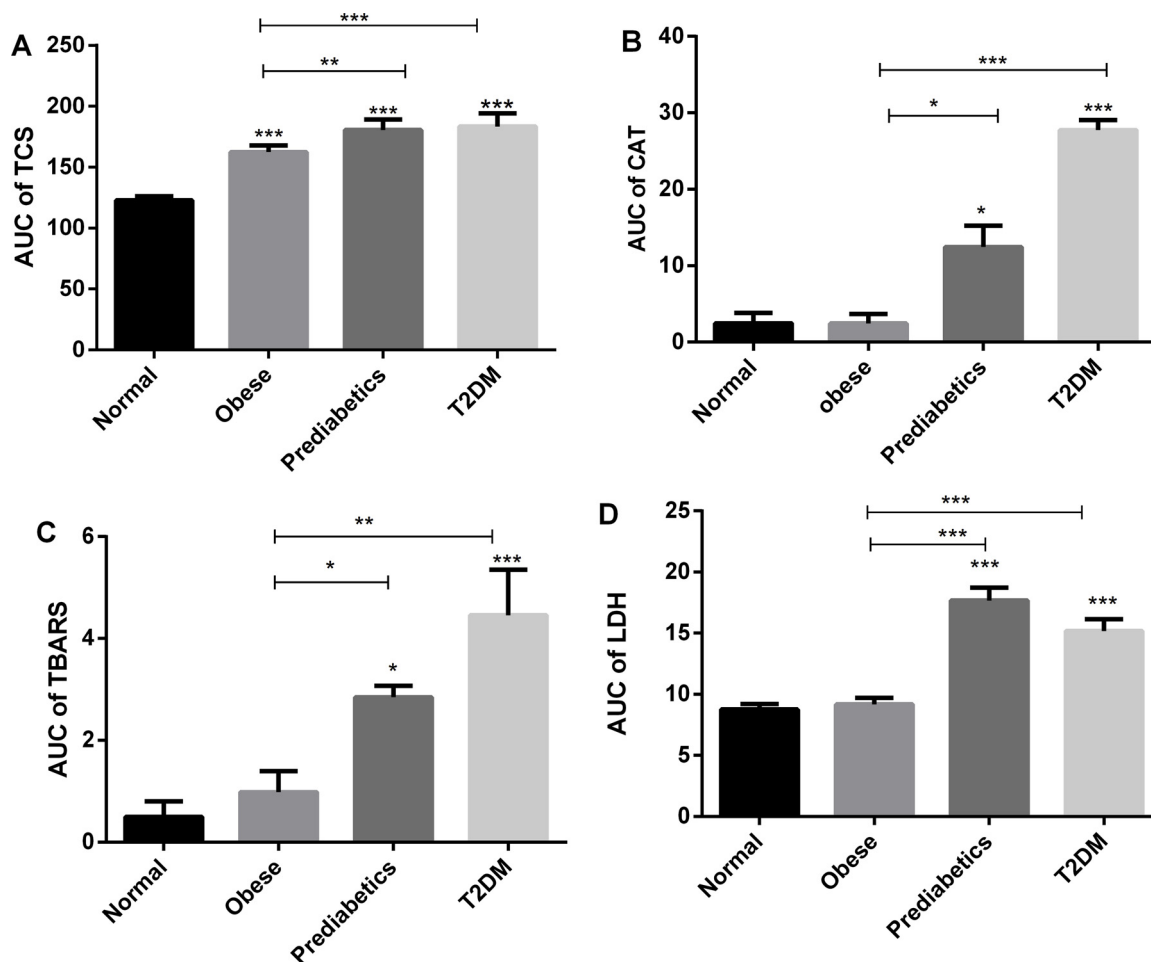


Fig. 5. Results of the analysis of area under the curve (AUC) – comparisons among groups for each of investigated parameters: (A) TCS in the Comet assay; (B) catalase; (C) TBARS and (D) LDH.

damaging effects of adrenaline. Indeed, there is an association between oxidative stress and obesity [36] and it is not surprising that obesity has contribution in the etiopathogenesis of diabetes mellitus [37].

There are experimental findings [38] that adrenaline causes greater increase of plasma glucose in obese than in non-obese mice, while plasma insulin level decreased only in obese animals after administration of adrenaline. This implies significant role of hyperglycemia in oxidative DNA damage. Indeed, there is a reduction of DNA damage after insulin treatment [39], whereas hyperglycemia may accelerate ROS through glucose oxidation pathway [40]. Bearing in mind that the T2DM group has higher blood glucose than prediabetics and obese groups it is not surprising that diabetics are most susceptible to oxidative DNA damage. This type of damage in our study is even more pronounced after adrenaline administration. Interestingly, despite harmful effects due to ROS production, adrenaline may have some beneficial effects in diabetic patients by increasing the glucagone secretion which may mitigate the sudden hypoglycemia [41] Hamilton et al., 2018).

