

# Reduced intake of dietary antioxidants can impair antioxidant status in type 2 diabetes patients

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## KEY WORDS

antioxidant status,  
dietary antioxidants,  
oxidative stress, type  
2 diabetes

## ABSTRACT

**INTRODUCTION** Oxidative stress plays a major role in the pathogenesis of type 2 diabetes.

**OBJECTIVES** The objective of this study was to investigate associations between dietary intake of antioxidants and antioxidant status in patients with type 2 diabetes.

**PATIENTS AND METHODS** In 80 patients with type 2 diabetes and 37 controls, total antioxidant status (TAS), activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD), malondialdehyde (MDA), and 4-hydroxyalkenals (4-HAE) were measured. The 24-hour food recall method and our own dietary database were used to calculate dietary total antioxidant capacity (DTAC), polyphenol content (DTPC), and flavonoid content (DTFC). Dietary antioxidant vitamins were calculated using national food composition databases.

**RESULTS** Serum TAS was 1.57 mmol/l in controls, 1.41 mmol/l in patients with newly diagnosed type 2 diabetes, and 1.23 mmol/l in patients with long-standing type 2 diabetes. Serum MDA and 4-HAE levels were 0.78  $\mu\text{mol/l}$  in controls, 1.45  $\mu\text{mol/l}$  in newly diagnosed diabetes, and 1.74  $\mu\text{mol/l}$  in long-standing diabetes. GPx and SOD activities were 42.6 and 1340 units/g hemoglobin (Hb), respectively, in controls, 47.3 and 2373 units/g Hb in long-standing diabetes, and 58.2 and 3093 units/g Hb in newly diagnosed diabetes. DTAC, DTPC, DTFC, and vitamin C content were 5697  $\mu\text{mol}$  Trolox equivalents (TE), 1031 mg gallic acid equivalents (GAE), 223 mg quercetine equivalents (QE), and 82 mg, respectively, in control diet. In patients with long-standing diabetes, the values were 4271  $\mu\text{mol}$  TE, 822 mg GAE, 173 mg QE, and 63 mg, respectively, and in those with newly diagnosed type 2 diabetes, they were 4545  $\mu\text{mol}$  TE, 839 mg GAE, 180 mg QE, and 65 mg, respectively.

**CONCLUSIONS** The diet of type 2 diabetes patients is poor in antioxidants despite increased demand.

**INTRODUCTION** Type 2 diabetes is considered to be one of the most common chronic diseases worldwide, including Poland.<sup>1</sup> Oxidative and nitrosative stresses play the main role in the pathogenesis of type 2 diabetes and long-term diabetic microvascular and macrovascular complications such as diabetic retinopathy, nephropathy, neuropathy, atherosclerosis, and coronary heart disease. Hyperglycemia can stimulate imbalance between the production and elimination of reactive oxygen species and reactive nitrogen species.<sup>2,3</sup>

In healthy subjects, exposure to reactive oxygen species or reactive nitrogen species, which are generated in normal metabolism, induces an antioxidant defense mechanism. Endogenous

antioxidants, including both enzymatic (eg, superoxide dismutase [SOD], catalase [CAT], glutathione peroxidase [GPx], and glutathione reductase [GRx]) and nonenzymatic systems (eg, glutathione, bilirubin, uric acid, coenzyme Q10, ferritin, metallothioneins, albumin, and ceruloplasmin) exert synergistic actions in scavenging free radicals by converting them to less harmful molecules.<sup>4</sup> Among antioxidant enzymes, SOD catalyzes the dismutation of the major reactive oxygen species—superoxide anion ( $\text{O}_2^{\bullet-}$ )—into hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and GPx converts  $\text{H}_2\text{O}_2$  into  $\text{H}_2\text{O}$ .<sup>5</sup>

Persistent hyperglycemia impairs the endogenous antioxidant defense system and can induce

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**TABLE 1** Baseline characteristics of the study groups

Variable	Long-standing type 2 diabetes (n = 45)	Newly diagnosed type 2 diabetes (n = 35)	Controls (n = 37)	P value	
age, y	60 ± 6	58 ± 8	56 ± 9	0.2	
female/male, n	21/24	16/19	17/20	0.5	
BMI, kg/m <sup>2</sup>	28 ± 6	27 ± 7	26 ± 5	0.1	
smoking, %	18	20	23	0.4	
family history of diabetes, %	46	43	25	0.01 <sup>a</sup>	
duration of diabetes, y	10 ± 6	–	–	–	
CHD, %	20	–	–	–	
HbA <sub>1c</sub>	mmol/mol	73 ± 31	54 ± 14	35 ± 5	0.01 <sup>b</sup>
	%	8.8 ± 5	7.1 ± 2	5.4 ± 1	0.01 <sup>c</sup> , 0.001 <sup>d</sup>
TC, mmol/l	5.7 ± 1.1	5.5 ± 1.2	5.3 ± 1.0	0.2	
TG, mmol/l	1.5 ± 0.7	1.4 ± 0.5	1.3 ± 0.4	0.2	
LDL-C, mmol/l	3.1 ± 0.5	3.0 ± 0.4	2.8 ± 0.3	0.2	
HDL-C, mmol/l	1.3 ± 0.3	1.3 ± 0.4	1.5 ± 0.5	0.3	
SBP, mm Hg	132 ± 17	130 ± 15	127 ± 14	0.3	
DBP, mm Hg	85 ± 12	83 ± 11	78 ± 9	0.2	

Data are presented as mean ± standard deviation or number or percentage.

