Effect of Vitamin E and Vitamin C Supplementation on Antioxidative State and Renal Glomerular Basement Membrane Thickness in Diabetic Kidney

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Abstract
The aim of this study was to analyze the effect of vitamins C and E on malondialdehyde (MDA) content and activities of key antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) as well as glomerular basement membrane (GBM) thickness in streptozotocin-induced diabetic kidney in rats. Wistar male rats were divided into following groups (12 rats each): the control, diabetic rats, diabetic rats whose drinking water was supplemented with vitamin C in a dose of 1.0 g/l or diet was supplemented with 200 mg of vitamin E/100 g fodder. Body weight, blood glucose and HbA1C levels and 24-hour urinary albumin excretion (UAE) were studied every week (0–12 weeks). After 6 and 12 weeks, MDA content and activities of SOD, CAT and GSH-Px were measured in the kidney homogenate supernatants. Electron micrographs of glomeruli were scanned and morphometric investigations were performed by means of computer image analysis system to compare GBM thickness. The blood glucose and HbA1C concentrations and UAE in diabetic rats were significantly higher than in the control group. An increase in the MDA level and decrease in the SOD, CAT and GSH-Px activities in the kidney of diabetic rats were observed after 6 and 12 weeks of experiment. Administration of vitamins C and E did not affect body weight, blood glucose and HbA1C levels. Both vitamin C and vitamin E decreased lipid peroxidation and augmented the activities of antioxidant enzymes studied in the kidneys of diabetic rats as well as reduced UAE, decreased kidney weight and GBM thickness. The results indicate the potential utility of antioxidant vitamins in the protection against the development of diabetic nephropathy.

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Introduction
Numerous theoretical premises and experimental and clinical studies point to the participation of oxidative stress in diabetes pathogenesis and its late vascular complications including diabetic nephropathy [1, 2].

Diabetes has been demonstrated to be an oxidative stress condition with significant predominance of oxida-
tive factors over antioxidative mechanisms. Hyperglycemia is one of the most important factors responsible for oxidative stress intensification in diabetes. This is confirmed by Ceriello’s thesis [3] on ‘glucose toxicity’, according to which monosaccharides’ auto-oxidation may be one of the sources of oxygen-reactive forms. The process of non-enzymatic glycation of proteins present in the course of hyperglycemia, or increased activation of the intracellular polyl pathway [4], may provide free radicals. Glucose metabolism proceeding along the polyl pathway is the main factor stimulating the increase of cytosol NADH/NAD⁺, that together with an increased content of diacylglycerol affects protein kinase C (PKC) activation. Renal glomeruli were demonstrated to be particularly sensitive to oxidative stress evoked by PKC activators. Increased glomerular PKC activity was observed in hyperglycemia, which suggests that hyperglycemia-induced activation of glomerular PKC may cause intensified lipid peroxidation. Moreover, PKC activation is thought to be responsible for oxidative stress initiation in diabetic glomeruli [2]. Reports of numerous authors confirm the intensification of lipid peroxidation processes both in experimental diabetes and in human beings. A decrease of antioxidant concentration was also observed in systemic fluids and in cells in diabetic patients [5]. In those patients, lower than in healthy subjects, plasma and blood cell ascorbate concentration, platelet and erythrocyte vitamin E concentration, as well as distribution and metal ions release disturbances were observed [6]. In diabetic patients, erythrocytes decreased the amount of glycated form Cu-Zn-superoxide dismutase (SOD) was found with accompanying decreased activity of this enzyme. Furthermore, a lower activity of other key antioxidative enzymes, such as catalase (CAT), peroxidase and glutathione reductase was observed [5]. Studies of numerous authors demonstrated favorable effects observed after antioxidative vitamin supplementation both in diabetic patients and in animals with experimental diabetes [6]. However, so far there have been discrepancies in regards to antioxidant effectiveness in the hazard of diabetic nephropathy. Kahler et al. [7] demonstrated that vitamin E administration to diabetic patients with albuminuria for 3 months reduced significantly urinary albumin excretion. Another group of authors stated that vitamin E administered to rats with streptozotocin-induced diabetes did not decrease albuminuria despite a significant effect on the decrease of glomerular size and glomerular reduction of TGF-β [8]. Vitamin E was found to decrease lipid peroxidation and production of advanced glycation end-products (AGE) and demonstrated antiproliferative properties in mesangium cell cultures in hyperglycemia [9]. Ascorbic acid was shown to be able to inhibit glyco-oxidation reaction and AGE production in plasma and tissue. However, vitamin C pro-oxidative properties in the presence of transient group ions of metals were also observed and as a result this vitamin in appropriate conditions may intensify oxidative stress [10]. Diabetic microangiopathy is the most characteristic morphological change in diabetic kidneys. Various forms of changes belong there from diffuse or nodular glomerulosclerosis to exudative changes or arteriole hyalinization. Diffused glomerulosclerosis is the most frequent change in diabetic nephropathy. It is usually characterized by the diffused increase of mesangium matrix and glomerular basement membrane (GBM) thickening. Østerby [11] thinks that the GBM thickness measurement is the most precise way of determining advancement in early diabetic changes in renal glomeruli. Simultaneously, even though morphological exponents of diabetic changes in kidneys are quite well known, the development of the pathomechanism of these structural disorders still remains unclear.

