Progress in Nutrition 2021; Vol. 23, N. 2: e2021050 DOI: 10.23751/pn.v23i2.9695

Original article

Dietary total antioxidant capacity and oxidative stress in patients with type-2 diabetes

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Abstract. *Background:* Reactive oxygen species can disrupt normal cellular functions by damaging DNA, protein, and lipid structures of the cell. Some antioxidant molecules may protect the body against reactive oxygen species. We aimed to investigate the relationship between the dietary intake of antioxidants and oxidative DNA damage in diabetic patients. *Material and Methods:* A total of 85 individuals were included in the study, of which 30 were newly diagnosed with type-2 diabetes, 30 were formerly diagnosed with type-2 diabetes, and 25 were healthy individuals. Twenty-four-hour dietary recalls were recorded for 3 consecutive days. Dietary total antioxidant capacity and dietary oxidative balance scores were calculated according to these records. Spot urine samples were collected and analyzed for 8-hydroxy-2′ deoxyguanosine/creatinine. *Results:* Dietary total antioxidant capacity, estimated via different methods, was higher in the controls than that in patients with type-2 diabetes ($p<0.05$). The urinary 8-hydroxy-2′-deoxyguanosine/creatinine ratio, a reliable predictor of oxidative DNA damage, was also higher in non-diabetic patients ($p<0.05$). The urinary 8-hydroxy-2′-deoxyguanosine/creatinine ratio was not related to dietary antioxidant intake ($p>0.05$). *Conclusion:* Urinary 8-hydroxy-2′-deoxyguanosine/creatinine concentration may not always reflect the current oxidative status of the body.

Keywords: Antioxidants; diet; DNA damage; type 2 diabetes; oxidative stress

Introduction

Oxidative stress, described as a physiological state resulting from a disruption of the balance between the production and degradation of reactive oxygen species (ROS) (1), is an important factor that may take part in the onset of both type-2 diabetes mellitus (T2DM) and diabetes-related complications. The data obtained from clinical studies suggest that systemic oxidative stress is closely related to metabolic syndrome and its components (2).

Chronic hyperglycemia is an important risk factor for ROS formation (3). An increased glucose flux causes an electron pressure on the mitochondrial electron transport system that may contribute to ROS formation, which in turn leads to an increased activity in alternative pathways for metabolizing glucose (4). Excessive ROS concentrations in cells may then disrupt nucleic acid, lipid, and protein structures (5). In the nuclear and mitochondrial DNA structure, guanosine is the most susceptible base for oxidation and it disintegrates into 8-hydroxy-2′-deoxyguanosine (8-OHdG)

in the presence of reactive oxygen derivatives (6). The measurement of this highly stable molecule in tissues and urine may impart information about DNA damage in an individual (7).

Dietary antioxidants may be protective against oxidative damage and T2DM, as substantiated by their effects on decreasing ROS concentrations in the body (8). To assess the antioxidant content of mixed diets, dietary total antioxidant capacity (DTAC) has been suggested as a tool that evaluates the efficacy of antioxidant molecules in foods against reactive compounds (9). Most studies revealed that DTAC decreased the risk of oxidative stress-related diseases (10,11).

In the light of this background, the present study aims to evaluate oxidative damage in subjects that were newly or formerly diagnosed with diabetes and to determine the relationship between DTAC and oxidative stress biomarker, 8-OHdG.

Materials and Methods

Study Design

This study included a total of 85 subjects recruited from October 2017 to October 2018 from the outpatient Department of Medicine, Hacettepe University. Overall, 30 subjects that were newly diagnosed with T2DM and additional 30 subjects that had been formerly diagnosed with T2DM (time since diagnosis was ≥5 years) as well as 25 control subjects were recruited. A questionnaire was administered by a trained dietitian via face-to-face interviews and spot urine samples were collected and analyzed for 8-OHdG/creatinine.

Ethical clearance

This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the Hacettepe University Non-Interventional Clinical Researches Ethics Board (Ref Code: GO 17/781). Informed consent was obtained from all participants before the survey began.

Participants

Individuals aged between 25 and 60 years were included in this study. The exclusion criteria included presence of a known inflammatory disease (rheumatoid arthritis, cancer, etc.). Pregnant or lactating women as well as smokers were also excluded. These criteria are of high importance as these presentations can cause a falsely high 8-OHdG/creatinine concentration, independent of the duration of diabetes or dietary habits of individuals.

Instruments

Demographic characteristics, general dietary habits, physical activity status, and anthropometric measurements were recorded within the scope of the questionnaire. Twenty-four-hour dietary recalls were taken from the participants over 3 consecutive days of which two days from weekdays and one day from weekend.