Significant differences between the study groups:

- a** long-standing type 2 diabetes and newly diagnosed type 2 diabetes vs. controls
- b** long-standing type 2 diabetes vs. newly diagnosed type 2 diabetes
- c** newly diagnosed type 2 diabetes vs. controls
- d** long-standing type 2 diabetes vs. controls

Abbreviations: BMI – body mass index, CHD – coronary heart disease, DBP – diastolic blood pressure, HbA<sub>1c</sub> – glycated hemoglobin, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, SBP – systolic blood pressure, TC – total cholesterol, TG – triglycerides

excessive oxidative and nitrosative stresses resulting in the development of insulin resistance and pancreatic  $\beta$ -cell failure.<sup>6</sup> Moreover, free radicals damage lipids, proteins, and nucleic acids leading to various diabetic complications. The well-known end products of polyunsaturated fatty acids peroxidation are malondialdehyde (MDA) and 4-hydroxyalkenals (4-HAE), which are used as indicators of oxidative stress. Higher levels of oxidative stress markers and lower levels of antioxidant enzymes were observed in patients with poorly controlled diabetes and diabetic-related complications.<sup>7,8</sup>

Dietary guidelines for the prevention and alleviation of type 2 diabetes symptoms are based on a healthy diet containing dietary fiber, polyunsaturated fatty acids, and other indispensable nutrients.<sup>9</sup> Dietary antioxidant intake should also be taken into consideration. Several studies have shown that a diet rich in exogenous antioxidants, including vitamins and flavonoids, can reduce the risk of type 2 diabetes.<sup>10,11</sup>

The objective of this study was to explore associations between intake of dietary antioxidants and antioxidant status in patients with type 2 diabetes.

**PATIENTS AND METHODS** **Patients** The study included 80 patients with type 2 diabetes and 37 matching healthy controls, aged from 40 to 65 years. Participants were selected and diagnosed in the Clinical Department of Endocrinology,

Diabetology and Internal Medicine, Medical University of Białystok, Białystok, Poland, between 2012 and 2013. Among type 2 diabetes subjects, 35 had newly diagnosed type 2 diabetes and were not receiving any medication, while 45 patients had long-standing type 2 diabetes and were treated with oral hypoglycemic drugs or insulin or both. In addition, 30 of those patients received anti-hypertensive drugs and 26, cholesterol-lowering drugs. Moreover, 9 patients with long-standing diabetes had coronary heart disease. Patients who were taking pharmaceutical supplements containing antioxidant vitamins and minerals were not included in the study. All patients were characterized with a moderate level of physical activity, middle economic status, and rare alcohol use. The baseline characteristics of the participants are shown in **TABLE 1**.

**Samples** Venous blood samples were drawn in heparinized vacuum tubes after overnight fasting. Plasma and erythrocytes were separated by centrifugation at 3000 rpm for 10 minutes at room temperature. Erythrocytes were washed 3 times with 0.9% NaCl solution and then hemolyzed with 4 volumes of cold deionized water. Freshly heparinized whole blood, serum, and erythrocyte lysates were used immediately for further analyses.

**Biochemical profile** The levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein

**TABLE 2** Total antioxidant status and antioxidant enzyme activity in the study groups

Variable	Long-standing type 2 diabetes (n = 45)	Newly diagnosed type 2 diabetes (n = 35)	Controls (n = 37)	P value
TAS, mmol/l	1.23 (0.93–1.29)	1.41 (1.19–1.46)	1.57 (1.48–1.76)	0.02 <sup>a</sup> , 0.02 <sup>b</sup> , 0.01 <sup>c</sup>
GPx, units/g Hb	47.3 (39.4–54.2)	58.2 (49.1–64.3)	42.6 (35.7–48.2)	0.02 <sup>a</sup> , 0.01 <sup>b</sup> , 0.02 <sup>c</sup>
SOD, units/g Hb	2373 (1765–2754)	3093 (2589–3495)	1340 (910–1527)	0.01 <sup>a</sup> , 0.001 <sup>b</sup> , 0.01 <sup>c</sup>
MDA+4-HAE, $\mu$ mol/l	1.74 (0.7–2.6)	1.45 (0.5–2.1)	0.78 (0.4–1.2)	0.02 <sup>a</sup> , 0.01 <sup>b</sup> , 0.01 <sup>c</sup>

Data are presented as median (range).