Thus, the aim of this study is to assess selected parameters of oxidative stress in rat kidneys in the course of streptozotocin-induced diabetes in various periods of its development as well as the effect of vitamins C and E on pro- and antioxidative processes in kidneys of the investigated animals in vivo. Furthermore, the morphometric analysis of GBM thickness in the investigated groups of animals is also subject to investigation.

**Material and Methods**

**Animals**

Wistar male rats of mean body weight 238 ± 32 g were investigated. Throughout the investigations the animals were fed a standardized diet (Murigram) and had free access to drinking water. The rats were 12 weeks old at the start of the study. In the quarantine period and in the course of investigations the animals were kept at 20 ± 1 °C and the duration of successive light-darkness cycles was 12 h. Diabetes was evoked by intraperitoneal administration of streptozotocin (Sigma, Deisenhofen, Germany) in a dose 65 mg/kg body weight, dissolved in 1 ml 0.1 M sodium citrate (pH 4.5) [12]. The control group of rats received intraperitoneally 1 ml 0.1 M sodium citrate (pH 4.5). Seven rats from the investigated group died within 24 h after streptozotocin administration, 9 rats were excluded from the study due to very low levels of glycemia (<250 mg/dl). A group of 11 rats died due to various reasons in the course of this experiment and thus were excluded from the study.

The remaining rats which survived were divided into four groups of 12 rats each: the control (C), rats with streptozotocin-induced diabetes (DR), diabetic rats on a standard diet supplemented with 200 mg of vitamin E (α-tocopherol acetate; Sigma) per 100 g fodder (DR+VE) (the animals consumed on average 200 mg/kg body weight/
24 h of vitamin E), diabetic rats were on a standard diet supplemented with vitamin C (L-ascorbic acid; Sigma) in a dose of 1 g/l in their drinking water (DR+VC) (the animals received on average 1 g/kg body weight/24 h of vitamin C).

The study was performed under the agreement of the Ethics Committee for Scientific Research at the Military Medical University of Łódź (No. 224/97).

Forty-eight hours after streptozotocin administration, blood was collected from the tail vein of all the animals taking part in the experiment to determine the glucose concentration in the blood. Animals which were administered streptozotocin and in which glucose concentration was <250 mg/dl were excluded from the study. After 3 days, 7 days, and then every week for 12 weeks, glucose concentration in blood was controlled with a Glucostix strip test in a glucometer (Glucometer GX Bayer Diagnostics GmbH, München, Germany), glycated hemoglobin concentration (HbA1c) in blood using the colorimetric method with the use of test kits (glycated hemoglobin; Sigma Diagnostics), body weight and 24-hour albumin excretion in urine.

In order to perform 24-hour urine collection, each rat from all the groups was kept in a metabolic cage. Albumin concentration in urine collected every 24 h was measured with the nephelometric method. Sheep anti-rat albumins (the binding site) were used in the experiment. Determination was performed with ICS Analyzer CC II (Beckman). After 6 and 12 weeks of the experiment, 6 randomly selected rats from every investigated group were anesthetized with inactin (sodic salt of 5-ethyl(methyl-propyl)-2-thiobarbituric acid; Byk-Gulden, Konstanz, Germany) and then blood and the right kidney were collected.