Mean daily nutrient intake was calculated using the Nutrition Information System (BEBIS) 7.1 software package (Hohenhim University, Stuttgart, Germany). Since the software does not include the antioxidant capacity of foods, the value of each food was assigned based on various previously published databases (12–15). According to these databases, the antioxidant capacity is estimated using four assays: ferric reducing antioxidant capacity (FRAP), trolox equivalent antioxidant capacity (TEAC), total radical trapping antioxidant potential (TRAP), and oxygen radical absorption capacity (ORAC). The ORAC values are reported for hydrophilic-ORAC (H-ORAC), lipophilic-ORAC (L-ORAC), Total-ORAC, and total phenolics (TP). FRAP values were estimated using two different databases. The FRAP values, estimated according to the database composed by Carlsen et al., are named as FRAP-1 and the FRAP values, estimated according to the database composed by Pellegrini et al., are named as FRAP-2 in the text. Regarding foods for which DTAC data were unavailable, the value of the nearest comparable food was assigned. Dietary Oxidative Balance Score (OBS) was also calculated for oxidant and antioxidant compounds in diet, as described by Agalliu (16).

Biochemical Parameters

Plasma fasting blood glucose (FBG), glycated hemoglobin % (HbA1c), total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, and triglyceride values were retrieved from patient records. Patients with missing records were excluded while evaluating these parameters.

In the collected spot urine samples, 8-OHdG (EIA Kit, Cayman, 589320) concentration was measured. This commercial kit is preferred because of its high sensitivity in urine analysis. Urine samples were collected from the participants according to the manufacturer's instructions, stored at –30°C in 2-mL centrifuge tubes until the day of analysis, and dissolved at +4°C the night before the analysis. Before running the ELISA test, the samples were centrifuged at 1500 × g for 5 min. A dilution ratio of 1:250 was preferred with the intention of falling within the range of the standard curve. Since the concentrations of oxidized guanine in spot urine samples could vary, the creatinine concentrations were also analyzed to standardize the level of 8-OHdG as recommended in the literature (17).

Statistical analysis

Data were evaluated using the statistical software package SPSS 23.0. General characteristics, energy intake, DTAC, OBS, and biochemical parameter variables were all reported for the newly diagnosed T2DM patients, formerly diagnosed T2DM patients, and healthy controls. For non-continuous variables, the data were presented in number (n) and percentage (%). For continuous variables, the data were presented as mean and standard deviation.

The differences between the three groups were tested using the following tests: F test was used for variables with a normal distribution and homogeneity of variances, Kruskal–Wallis H test was used for variables with a non-normal distribution, and X2 test was used for categorical variables. For post hoc analysis, Tukey test was used for variables with a normal distribution and homogeneity of variances and the Bonferroni test was used for non-normally distributed variables.

A two-way ANOVA test was used to identify the variations in 8-OHdG/creatinine values within the three groups after making adjustments for age, physical activity, BMI, and energy intake. To evaluate the effect of each DTAC value and OBS on urinary 8-OHdG/creatinine values, separate logistic regression models were applied after making adjusted for age, BMI, physical activity level, diabetes status, and total energy intake as potential confounders. Both 8-OHdG/creatinine and dietary components were grouped as low tertile and high tertile according to their median values. Low tertile 8-OHdG/creatinine group was set as reference group, whereas high tertile DTAC and OBS values were taken as reference groups while performing logistic regression analysis.

In all tests, p value of <0.05 was considered significant.

Results

In Table 1, the main descriptive characteristics of the participants are demonstrated separately for each group. The mean ages (±SD) of newly diagnosed T2DM, formerly diagnosed T2DM, and healthy controls were 50.8±6.8, 51.8±7.4, and 45.6±8.6 years, respectively. Distribution of women was higher in each group (range: 53.3%–56.7%). Obesity, hypertension, and hyperlipidemia were the most common diseases in both newly and formerly diagnosed diabetic patients (range: 33.3%–63.3%). Compared with non-diabetic patients, both formerly and newly diagnosed diabetic patients had significantly higher BMI levels ($p<0.001$).

Table 2 outlines DTAC and OBS of the participants in each group. Accordingly, DTAC, estimated by FRAP-1, FRAP-2, TRAP, TEAC, and L-ORAC assays, was greater in non-diabetic patients than in diabetic patients. DTAC, estimated by FRAP-2, TRAP, and TEAC assays, was approximately two-fold higher in non-diabetic patients. Nevertheless, OBS within three groups were similar ($p=0.253$; Table 2).