Significant differences between the study groups:

**a** long-standing type 2 diabetes vs. newly diagnosed type 2 diabetes

**b** newly diagnosed type 2 diabetes vs. controls

**c** long-standing type 2 diabetes vs. controls

Abbreviations: GPx – glutathione peroxidase, 4-HAE – 4-hydroxyalkenals, MDA – malondialdehyde, SOD – superoxide dismutase, TAS – total antioxidant status

(HDL) cholesterol were determined in a hospital laboratory with standard laboratory methods using commercial kits. Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) was measured by high-performance liquid chromatography (D-10 Hemoglobin Testing System, Bio-Rad Hercules, United States).

**Total antioxidant status** Total antioxidant status (TAS) was measured using spectrophotometry in serum samples using a commercially available TAS kit (Randox Laboratories Ltd, Crumlin, United Kingdom). The assay is based on the reduction of free radicals, as a decrease of absorbance at 600 nm at 3 minutes by antioxidants present in a sample. The radical cation ABTS<sup>+</sup> is formed during incubation of ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulphonate]) with peroxidase (methmyoglobin) and H<sub>2</sub>O<sub>2</sub> in 37°C to form a blue-green dye. The results were expressed as mmol/l in serum.

**Antioxidant enzyme activity** GPx activity was measured in whole blood samples using the RANSEL kit (Randox Laboratories Ltd, Crumlin, United Kingdom). GPx catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide to oxidized glutathione (GSSG). Next, GSSG is converted to GSH with a concomitant oxidation of NADPH to NADP<sup>+</sup>. Absorbance was read in a spectrophotometer at 340 nm. The results were calculated in GPx units/g of hemoglobin (Hb). A unit of GPx activity was defined as the amount of enzyme necessary to catalyze the oxidation of 1  $\mu$ mol of NADPH to NADP<sup>+</sup> per minute at 37°C.

SOD activity was measured in erythrocyte lysates using the RANSOD kit (Randox Laboratories Ltd). In this assay, SOD catalyses the dismutation of the toxic superoxide radicals, generated by xanthine and xanthine oxidase, to hydrogen peroxide and molecular oxygen. Superoxide radicals react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form red dye. SOD activity was measured by the degree of inhibition of this reaction. The results were expressed as SOD units/g Hb. One unit of SOD causes a 50%

inhibition of the rate of reduction of INT measured in a spectrophotometer at 505 nm and 37°C.

**Oxidative stress markers** MDA together with 4-HAE were measured in serum using the spectrophotometric assay for lipid peroxidation (Bioxytech LPO-586, Oxis Research, Portland, United States). Polyunsaturated fatty acid peroxides generate MDA and 4-HAE upon decomposition. The assay is based on the reaction of a chromogenic reagent (N-methyl-2-phenylindole) with MDA and 4-HAE at 45°C. Absorbance was measured in a spectrophotometer at 586 nm. The results were expressed as  $\mu$ mol MDA and 4-HAE/l of serum.

**Dietary survey** Daily food consumption was estimated by the 24-hour dietary recall method in a direct interview of a dietitian with a patient immediately after venous blood samples were obtained between 7.00 and 8.00 AM in the laboratory of the Department of Endocrinology, Diabetology and Internal Medicine of the Medical University of Białystok, Białystok, Poland. Food portion sizes were estimated using an album of food photographs.<sup>12</sup>

Our previously published dietary database,<sup>13–16</sup> containing the ferric reducing antioxidant power total antioxidant capacity of foods, total polyphenol and flavonoid contents in beverages and plant food items, was used to calculate the dietary total antioxidant capacity (DTAC), dietary total polyphenol content (DTPC), and dietary total flavonoid content (DTFC). This database contains over 150 beverages and plant foods analyzed in the Department of Food Commodities Science and Technology. For some beverages and plant foods, which were found to be consumed by study participants, but were not included in the database (such as different types of tea), additional measurements were performed. Methods used to measure the total antioxidant capacity, total polyphenol content, and total flavonoid content in beverages and plant foods were described in detail elsewhere.<sup>13–16</sup>

**TABLE 3** Total antioxidant capacity and per capita daily intake of total polyphenols, flavonoids, and antioxidant vitamins in the study groups

Variable	Long-standing type 2 diabetes (n = 45)	Newly diagnosed type 2 diabetes (n = 35)	Controls (n = 37)	P value
DTAC, $\mu\text{mol TE}$	4271 (3767–4840)	4545 (3870–4962)	5697 (5134–6265)	0.01 <sup>a</sup>
DTPC, mg GAE	822 (740–951)	839 (778–988)	1031 (915–1231)	0.01 <sup>a</sup>
DTFC, mg QE	173 (161–213)	180 (172–221)	223 (192–269)	0.02 <sup>a</sup>
vitamin E, mg $\alpha$ -tocopherol equivalents	6 (4–8)	6 (4–7)	7 (5–8)	0.5
vitamin C, mg	63 (44–82)	65 (43–85)	82 (55–97)	0.02 <sup>a</sup>
$\beta$ -carotene, $\mu\text{g}$	1785 (1511–2346)	1812 (1632–2454)	1871 (1668–2498)	0.2

Data are presented as median (range).