Having dissected the kidneys and washed them with a solution of normal saline, their mass was measured and then they were subjected to biochemical and histopathological examinations. The kidneys were homogenized in 10 volumes of 50 mm/l Tris-0.1 mmol/l EDTA (pH 7.6) at +4 °C for about 30 s with homogenizer Ultra-Turrax® T 25 (Janke Kunkel IKA Labortechnik). Next, the homogenates were centrifuged at 12,000 g for 20 min at +4 °C in order to eliminate tissue debris, nuclear and mitochondrial fraction. To determine CAT and glutathione peroxidase (GSH-Px) activity, the supernatant was centrifuged at +4 °C at 105,000 g for 20 min in a Beckman C7-35 ultracentrifuge. SOD activity (EC 1.15.1.1) in the cytoplasmic fraction was determined with the Misra and Fridovich method [13]. CAT activity (EC 1.11.1.16) in the post-mitochondrial fraction was determined with the Beers and Sizer method [14]. GSH-Px activity (EC 1.11.1.9) in the cytoplasmic fraction was determined with the Little and O’Brien method [15].

Malondialdehyde (MDA) concentration in kidney homogenates was determined according to the method of Placer et al. [16]. Proteins were measured by the method of Lowry et al. [17]. Plasma vitamin C concentration was determined according to Kyaw’s method [18]. Plasma vitamin E concentration was determined with the spectrophotometric method [19]. Kidney specimens for electron microscopy were fixed in 3.6% solution of glutaraldehyde in 0.13 M cacodylate buffer with pH 7.2. They were then fixed in 2% aqueous solution of osmium tetroxide and embedded in Epon. The specimens were dehydrated in a series of ethanol solutions and embedded in Epon. Sections were cut with an Ultracut R microscope (Leica) and stained with uranyl acetate and lead citrate. The sections were examined with a transmission electron microscope (EM 109, Zeiss).

Fig. 1. Rats from the control group: the structure of glomerular basement membrane (BP) is preserved. Slit pores (V) are visible between foot processes (P). A monolayer line of endothelium cells is visible in capillary vessels (K). × 5,000.

Fig. 2. Rats from the control group: capillary loop vessels (K) are lined with monolayer endothelium (S) on one side and on the other they are adjacent to GBM (BP). Primary urine space (PMP) is clear. × 3,200.
were cut with an LKB-5 ultramicrotome; the networks were contrasted in an ultrastatin automat (LKB).

Morphometric estimation was performed by means of the computer image analysis Micro Image for Windows (Olympus). The analysis included preparation of electron micrographs from 5 glomeruli in which a photographic picture was transformed into a digital image with Agfa Snap Scan 1236. After initial processing, brightness and contrast parameters were added and elements of the image of required brightness (with the use of histogram function) were selected. Morphometric measurements were performed after filtering of noise and disturbances. GBM thickness within capillaries was estimated. Due to the comparative character of the above-mentioned investigations, the program calibration was not performed with calibration net and GBM thickness was expressed in pixels. Typical electron micrographs from various experimental groups are illustrated in figures 1–5.

Statistical significance of differences was assessed with one-way ANOVA with the use of SPSS for Windows (version 6.1); p < 0.05 was assumed as statistically significant.

**Fig. 4.** Streptozotocin-diabetic rats receiving vitamin C: Decrease in the thickness of GBM (BP) with evident podocyte foot processes. Expansion of endoplasmic reticulum tubules with single lamellated corpuscles visible in the cytoplasm of podocytes (P). Indentation of mesangial cells is present in some capillary vessels (K). Mesangial area is expanded. × 3,200.

**Fig. 5.** Streptozotocin-diabetic rats receiving vitamin E: decrease in the thickness of GBM (BP), standardization of podocyte structure (P), continuing expansion of mesangial area, indentation of mesangial cells in some capillary vessels. × 3,200.

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Antioxidative Vitamins and Diabetic Kidney

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e137
Results

Concentration of Glucose and Glycated Hemoglobin (HbA1c) (table 1)

In the group of rats with streptozotocin-induced diabetes after 6 and 12 weeks’ observation, a significant increase of glucose concentration in their blood was demonstrated in comparison to the control group. The value of glycemia in the group of diabetic rats administered vitamins E and C did not differ significantly from those with streptozotocin-induced diabetes in which these vitamins were not administered both after 6 and 12 weeks of the experiment. Glycated hemoglobin concentration in the group of rats with streptozotocin-induced diabetes was significantly higher both after 6 and 12 weeks of the experiment compared to the control group. HbA1c concentration in diabetic rats which were administered vitamin E or C did not differ significantly after 6 and 12 weeks of observation from that observed in diabetic rats which were not administered the vitamins.