Table 3 shows the biochemical markers of the participants in each group. Accordingly, formerly diagnosed diabetic patients had the highest FBG, VLDL cholesterol, and triglyceride concentrations, whereas

Table 1. General characteristics of participants

	Diabetic patients		Control group	*p*
	Newly diagnosed (n = 30)	Formerly diagnosed (n =30)	(n = 25)	
Age, years	50.8 ±6.8	51.8 ±7.4	45.6 ±8.6	**0.05**[a]
Gender				
Men	13 (43.3%)	14 (46.7%)	11 (44.0%)	0.963
Women	17 (56.7%)	16 (53.3%)	14 (56.0%)	
Marital status				
Single	1 (3.3%)	5 (16.7%)	5 (20.0%)	0.139[b]
Married	29 (96.7%)	25 (83.3%)	20 (80.0%)	
Total length of education, years	10.0 ±4.2	8.9 ±4.8	15.1 ±3.1	**<0.001**[a]
Treatment method				
Diet	3 (10.0%)	-		
Diet + Oral Antidiabetic Drugs	16 (53.3%)	13 (43.3%)	NQ	0.119[b]
Diet + insulin	11 (36.7%)	17 (56.7%)		
Family diabetes history †				
None	5 (16.7%)	7 (23.3%)		0.747
Mother or Father	14 (46.7%)	12 (40.0%)	NQ	0.794
Mother and Father	3 (10.0%)	7 (23.3%)		0.299
Siblings	9 (30.0%)	16 (53.3%)		0.116
Existing diseases †				
None	2 (6.7%)	3 (10.0%)	16 (64.0%)	**<0.001**
Hypertension	10 (33.3%)	16 (53.3%)	4 (16.0%)	**0.015**
Obesity	16 (53.3%)	19 (63.3%)	3 (12.0%)	**<0.001**
Hyperlipidemia	11 (36.7%)	12 (40.0%)	1 (4.0%)	**0.006**
Cardiovascular diseases	3 (10.0%)	3 (10.0%)	-	0.270[b]
Kidney diseases	2 (3.3%)	5 (16.7%)	1 (4.0%)	0.167[b]
Thyroid diseases	7 (23.3%)	7 (23.3%)	3 (12.0%)	0.492
Nutritional Habits				
Meal skipping				
Usually	4 (13.3%)	6 (20.0%)	1 (4.0%)	
No	21 (70.0%)	14 (46.7%)	16 (64.0%)	0.192[b]
Sometimes	5 (16.7%)	10 (33.3%)	8 (32.0%)	
Number of regular meals	2.9 ±0.4	2.9 ±0.4	3.00 ±0.2	0.448[a]
Number of snacks	2.0 ±1.0	2.1 ±1.1	1.7 ±1.1	0.248[a]
Energy Intake	1778.4 ±355.1	1670.3 ±479.6	1954.7 ±405.1	**0.046**[c]
Body Mass Index (kg/m²)	31.8 ±6.4	32.9 ±5.5	26.1 ±3.8	**<0.001**[c]
Physical Activity Level	1.75 ±0.23	1.72 ±0.12	1.69 ±0.14	0.635[a]

NQ: Not Questioned.
†: Multiple options marked.
Analyzed with Chi-square test.
a: Analyzed with Kruskal Wallis test,
b: Analyzed with Fisher's Exact test,
c: Analyzed with One-way Anova test,
p<0.05.

Table 2. Dietary total antioxidant capacity and oxidative balance score values of participants ($\bar{X}\pm SD$)

	Diabetic patients						Control group			Post-hoc analysis			
	Newly diagnosed			Formerly diagnosed									
	n	$\bar{X}\pm SD$	Median (Min-Max)	n	$\bar{X}\pm SD$	Median (Min-Max)	n	$\bar{X}\pm SD$	Median (Min-Max)	p	p_1	p_2	p_3
DTAC													
FRAP - 1	30	5.6±1.9	5.1 (2.4 - 9.4)	30	5.9±2.1	5.9 (2.7 - 10.5)	25	8.0±3.1	7.1 (2.6 - 15.5)	**0.001**[a]	0.903	**0.001**	**0.004**
FRAP - 2	30	14.9±7.0	13.5 (5.4 - 41.1)	30	15.5±5.3	14.7 (6.3 - 29.9)	25	31.0±21.1	24.4 (8.5 - 104.9)	**<0.001**	1.000	**<0.001**	**<0.001**
TRAP	30	5.4±3.3	4.7 (1.5 - 18.5)	30	5.5±1.9	5.3 (2.2 - 9.7)	25	12.5±10.3	9.6 (3.2 - 50)	**<0.001**	1.000	**<0.001**	**0.001**
TEAC	30	4.8±2.3	4.4 (1.5 - 12.9)	30	5.1±1.7	5.0 (1.9 - 9.4)	25	9.7±6.2	7.8 (2.7 - 31)	**<0.001**	0.972	<0.001	<0.001
H-ORAC	30	20246.1±7800.9	19453.4 (9342.1 - 38505.1)	30	19749.6±7442.2	18409.9 (9673.5 - 38702.2)	25	21431.4±7980.1	19435 (10827.9 - 40869.5)	0.700			
L–ORAC	30	515.8±155.5	488.7 (248.6 - 905.1)	30	523.3±291.3	435.5 (218.7 - 1839.4)	25	672.7±345.1	604.7 (289.8 - 1801.2)	**0.033**	1.000	0.171	**0.033**
Total ORAC	30	20876.3±7923.9	19938.5 (9670 - 38937.9)	30	20372.8±7681.0	18979.3 (10068 - 39140.1)	25	22305.7±8019.1	20037.8 (11486.3 - 41603.2)	0.633			
TP	30	1494.0±475.0	1393.1 (647.6 - 2607.3)	30	1490.2±560.7	1382.8 (497.9 - 2407.9)	25	1403.2±464.1	1401.5 (858.2 - 2877.2)	0.724			
Oxidative balance score	30	31.2±5.6	32 (17.0 – 39.0)	30	29.5±6.3	29.5 (17.0 – 44.0)	25	28.8±4.8	29.0 (21.0 – 37.0)	0.253[a]			