Significant differences between the study groups:

**a** long-standing type 2 diabetes and newly diagnosed type 2 diabetes vs. controls

Abbreviations: DTAC – dietary total antioxidant capacity, DTFC – dietary total flavonoid content, DTPC – dietary total polyphenol content, GAE – gallic acid equivalents, QE – quercetine equivalents, TE – Trolox equivalents

Antioxidant vitamin (C, E, and  $\beta$ -carotene) contents in diet were calculated using the Dieta 5.0 computer program based on the national food composition (National Food and Nutrition Institute in Warsaw).<sup>17</sup> This program takes into account vitamin losses during culinary processes.

The study was approved by the local Ethics Committee Medical University of Białystok (approval number: R-I-002/264/2012), and all participants gave written informed consent to participate in the study.

**Statistical analysis** Statistical analysis was performed using the Statistica 10.0 software (StatSoft, Inc.). The results were expressed as number, percentage for categorical variables, and mean  $\pm$  standard deviation or median with interquartile range for continuous variables according to the normal or nonnormal distribution of data. Categorical variables were compared with the  $\chi^2$  test. Normality of continuous data distribution was verified with the Shapiro–Wilk test. The one-way analysis of variance followed by the Tukey's post hoc test was used for normally distributed variables and the Kruskal–Wallis test followed by the Dunn's post hoc test was used for variables without normal distribution. Associations between variables were calculated with the Spearman's rank correlation test. A *P* value of less than 0.05 was considered to be statistically significant.

**RESULTS** Clinical and biochemical data of the participants are given in **TABLE 1**. The mean disease duration in patients with long-standing diabetes was 10 years. Although all patients were treated with oral hypoglycemic drugs or insulin or both, they had poorly controlled diabetes and had a significantly higher level of  $\text{HbA}_{1c}$  compared with patients with newly diagnosed diabetes and controls. A family history of diabetes was more common in patients with type 2 diabetes than in controls. Patients were well-matched for age, sex, body mass index, smoking status, TC, TG, LDL

cholesterol, HDL cholesterol, systolic blood pressure, and diastolic blood pressure.

**TABLE 2** shows that serum TAS was significantly elevated in controls (1.57 mmol/l) compared with patients with newly diagnosed diabetes (1.41 mmol/l) and those with long-standing diabetes (1.23 mmol/l). A significant inverse correlation was observed for the serum MDA+HAE level, which was lower in controls (0.78  $\mu\text{mol/l}$ ) compared with patients with newly diagnosed diabetes (1.45  $\mu\text{mol/l}$ ) and those with long-standing diabetes (1.74  $\mu\text{mol/l}$ ). GPx and SOD activities were significantly lower in controls (42.6 units/g Hb and 1340 units/g Hb, respectively) compared with patients with long-standing diabetes (47.3 units/g Hb and 2373 units/g Hb, respectively) and those with newly diagnosed diabetes (58.2 units/g Hb and 3093 units/g Hb, respectively).

The dietary analysis of total antioxidant capacity as well as intake of polyphenols, flavonoids, and antioxidant vitamins showed that DTAC, DTPC, DTFC, and vitamin C content were significantly higher in control diet (5697  $\mu\text{mol}$  Trolox equivalents [TE], 1031 mg gallic acid equivalents [GAE], 223 mg quercetine equivalents [QE], and 82 mg, respectively) than in patients with long-standing diabetes (4271  $\mu\text{mol TE}$ , 822 mg GAE, 173 mg QE, and 63 mg, respectively) and those with newly diagnosed diabetes (4545  $\mu\text{mol TE}$ , 839 mg GAE, 180 mg QE, and 65 mg, respectively). No significant differences were found between the diabetic groups. Daily intakes of vitamin E and  $\beta$ -carotene were similar in all studied groups (**TABLE 3**).

Serum TAS in controls, patients with newly diagnosed diabetes, and those with long-standing diabetes were correlated only with DTAC ( $r = 0.55$ ,  $P = 0.02$ ;  $r = 0.49$ ,  $P = 0.03$ ;  $r = 0.37$ ,  $P = 0.04$ ; respectively). No significant associations were found between serum TAS and intake of single dietary antioxidants, namely, DTPC, DTFC, vitamins E and C, and  $\beta$ -carotene. GPx and SOD activities as well as the MDA+HAE level were independent of dietary antioxidant intake in all study groups.