Body Weight, Kidney Weight, the Ratio of Kidney Weight to Body Weight

In rats with streptozotocin diabetes, body weight after 6 and 12 weeks of observation was statistically significantly lower compared to the control group. After 6 and 12 weeks of observation a statistically significantly lower body weight was found in diabetic rats supplemented with vitamin C or E in comparison to the control group.

The body weight of rats with streptozotocin diabetes, which were administered vitamin C or E after 6 and 12 weeks of observation, did not differ from the body weight of diabetic rats not taking vitamins. In the group of diabetic rats, kidney weight after 6 and 12 weeks was statistically significantly higher compared to the control group. The value of glycemia in the group of diabetic rats administered vitamin E or C did not differ significantly after 6 and 12 weeks of observation from that observed in diabetic rats which were not administered the vitamins.

Twenty-Four-Hour Albumin Excretion in Urine (table 1)

In the group of rats with streptozotocin diabetes after 6 and 12 weeks of the experiment, a significantly increased 24-hour albumin excretion in urine was observed in comparison to the control group. After 6 and 12 weeks of the experiment a significantly increased 24-hour albumin excretion in urine was observed in comparison to the control group. However, in comparison to the control group after 12 weeks of observation, the ratio of kidney weight to body weight was found to be significantly higher in rats receiving vitamin E or C.

Table 1. Characteristics of the animals studied (mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C 6 weeks</th>
<th>12 weeks</th>
<th>DR 6 weeks</th>
<th>12 weeks</th>
<th>DR+VC 6 weeks</th>
<th>12 weeks</th>
<th>DR+VE 6 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>114.0 ± 7.9</td>
<td>116.7 ± 9.4</td>
<td>536.5 ± 52.6a</td>
<td>536.2 ± 25.3a</td>
<td>520.0 ± 27.7a</td>
<td>527.1 ± 25.49a</td>
<td>532.5 ± 18.9a</td>
<td>540.16 ± 23.1a</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>1.27 ± 0.54</td>
<td>1.17 ± 0.23a</td>
<td>3.28 ± 1.11a</td>
<td>3.69 ± 1.08a</td>
<td>3.1 ± 0.4a</td>
<td>3.54 ± 0.34a</td>
<td>3.34 ± 0.47a</td>
<td>3.69 ± 0.24a</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>350 ± 15</td>
<td>364 ± 15a</td>
<td>158 ± 14a</td>
<td>163 ± 5a</td>
<td>164 ± 15a</td>
<td>182 ± 6a</td>
<td>157 ± 14a</td>
<td>171 ± 10a</td>
</tr>
<tr>
<td>Kidney weight, mg</td>
<td>836 ± 63</td>
<td>969 ± 27a</td>
<td>963 ± 73a</td>
<td>1,189 ± 78a</td>
<td>958 ± 43.0b</td>
<td>996 ± 12.0a</td>
<td>943 ± 21.0a</td>
<td>1,008 ± 46a</td>
</tr>
<tr>
<td>Kidney weight/body weight</td>
<td>0.24 ± 0.08</td>
<td>0.27 ± 0.06a</td>
<td>0.61 ± 0.09a</td>
<td>0.73 ± 0.19a</td>
<td>0.58 ± 0.09ab</td>
<td>0.54 ± 0.09a</td>
<td>0.60 ± 0.07ab</td>
<td>0.59 ± 0.08</td>
</tr>
<tr>
<td>24-Hour urinary albumin excretion, mg/24 h</td>
<td>0.77 ± 0.06</td>
<td>0.79 ± 0.05a</td>
<td>5.28 ± 0.46a</td>
<td>19.5 ± 0.98a</td>
<td>4.84 ± 0.42ab</td>
<td>14.14 ± 2.57a</td>
<td>4.96 ± 0.22ab</td>
<td>11.93 ± 1.37</td>
</tr>
</tbody>
</table>

C = Control rats; DR = rats with ST2 diabetes; DR+VC = diabetic rats supplemented with vitamin C; DR+VE = diabetic rats supplemented with vitamin E.

a p < 0.05 (DR, DR+VC, DR+VE vs. C).
b p < 0.05 (DR+VC, DR+VE vs. DR).
creatinine was found in the groups of diabetic rats which were administered vitamin E or C compared to the control group. After 6 weeks of observation of diabetic rats which received vitamin E or C, a decreased 24-hour albumin excretion in urine was observed in comparison to diabetic rats not receiving vitamins; however, these differences were not statistically significant.