Adrenaline caused increased DNA damage possibly by production of reactive oxygen species (ROS), as demonstrated in our previous investigations [16,33]. The possible mechanisms underlying this ROS production may include redox cycling of adrenaline [19] and/or stimulation of β_2 adrenoceptors [18].

In order to determine the effect of adrenaline on antioxidative status in all tested group we evaluated activity of CAT. Analyzing the PBMCs catalase activity, we noticed a weak increase of the activity of this enzyme in control and obese groups. A high level of catalase enables lower hydrogen peroxide production in cells and therefore prevents

oxidative damage [42]. A decrease in catalase activity can be the result of inactivation of the enzyme. Decreased activity of catalase can lead to a numerous harmful effects due to accumulation of superoxide anion and hydrogen peroxide [43].

By comparison of the values between control and obese subjects, we observed a higher initial levels of activities of enzymes in prediabetic and diabetic groups. These results are in accordance with a study of Cericlo et al. [44] showing that biomarkers of oxidative stress, including the activity of catalase, are increased in T2DM patients. The hyperglycemia itself is gradually established during the period of prediabetes and it causes an additional increase of free radical production.

In this study, we showed that after the treatment of PBMCs with various concentrations of adrenaline or H_2O_2 activity of catalase is decreased. Therefore, the antioxidative capacity of the cell is lowered and production of free radicals is increased, and all of that results in redox homeostasis disorder [4].

The end products of lipid peroxidation are extremely reactive and cause a consecutive chain propagation and oxidation of nearby polyunsaturated fatty acid (PUFA). It has been shown that products of lipid peroxidation can be transported from the site of their formation to the other sites in organism, acting as secondary cytotoxic signalling molecules – signals that transfer an information about the origin of primary oxidative reaction [45]. An extent of lipid peroxidation in examined patients was monitored by concentration TBARS including of MDA, one of the end products of cyclic reaction of lipid peroxidation as a marker of oxidative damage in cells. MDA is especially important product because it has mutagenic effects in mammalian cells and carcinogenic effect in rodents [3]. Our results have shown increased levels of TBARS

at all three concentrations of adrenaline in vitro, and in all examined groups of patients in comparison to the negative control, except for the control group. The most profound changes were detected in T2DM group, at all concentrations of adrenaline (Fig. 3). Increased TBARS were noticed in T2DM in correlation with duration of the illness and development of complications [46]. Alvarez-Diduk and Galano [47] suggest that catecholamines (adrenaline and noradrenaline) play role as protectors and molecular targets of oxidative stress.

PBMCs from T2DM patients exhibited production of ROS and damage of cell membranes [48]. Adrenaline exerts a similar effect in regard to TBARS level. In this investigation all tested concentrations of adrenaline in D2MT subjects induced significant enhancement of level of TBARS in comparison to control. In addition, the significant increase in TBARS level was found in prediabetic and T2DM group after treatment with highest concentration of adrenaline. Previous studies have reported increased levels of lipid peroxide in nonobese T2DM subject [49]. On other hand, there is evidence that treatment with adrenaline may result in significant increment of lipid peroxidation [50]. Therefore increased TBARS level signifies on disrupted of redox homeostasis as result of adrenaline influence. Besides, the observed DNA damage may derive from lipid peroxidation, since MDA has the ability to react with DNA and form exocyclic adducts such as mutagenic pyrimidopurine. Respectively, lipid peroxidation may be important mediators of DNA damage related with oxidative stress caused by reactions of adrenaline and higher level of glucose.

The PBMCs membrane damage in this study was indirectly also monitored through the amount of release of the enzyme lactate dehydrogenase from cells. LDH is a strictly intracellular enzyme, the damage of cell membranes in tissues causes leak of cytoplasmic enzyme LDH from cells. Therefore, spontaneous LDH release and the level of LDH extracellular activity can be used to estimate which tissue is damaged and to what extent [51]. In all experimental groups in this investigation, we observed increased amounts of extracellular LDH, pointing to unambiguously increased damage of PBMCs membranes.

The produced reactive oxygen species start the lipid peroxidation interacting with PUFA, which concentration is the highest in cell membranes of parenchymatic organs in T2DM [5]. That is why it is important to monitor parameters of oxidative damage such as hydrogen peroxide content, which directly give an answer about formation of hydroxyl radical that can lead to disturbed cell functioning resulting with mutagenesis and carcinogenesis [52]. However, further studies are needed to unravel the exact mechanisms underlying adrenaline induced genotoxicity.

5. Conclusions

PBMCs of obese, prediabetic and T2DM persons are more prone to DNA damage after in vitro treatment with adrenaline in comparison to those of healthy individuals. Also, in these groups of patients adrenaline causes a higher increase in oxidative stress and damage of cell membrane in vitro in comparison to the healthy group.

Conflict of interest

The authors declare no conflicts of interests.

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