**TABLE 4** Mean daily intake of plant food and beverages in the study groups (g [ml] fresh edible mass/person/day)

Plant food and beverages	Long-standing type 2 diabetes (n = 45)	Newly diagnosed type 2 diabetes (n = 35)	Controls (n = 37)	P value
beverages (total) <sup>A</sup>	342 (223–435)	386 (272–486)	537 (377–674)	0.01 <sup>a</sup>
tea infusion <sup>B</sup>	245 (134–295)	264 (156–342)	355 (215–423)	0.02 <sup>a</sup>
coffee infusion <sup>C</sup>	87 (54–163)	102 (72–186)	135 (114–235)	0.02 <sup>a</sup>
others <sup>D</sup>	10 (5–17)	20 (14–28)	47 (31–65)	–
vegetables, mushrooms (total)	375 (255–437)	396 (267–475)	415 (320–512)	0.4
potatoes	190 (155–234)	219 (170–295)	247 (188–310)	0.02 <sup>a</sup>
cabbage <sup>E</sup>	40 (31–64)	41 (28–62)	49 (21–71)	0.03 <sup>a</sup>
tomatoes	37 (15–48)	42 (24–53)	48 (33–72)	0.03 <sup>a</sup>
cucumbers	23 (15–27)	21 (12–25)	20 (10–19)	0.5
carrots	15 (8–20)	15 (6–23)	15 (7–20)	0.6
beetroots	15 (5–20)	12 (4–18)	11 (4–16)	0.1
cauliflower	14 (7–21)	8 (5–13)	5 (3–8)	0.03 <sup>b</sup>
others <sup>F</sup>	41 (28–47)	38 (21–44)	20 (14–31)	–
fruit and fruit jams (total)	175 (154–223)	186 (165–235)	230 (189–271)	0.03 <sup>a</sup>
apples	125 (70–167)	126 (85–175)	156 (125–205)	0.03 <sup>a</sup>
citrus fruits <sup>G</sup>	27 (18–42)	25 (14–41)	35 (16–48)	0.03 <sup>a</sup>
others <sup>H</sup>	23 (8–22)	35 (15–35)	39 (17–48)	–
cereal products (total)	178 (165–192)	190 (171–224)	198 (182–233)	0.08
white bread, rolls	55 (33–68)	121 (76–144)	108 (75–165)	0.01 <sup>c</sup>
				0.03 <sup>d</sup>
				0.02 <sup>e</sup>
wholegrain bread	83 (69–124)	23 (10–45)	47 (28–55)	0.01 <sup>c</sup>
				0.02 <sup>d</sup>
				0.02 <sup>e</sup>
wheat flour	21 (16–27)	26 (18–33)	27 (19–35)	0.4
others <sup>I</sup>	19 (12–26)	20 (10–24)	16 (7–22)	–
nuts and seeds (total) <sup>J</sup>	<1	<1	<1	0.9
vegetable oils (total) <sup>K</sup>	5 (3–12)	10 (5–16)	10 (4–18)	0.5

Data are presented as median (range).

Significant differences between the study groups:

**a** long-standing type 2 diabetes and newly diagnosed type 2 diabetes vs. controls

**b** long-standing type 2 diabetes vs. newly diagnosed type 2 diabetes and controls

**c** long-standing type 2 diabetes vs. newly diagnosed type 2 diabetes

**d** newly diagnosed type 2 diabetes vs. controls

**e** long-standing type 2 diabetes vs. controls

**A** tea infusion, coffee infusion: 1 g dry sample per 100 ml boiling distilled water; **B** tea infusion: black tea, green tea, red tea, rooibos tea, white tea; **C** coffee infusion: ground coffee, instant coffee; **D** others: beer, juices (apple, black currant, orange), wine (red, white); **E** cabbage: Chinese, red, white; **F** others: beans, celery roots, chives, fennel, leeks, lettuce, mushrooms, onions, parsley roots, peas, pepper (green, red), radish, sorrel; **G** citrus fruits: grapefruits, mandarins, oranges; **H** others: apricots, bananas, bilberries, grapes, kiwi fruits, lingonberries, nectarines, northern cranberries, peaches, pears, plums, raspberries, red currants, sour cherries, strawberries, sweet cherries, watermelon, woodland strawberries, fruit jams; **I** others: barley groats, buckwheat groats, extruded bread, noodles, oats, rice; **J** nuts and seeds: hazelnuts, peanuts, pistachios, pumpkin seeds, sunflower seeds, walnuts; **K** vegetable oils: olive, rape oil, sunflower oil

The daily intake of beverages and plant food in the study groups is presented in **TABLE 4**. The highest amount of plant-derived beverages, particularly tea and coffee, was consumed by controls. Vegetable intake was similar in all studied groups with the highest percentage of potatoes, cabbage, and tomatoes, especially in controls. Patients with long-standing diabetes consumed more cauliflower in comparison with the remaining groups. Fruit intake (particularly apples and citrus fruits) was the highest in controls. Of note, despite a similar total consumption of cereal products in individual groups, the groups differed in terms of types of cereal. Compared with the other groups, patients with long-standing diabetes

consumed more wholegrain bread and less white bread and rolls. Patients with newly diagnosed diabetes showed the highest intake of white bread and rolls and the lowest intake of wholegrain bread. The remaining plant foods, namely, nuts and seeds and vegetable oils, were consumed in insignificant amounts.

**TABLE 5** shows the contribution of tea, coffee, and apples to DTAC, DTPC, DTFC in the study groups: 53%, 58%, 65% of DTAC, 43%, 46%, 49% of DTPC, and 52%, 51%, 55% of DTFC in patients with long-standing diabetes, newly diagnosed diabetes, and controls, respectively.