After 12 weeks of the experiments a statistically significantly lower 24-hour albumin excretion in urine was found in diabetic rats receiving vitamin E or C in comparison to the groups of diabetic rats not receiving these vitamins.

**Plasma Vitamin E and Vitamin C Concentration**

Plasma vitamin E and vitamin C concentration in diabetic rats after 6 and 12 weeks of the experiment was significantly decreased in comparison to the control group. In diabetic rats receiving vitamin E or C after 6 and 12 weeks of observation, a significantly higher concentration of these vitamins was observed in comparison to diabetic rats not receiving vitamin supplementation. In diabetic rats receiving vitamin C a statistically higher vitamin concentration was found in comparison to the control group.

**Renal MDA Concentration**

Renal MDA concentration in rats with streptozotocin diabetes after 6 and 12 weeks of the experiment was statistically significantly higher in comparison to the control group. After 6 and 12 weeks of the experiment, a significantly lower MDA concentration was observed in the kidneys of rats receiving vitamin E or C in comparison to the groups of diabetic rats not receiving these vitamins.

**SOD Activity in Rat Kidneys**

In diabetic rats after 6 and 12 weeks of the experiment a significantly decreased SOD activity was observed in the kidneys in comparison to the control group. After 6 and 12 weeks of the experiment a significantly higher SOD activity was found in the kidneys of rats receiving vitamin E or C in comparison to the group of diabetic rats not receiving these vitamins.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>6 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
</table>
| Plasma vitamin E
(n = 12) | 10.32 ± 0.85<sup>a</sup> | 10.86 ± 0.25<sup>a</sup> |
| DR (n = 12) | 8.62 ± 0.61<sup>b</sup> | 8.21 ± 1.15<sup>b</sup> |
| DR+VE (n = 12) | 9.62 ± 0.84 | 9.90 ± 0.59 |
| Plasma vitamin C
C (n = 12) | 1.83 ± 0.15<sup>a</sup> | 1.80 ± 0.22<sup>a</sup> |
| DR (n = 12) | 1.50 ± 0.11<sup>a,b</sup> | 1.46 ± 0.16<sup>a,b</sup> |
| DR+VC (n = 12) | 2.48 ± 0.21 | 2.60 ± 0.29 |

<sup>a</sup> p < 0.05 (DR, DR+VE, DR+VC vs. C).
<sup>b</sup> p < 0.05 (DR+VE, DR+VC vs. DR).

**Table 2.** Malondialdehyde content (MDA), superoxide dismutase activity (SOD-1), catalase activity (CAT) and glutathione peroxidase activity (GSH-Px) in the kidney of control rats (C), rats with STZ-induced diabetes (DR), diabetic rats supplemented with vitamin E (DR+VE) or vitamin C (DR+VC) (mg/dl) (mean ± SD)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>MDA nmol/g protein</th>
<th>SOD-1 U/mg protein</th>
<th>CAT, Bergmeyer units/g protein</th>
<th>GSH-Px U/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n = 12)</td>
<td>45.8 ± 2.3</td>
<td>9.56 ± 1.5</td>
<td>11.86 ± 0.96</td>
<td>175.4 ± 25.9</td>
</tr>
<tr>
<td>6 weeks</td>
<td>44.6 ± 4.1</td>
<td>9.01 ± 1.23</td>
<td>12.05 ± 0.61</td>
<td>176.3 ± 17.8</td>
</tr>
<tr>
<td>12 weeks</td>
<td>104.7 ± 7.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7 ± 1.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.5 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.32 ± 19.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DR (n = 12)</td>
<td>110.6 ± 7.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.67 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.04 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>101.94 ± 25.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6 weeks</td>
<td>65.34 ± 5.26&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.99 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.78 ± 0.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>185.33 ± 15.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 weeks</td>
<td>67.25 ± 2.8&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>8.28 ± 1.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.41 ± 1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>189.98 ± 17.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DR+VE (n = 12)</td>
<td>6 weeks</td>
<td>67.46 ± 5.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10.01 ± 1.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.57 ± 0.45&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12 weeks</td>
<td>66.60 ± 4.7&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>9.73 ± 1.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.05 ± 0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>182.46 ± 11.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> p < 0.05 (DR, DR+VE, DR+VC vs. C).
<sup>b</sup> p < 0.05 (DR+VE, DR+VC vs. DR).
**Table 4.** Results of morphometric studies of the basal membrane of renal glomeruli (GBM) in control rats (C), rats with STZ diabetes (DR) and diabetic rats supplemented with vitamin C (DR+VC) or vitamin E (DR+VE) (pixels) (mean ± SD)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>C</th>
<th>DR</th>
<th>DR+VC</th>
<th>DR+VE</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 12 weeks</td>
<td>54.0±18.07</td>
<td>85.43±22.3(^a)</td>
<td>68.82±28.2(^{a,b})</td>
<td>67.73±20.48(^{a,b})</td>
</tr>
</tbody>
</table>