**TABLE 5** Total antioxidant capacity, and per capita daily intake of total polyphenol and flavonoid in the study groups

Beverages and plant foods	DTAC, µmol TE (% contribution)				DTPC, mg GAE (% contribution)				DTFC, mg QE (% contribution)			
	long-standing diabetes	newly diagnosed diabetes	controls	P value	long-standing diabetes	newly diagnosed diabetes	controls	P value	long-standing diabetes	newly diagnosed diabetes	controls	P value
beverages	2359 (55)	2640 (58)	3485 (61)	0.01 <sup>a</sup>	291 (35)	327 (39)	441 (43)	0.02 <sup>a</sup>	76 (44)	84 (47)	108 (48)	0.03 <sup>a</sup>
tea infusion <sup>A</sup>	1495 (28)	1610 (31)	2165 (36)	0.03 <sup>a</sup>	176 (21)	190 (23)	256 (25)	0.03 <sup>a</sup>	39 (23)	42 (23)	57 (26)	0.03 <sup>a</sup>
coffee infusion <sup>B</sup>	667 (12)	785 (15)	1039 (17)	0.02 <sup>a</sup>	81 (10)	95 (11)	126 (12)	0.03 <sup>a</sup>	13 (8)	15 (8)	20 (9)	0.02 <sup>a</sup>
others <sup>C</sup>	197 (4)	245 (4)	281 (5)	–	34 (4)	42 (5)	59 (6)	–	24 (14)	27 (15)	31 (14)	–
vegetables	597 (14)	570 (13)	579 (10)	0.46	203 (25)	206 (25)	226 (22)	0.08	33 (19)	33 (18)	37 (17)	0.32
potatoes	114 (2)	131 (3)	148 (2)	0.06	59 (7)	68 (8)	77 (7)	0.06	8 (5)	9 (5)	10 (5)	0.24
cabbage <sup>D</sup>	128 (2)	135 (3)	155 (2)	0.03 <sup>a</sup>	56 (7)	58 (7)	69 (7)	0.05	10 (6)	10 (6)	12 (5)	0.37
tomatoes	56 (1)	63 (1)	72 (1)	0.05	22 (3)	25 (3)	29 (3)	0.17	5 (3)	5 (3)	6 (3)	0.56
cucumber	5 (<1)	4 (<1)	4 (<1)	0.74	4 (<1)	4 (<1)	3 (<1)	0.53	1 (<1)	1 (<1)	1 (<1)	0.95
carrots	23 (<1)	23 (<1)	23 (<1)	0.87	5 (<1)	5 (<1)	5 (<1)	0.87	1 (1)	1 (1)	1 (1)	0.89
beetroots	153 (3)	122 (2)	112 (2)	0.03 <sup>b</sup>	27 (3)	21 (3)	20 (2)	0.78	4 (2)	3 (2)	3 (1)	0.77
cauliflower	76 (1)	43 (<1)	27 (<1)	0.03 <sup>b</sup>	13 (2)	7 (<1)	5 (<1)	0.03 <sup>b</sup>	1 (<1)	1 (<1)	1 (<1)	0.94
others <sup>E</sup>	42 (<1)	49 (<1)	38 (<1)	–	17 (2)	18 (2)	18 (2)	–	3 (1)	3 (1)	3 (1)	–
fruits	954 (22)	1013 (22)	1271 (22)	0.03 <sup>a</sup>	159 (19)	162 (19)	205 (20)	0.03 <sup>a</sup>	50 (29)	50 (28)	63 (28)	0.05
apples	550 (13)	554 (12)	686 (12)	0.03 <sup>a</sup>	96 (12)	97 (12)	120 (12)	0.02 <sup>a</sup>	36 (21)	36 (20)	45 (20)	0.03 <sup>a</sup>
citrus fruits <sup>F</sup>	229 (5)	213 (5)	298 (5)	0.02 <sup>a</sup>	39 (5)	36 (4)	50 (5)	0.03 <sup>a</sup>	8 (5)	7 (4)	10 (5)	0.09
others <sup>G</sup>	175 (4)	246 (5)	287 (5)	–	24 (3)	29 (3)	35 (3)	–	6 (3)	7 (4)	8 (4)	–
cereal products	361 (9)	322 (7)	362 (7)	0.15	149 (18)	139 (17)	154 (15)	0.09	14 (8)	13 (7)	15 (7)	0.67
white bread, rolls	94 (2)	206 (5)	184 (3)	0.02 <sup>b</sup>	41 (5)	91 (11)	81 (8)	0.03 <sup>b</sup>	3 (2)	8 (4)	7 (3)	0.05
wholegrain bread	199 (5)	55 (1)	113 (2)	0.01 <sup>b</sup>	81 (10)	23 (3)	46 (4)	0.03 <sup>b</sup>	7 (4)	2 (1)	4 (2)	0.05
others <sup>H</sup>	68 (2)	61 (1)	65 (1)	–	27 (3)	25 (3)	27 (3)	–	4 (2)	3 (2)	4 (2)	–
nuts and seeds <sup>I</sup>	<1 (<1)	<1 (<1)	<1 (<1)	0.82	20 (2)	5 (<1)	5 (<1)	0.02 <sup>b</sup>	<1 (<1)	<1 (<1)	<1 (<1)	0.75
vegetable oils <sup>J</sup>	<1 (<1)	<1 (<1)	<1 (<1)	0.56	<1 (<1)	<1 (<1)	<1 (<1)	0.64	<1 (<1)	<1 (<1)	<1 (<1)	0.69
total	4271	4545	5697	0.01 <sup>a</sup>	822	839	1031	0.01 <sup>a</sup>	173	180	223	0.04 <sup>a</sup>

Data are presented as median (with percent contribution of the food categories and main food products to DTAC, DTPC and DTFC).

DTAC and DTFC were calculated according to Zujko and Witkowska<sup>13,15</sup>, and DTPC, according to Zujko et al.<sup>16</sup>

Significant differences between the study groups:

- a** long-standing diabetes and newly diagnosed diabetes vs. controls
- b** long-standing diabetes vs. newly diagnosed diabetes and controls

For **A–J**, see **TABLE 4**; abbreviations: see **TABLE 3**

**DISCUSSION** In this study, we investigated associations between TAS, antioxidant enzyme activities, and lipid peroxidation end products and the course of diabetes, and whether dietary intake of antioxidants might affect antioxidant status in studied subjects.

Compared with controls, patients with newly diagnosed diabetes had significantly reduced TAS and an increased level of MDA+HAE, which indicates possible damage to the antioxidant system. Higher SOD and GPx activities in newly diagnosed diabetes may be interpreted as a protective mechanism due to increased superoxide radical generation and oxidative stress. An increase in SOD activity, resulting in  $H_2O_2$  formation, is accompanied by a concomitant increase in GPx, which removes  $H_2O_2$ . However, with longer disease duration, the threshold of antioxidant protection by endogenous antioxidant mechanisms progressively decreases; therefore, the TAS level and SOD and GPx activities are reduced, while the MDA+HAE level is increased in long-standing diabetes. Our findings are in line with those of other authors, who demonstrated that oxidative stress occurred early in diabetes and increased in the course of disease, especially in diabetic complications.<sup>18-20</sup>

Interestingly, all patients with long-standing diabetes used oral hypoglycemic drugs or insulin or both, and most of them were taking antihypertensive and cholesterol-lowering drugs, they showed the highest imbalance between the antioxidant status and increased concentrations of oxidative damage markers. Previous in-vitro and in-vivo studies demonstrated possible antioxidant properties of some medications used in the treatment of diabetes patients, such as metformin,<sup>21</sup> insulin,<sup>22</sup> statins,<sup>23</sup> and irbesartan.<sup>24</sup> Metformin alone or in combination is the most commonly used drug in patients with type 2 diabetes in Poland.<sup>25</sup> The beneficial effect of metformin added to insulin therapy was observed also in patients with type 1 diabetes.<sup>26</sup> The present study and other studies<sup>27,28</sup> have demonstrated that oxidative stress parameters were elevated in patients with long-standing diabetes, which suggests that regular use of antioxidant pharmaceuticals does not protect against oxidative stress.

A number of investigators<sup>29,30</sup> emphasized the key role of modifiable lifestyle factors, mainly weight loss, physical activity, and well-balanced diet in the prevention and treatment of type 2 diabetes. Epidemiological studies showed that dietary antioxidants, including flavonoids,<sup>11,31</sup> vitamin C,<sup>32</sup> vitamin E, and some carotenoids,<sup>10</sup> can protect cells from oxidative stress and may reduce the risk of type 2 diabetes. However, other authors<sup>33</sup> reported that vitamin E supplementation could not improve the antioxidant endogenous system in diabetic patients. In contrast, a few studies<sup>34,35</sup> indicated that an increase in plasma antioxidant capacity after an intake of flavonoid-rich foods was only minimally caused by flavonoids, and was rather a consequence of

urate production through fructose, caffeine, theobromine, and other components of such foods as fruit, tea, coffee, and chocolate, which are rich in flavonoids.

A significant decrease of the DTAC, as well as DTPC, DTFC, and vitamin C contents in our study was observed in patients with long-standing and newly diagnosed diabetes compared with controls. These findings indicate that the failure of the defensive system may occur early in diabetes, persist with longer diabetes duration, and is associated with reduced antioxidant intake in a diet. The serum TAS in all groups correlated only with the DTAC, but not with DTPC, DTFC, and antioxidant vitamin content. This suggests that the positive effect of dietary antioxidants on TAS may not be caused by individual antioxidants, but may be elicited through synergistic enhancement of different food antioxidants. The absence of associations between dietary antioxidants and antioxidant enzyme activities and the level of oxidative stress markers can be explained by an individual diversity in cell response to exogenous antioxidants.