\(^a\) p < 0.05 (DR, DR+VC, DR+VE vs. C).
\(^b\) p < 0.05 (DR+VC, DR+VE vs. DR).

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**CAT Activity in Rats Kidneys** (table 3)

After 6 and 12 weeks of the experiment, renal CAT activity in rats with streptozotocin diabetes was significantly lower compared to the control group. A statistically significantly higher renal CAT activity was found after 12 weeks in diabetic rats receiving vitamin E or C compared to diabetic rats not receiving vitamins; however, no significant differences were observed between these groups after 6 weeks of the experiment.

**GSH-Px Activity in Rats Kidney** (table 3)

After 6 and 12 weeks of observation a significantly lower renal GSH-Px activity was observed in rats with streptozotocin diabetes compared to the control group. A significantly higher GSH-Px activity was found in the kidneys of rats receiving vitamin E or C after 6 and 12 weeks of the experiment in relation to diabetic rats not receiving vitamins.

**Results of Morphometric Investigations** (table 4)

The performed quantitative examination of renal glomeruli GBM thickness demonstrated that during 12 weeks of observation there is a statistically significant increase of renal glomeruli GBM thickness in rats with streptozotocin diabetes compared to the control group. A significantly higher GBM thickness was found in the kidneys of rats receiving vitamin E or C after 12 weeks a statistically significant decrease of GBM thickness was observed in comparison to diabetic rats not receiving vitamins.

**Discussion**

Hyperglycemia is one of the primary causes of a majority of diabetic complications. Increased glucose concentration results in the occurrence of systematic oxidative stress in diabetes [1].

In our presented studies with the use of the model streptozotocin-induced diabetes, intensification of oxidative stress was observed in the kidneys of the investigated animals together with advancement of the disease. An increase of renal MDA, a decrease of plasma concentration of the so-called antioxidative vitamins (E and C), and a decrease of renal enzymatic antioxidative systems in rats with streptozotocin-induced diabetes were the exponents of this condition.

The so far carried out studies have demonstrated that kidneys are particularly sensitive to oxidative stress activity, which suggests their role in renal diabetes pathogenesis [2]. MDA concentration, manifesting intensification of peroxidation processes as well as oxygen-reactive forms of cytotoxic activity, is a good exponent of oxidative stress severity in tissues. In our investigations already in the 6 weeks of streptozotocin diabetes development, an increased level of MDA was observed in diabetic rat kidneys. After the next 6 weeks of the experiment a further significant increase of this factor was not observed, which would suggest significant intensification of peroxidation process in the kidneys already in the early stage of streptozotocin-induced diabetes development. Microalbuminuria is considered to be an important prognostic indicator of increased risk of evident diabetic nephropathy development [20]. Increased albumin excretion in urine is caused by renal glomeruli filtration barrier damage, but the mechanism of this phenomenon has not been thoroughly recognized. Disturbed basement membrane metabolism evoked among others by the severity of pro-oxidative stress in diabetes may be one of the possible mechanisms. It has been stated that AGE interaction with AGE receptor and oxidative stress may affect increased permeability of vessels in diabetes. Increased lipid peroxidation of cell membranes evoked by oxygen-reactive forms may be the reason for these disturbances [2, 3].
In our investigations of diabetic rats, an increased 24-hour albumin excretion in urine was observed after 6 weeks of the experiment and a fourfold increase of albumin excretion in urine after the next 6 weeks. It was connected with the increase of MDA concentration in the kidney tissue of diabetic rats.

Our observations find confirmation in the studies of Ha and Kim [2] who observed an increased concentration of lipid peroxides in plasma and urine of rats with streptozotocin-induced diabetes and microalbuminuria. The same authors demonstrated that hyperglycemia may evoke lipid peroxidation in rat isolated renal glomeruli [21]. Elevated glucose concentration activates the system of PKC, which in consequence leads to intensified lipid peroxidation in diabetes. It is suggested that PKC activation initiates oxidative stress in renal glomeruli in diabetes, whereas other mechanisms such as glucose autoxidation, polyol pathway activation and glycation play a significant role only in later periods of peroxidative damage of renal glomeruli [2].

It should be emphasized that lipid peroxidation products such as lipid peroxides, hydroxynonenol, and F2-isoprostanoid are of importance in diabetic complications progression particularly in microangiopathy. Moreover, lipid peroxidation may also play a significant role in renal glomerulosclerosis pathogenesis, and thus in the development of renal insufficiency [22].

In numerous clinical and experimental examinations, changes in antioxidative enzymatic systems activity were revealed in various tissues in the course of diabetes. In our earlier performed studies [23] a decrease of key antioxidative enzymes activity was observed in erythrocytes of patients with diabetes type 2 which were more severe in patients with diabetic nephropathy.

In the performed investigations of diabetic rats a decreased activity of key antioxidative cellular enzymes was observed, that is of SOD, CAT and GSH-Px in the kidneys.

Our observations are in agreement with the studies of other authors. Kakkar et al. [24] revealed a decreased SOD activity in the kidneys of rats with streptozotocin-induced diabetes. Asayama et al. [25] demonstrated in rats with 14-day streptozotocin-induced diabetes, an increased CAT activity in the heart, whereas it was decreased in the liver and kidneys. A decrease of renal antioxidative enzymes activity in the course of experimental diabetes is connected by some authors with a significant reduction of body weight [25]. A significant reduction of body weight compared to the control group, accompanied in our investigations changes of renal antioxidative enzymes activity in diabetic rats. Other indicators which accompanied intensified renal lipid peroxidation and decreased antioxidative enzymes activity in diabetic rats were elevated glucose and glycolated hemoglobin concentration, as well as increasing the disease advancement, albumin excretion in urine, significant increase of kidney weight and the ratio of kidney weight to body weight compared to the control group. Furthermore, the results of morphometric investigations of renal glomerular capillaries’ basement membrane thickness point to a significant increase of basement membrane thickness in renal glomeruli with streptozotocin-induced diabetes in comparison to the control group.

Development of renal hypertrophy in rats with streptozotocin-induced diabetes was also observed by other authors. Ku et al. [26] found an increase of mass and protein content in the kidneys of rats with 7-day streptozotocin diabetes compared to the control group. Another group of authors demonstrated in a morphometric analysis that the renal hypertrophy observed by them resulted from the increase of renal glomeruli size. Mechanisms which may cause renal hypertrophy in diabetes have not been unequivocally determined yet. Recently, attention has been paid to the role of TGF-ß in collecting extracellular matrix proteins in diabetic kidneys. On the other hand, oxygen-reactive forms and/or antioxidative enzyme insufficiency are among others made responsible for induction of TGF-ß in diabetes [22].

Deficit or decrease of plasma antioxidant activity including α-tocopherol or ascorbic acid may also be the effect of hyperglycemia accompanying diabetes. In our studies a significant decrease of antioxidative vitamins (such as C and E) concentration was observed in diabetic rat plasma.

A decreased concentration of vitamins C and E in plasma and tissues of diabetic patients and in animals with experimental diabetes was also observed by other authors. A decreased ascorbic cellular uptake is explained by competition with glucose, increased ascorbic acid excretion in urine, and ascorbic acid enzymatic regeneration from dehydroascorbian caused by decreased glutathione concentrations [6, 8].

Impairment of α-tocopherol reproduction is also connected with a decreased ascorbate concentration. Vitamin C is the strongest physiological antioxidant acting in the organism’s aqueous environment, thus it plays an important role in protein thiol group protection against oxidation. Oxidized proteins may activate PKC, which in consequence can lead to an increased TGF-ß release, increased extracellular matrix protein synthesis, and me-
sangium proliferation. An increase of vitamin C concentration in renal cortex was found to be able to inhibit these processes by decreasing PKC activity [8]. Vitamin C is a known free radical scavenger and may inhibit glycooxidation reaction and prevent AGE development in plasma and tissues. On the other hand, vitamin E is in physiological conditions an important antioxidant with lipophil properties acting in cellular membranes and plasma lipoproteins. It has an inhibitory effect on lipid peroxidation and AGE production; moreover, it demonstrates antiproliferative activity in mesangial cell cultures in medium to high glucose concentration [27]. α-Tocopherol was found to be able to inhibit increased TGF-β release in renal glomeruli of diabetic rats through the decrease of PKC activity. Taking into consideration the given data here, the observed decreased concentration of antioxidative vitamins in plasma of diabetic rats may play an essential role in the development of systemic oxidative stress in diabetes and free radical pathogenesis of diabetic nephropathy. In the literature, numerous favorable effects observed after administration of antioxidants both in diabetic patients and experimental diabetes have been described. They were found to beneficially affect utilization and glucose metabolism. They decrease the glycated protein concentration, decrease oxidative stress severity, and also improve endothelial function [10, 28].