The DTAC was dependent on the dietary habits of the subjects as well as antioxidant capacity, polyphenol, flavonoid, vitamins C and E, and  $\beta$ -carotene contents in food products consumed by the participants. The significant sources of the DTAC, DTPC, and DTFC in all studied groups were tea, coffee, and apples. Compared with type 2 diabetes patients, controls consumed the highest amounts of those foods, which might be positively reflected by the antioxidant capacity of the diet. These findings are in agreement with our previous cross-sectional studies, conducted in a representative sample of Polish adult population.<sup>14,16</sup> In the present study, healthy subjects consumed more apples, citrus fruits, potatoes, cabbage, and tomatoes than diabetic patients, which was associated with the largest intake of vitamin C. The diet of the studied groups was poor in olive oil, legumes, nuts, fruits (except apples), and varied vegetables (except potatoes, cabbage, and tomatoes), which are present in the Spanish Mediterranean diet recommended in oxidative stress-related diseases.<sup>36</sup> As a result, our patients showed low vitamin E and  $\beta$ -carotene intake. Of note, patients with long-standing diabetes consumed more wholegrain bread and less white bread and rolls in comparison with the other groups, which shows that their dietary knowledge has improved considerably.

In conclusion, our data indicate that the diet of type 2 diabetic patients was poor in antioxidants despite increased demand, especially in patients with long-lasting disease. Our findings encourage to continue research on a larger group of patients, including those with well-controlled type 2 diabetes, to better understand associations between dietary antioxidants and antioxidant status and to design practical guidelines for diabetic patients, including recommendations on food products rich in antioxidants.

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**Contribution statement** MEZ designed the research study, collected the samples and diet questionnaires, performed laboratory and statistical analyses, analyzed the data, and wrote the manuscript. AMW contributed to the design of the research and revised the manuscript. MG coordinated funding for the project. JW took part in sample collection. AK conceived the idea of the study.

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# Obniżone spożycie antyoksydantów w diecie może niekorzystnie wpływać na stan antyoksydacyjny u pacjentów z cukrzycą typu 2

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## SŁOWA KLUCZOWE

antyoksydanty  
w diecie, cukrzyca  
typu 2, stan  
antyoksydacyjny,  
stres oksydacyjny

## STRESZCZENIE

**WPROWADZENIE** W patogenezie cukrzycy typu 2 podstawową rolę odgrywa stres oksydacyjny.

**CELE** Celem pracy było zbadanie zależności między spożyciem antyoksydantów w diecie a stanem antyoksydacyjnym u pacjentów z cukrzycą typu 2.

**PACJENCI I METODY** U 80 pacjentów z cukrzycą typu 2 i 37 osób z grupy kontrolnej oznaczono całkowity stan antyoksydacyjny (*total antioxidant status* – TAS), aktywność peroksydazy glutationowej (*glutathione peroxidase* – GPx) i dysmutazy ponadtlenkowej (*superoxide dismutase* – SOD), dialdehyd malonowy (MDA) oraz 4-hydroksyalkeny (4-HAE). Do wyliczenia całkowitej aktywności antyoksydacyjnej diety (*dietary total antioxidant capacity* – DTAC), zawartości polifenoli (*dietary total polyphenol content* – DTPC) i flawonoidów (*dietary total flavonoid content* – DTFC) w diecie zastosowano 24-godzinny wywiad żywieniowy i własną bazę danych. Zawartość witamin antyoksydacyjnych w diecie wyliczono za pomocą tabel składu i wartości odżywczej produktów spożywczych.

**WYNIKI** TAS w surowicy wynosił 1,57 mmol/l w grupie kontrolnej, 1,41 mmol/l u nowo zdiagnozowanych pacjentów z cukrzycą i 1,23 mmol/l a u pacjentów z wieloletnią cukrzycą. Stężenie MDA+HAE w surowicy wyniosło 0,78 μmol/l w grupie kontrolnej, 1,45 μmol/l u nowo zdiagnozowanych pacjentów i 1,74 μmol/l u pacjentów z wieloletnią cukrzycą. Aktywność GPx i SOD wyniosła odpowiednio: 42,6 i 1340 jednostek/g Hb w grupie kontrolnej, 47,3 i 2373 jednostek/g Hb u pacjentów z wieloletnią cukrzycą oraz 58,2 i 3093 jednostek/g Hb u nowo zdiagnozowanych pacjentów. DTAC, DTPC, DTFC i zawartość witaminy C w diecie grupy kontrolnej wyniosły odpowiednio: 5697 μmol ekwiwalentów Troloksu (*Trolox equivalents* – TE), 1031 mg ekwiwalentów kwasu gallusowego (*gallic acid equivalents* – GAE), 223 mg ekwiwalentów kwercetyny (*quercetine equivalents* – QE) i 82 mg. Wartości te u pacjentów z wieloletnią cukrzycą wyniosły odpowiednio: 4271 μmol TE, 822 mg GAE, 173 mg QE i 63 mg, a u pacjentów nowo zdiagnozowanych – 4545 μmol TE, 839 mg GAE, 180 mg QE i 65 mg.

**WNIOSKI** Dieta pacjentów z cukrzycą typu 2 jest uboga w związki antyoksydacyjne mimo zwiększonego na nie zapotrzebowania.

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