Despite many studies concerning the participation of free radical processes in diabetes pathogenesis, the number of reports concerning the protective effect of antioxidants on oxidative stress development in diabetes and its renal complications is still limited. In our studies, administration of vitamin E or C to diabetic rats was found to lead to an increase of vitamin concentration in plasma and also a decreased lipid peroxidation severity in kidneys of rats compared to the group with diabetes but not receiving these vitamins. However, it did not affect the glycated hemoglobin concentration.

In diabetic rats which additionally received α-tocopherol or ascorbic acid, differences in body weight were not detected compared to the group of diabetic animals not receiving vitamins. Administration of vitamin C or E however had a beneficial effect on kidney weight reduction in diabetic rats and significantly decreased albumin excretion in urine after 12 weeks of the experiment, which is in accordance with observations of Craven et al. [8]. On the other hand, other authors having administered vitamin E to rats with streptozotocin-induced diabetes did not notice any beneficial effect on microalbuminuria or intensified lipid peroxidation. Maybe the differences between our observations and the results of studies presented by Ha and Kim [2] may result from the differences in the course of observations because these authors carried out their experiment three times shorter than ours. It has been demonstrated that administration of vitamin C or E to diabetic rats has an inhibitory effect on PKC activity in retina, aorta and also mesangium cell cultures in medium to high glucose concentration and in renal glomeruli isolated from diabetic rats. In this way these vitamins decrease oxidant concentration and prevent functional protein sulfhydryl groups oxidation. This may confirm the beneficial effect of vitamins C and E on lipid peroxidation in rat kidneys which was accompanied by inhibition of renal hypertrophy and a decrease of albumin excretion in urine. Moreover, in our studies, administration of these vitamins appeared to have favorable effects on the increase of the investigated antioxidative enzymes activity in kidneys of diabetic rats. It seems that the observed increased activity of antioxidative enzymes in the examined tissue should not be associated only with the decreased generation of oxygen-reactive forms; indirect proof is the decrease of lipid peroxidation, but also the decreased direct damaging effect of oxygen-reactive forms on enzymatic proteins which was not observed in diabetic animals which were not administered any drugs.

The beneficial effect of oxidative vitamins E and C on renal structure is confirmed by morphometric analysis of renal glomeruli capillaries basal membrane in which a significant decrease of its thickness was observed in diabetic rats receiving vitamin E or C compared to diabetic rats not receiving these vitamins. The beneficial effect of vitamins E and C on renal structure was also observed by Craven et al. [8]. Recently published research by Molyneux et al. [29] also confirms the positive influence of Trolox (hydrophilic analog of α-tocopherol) and ascorbic acid on alleviating the effects of increased activity of reactive oxygen species. Antioxidative vitamins used by the researchers not only decreased the processes of lipid peroxidation but also reduced structure damage and the impairment of heart’s microcirculation.

In the discussion concerning antioxidative vitamin activity, besides the mechanism of their action, the applied dose seems to be an essential element. In the case of vitamin C it was found that it may be in small doses and in the presence of transient group metal exert a prooxidative effect. Furthermore, ascorbic acid may intensify collagen synthesis in vitro in the mechanism dependent on intensified lipid peroxidation, and this process may be inhibited by α-tocopherol [30]. In our investigations, high doses of both vitamin C and vitamin E were applied. A high dose of vitamin E not only prevented lipid oxidation...
and intensified the organism’s antioxidative potential, but also restored the balance between thromboxane A₂ and prostacyclin as well as decreased blood platelet aggregation which is of significant importance in vascular complication inhibition including diabetic nephropathy.

In conclusion, it may be stated that in experimental streptozotocin-induced diabetes in rats, renal oxidative stress develops already in its early stage. Furthermore, on the basis of the performed investigations, vitamins E and C seem to be essential antioxidative substances which are able to modulate prooxidative and antioxidative processes in diabetes and have a protective effect on renal structure and function in the course of diabetes.

References