DTAC, Dietary total antioxidant capacity; FRAP-1, Ferric Reducing Antioxidant Power (Carlsen Database); FRAP-2, Ferric Reducing Antioxidant Power (Pellegrini Database); H-ORAC, Hydrophilic Oxygen Radical Absorbing Capacity (USDA); L-ORAC, Lipophilic Oxygen Radical Absorbing Capacity (USDA); TEAC, Trolox Equivalent Antioxidant Capacity (Pellegrini Database); Total ORAC, Total Oxygen Radical Absorbing Capacity (USDA); TP, Total Phenolics (USDA); TRAP, Total Radical Trapping Antioxidant Potential (Pellegrini Database),.

p1: Newly diagnosed diabetic patients – formerly diagnosed diabetic patients, p2: Newly diagnosed diabetic patients – controls, p3: Formerly diagnosed diabetic patients – controls.

Analyzed with Kruskal Wallis test, post-hoc analysis performed with Bonferroni test,

a: Analyzed with One-way Anova test, post-hoc analysis performed with Tukey test,

p<0.05.

non-diabetic patients had the highest total cholesterol, LDL, and HDL cholesterol concentrations; 8-OHdG/creatinine concentrations were 158.5±129.5 ng/mg, 129.5±86.4 ng/mg, and 251.0±147.3 ng/mg in the newly diagnosed diabetic patients, formerly diagnosed diabetic patients, and non-diabetic patients, respectively. 8-OHdG/creatinine value was statistically higher in non-diabetic patients than in diabetic patients after adjustments for age, BMI, physical activity level, and energy intake ($p<0.05$; Table 3).

Separate logistic regression models explaining the effect of DTAC and OBS on urinary 8-OHdG/creatinine concentrations are summarized in Table 4. Lower DTAC values, estimated by eight different assay methods, did not pose a significant risk for having higher urinary 8-OHdG/creatinine ratio, after being corrected for potential cofounders ($p<0.05$). Likewise, lower dietary OBS did not have a considerable effect on increasing urinary 8-OHdG/creatinine concentrations (OR= 1.399 %95 CI: 0.461 – 4.247, p=0.553; Table 4).

Discussion

The results of this study indicate that DTAC of diabetic patients is significantly lower than that of non-diabetic patients (Table 2). These results are consistent with previously published studies (11,18). Lower levels of DTAC may be considered as a potential predictor of the risk of diabetes development. In the French EPIC study, it has been emphasized that a higher dietary total antioxidant intake was associated with a lower T2DM risk (11); in that study, DTAC levels of <15 mmol/day (according to the database composed by Pellegrini et al.) were associated with a higher risk of diabetes (11). Likewise, in the ATTICA study, DTAC estimated using FRAP, TRAP, and TEAC methods were negatively correlated with plasma glucose, insulin, and HOMA-IR levels (18).

Biochemical markers of the individuals differ among three groups as predicted (Table 3). In line with the previously published data (19), the concentrations of FBG and HbA1c increase with the duration of diabetes. Surprisingly, in this study, total cholesterol and LDL cholesterol concentrations were found to be higher in non-diabetic patients than those in diabetic patients (Table 3). Since insulin resistance increases non-esterified fatty acid secretion from adipose tissues, dyslipidemia is common among diabetic patients, whereas plasma cholesterol concentrations can be affected by a number of factors including individuals' dietary patterns, genetic factors, age, and sex, apart from diabetes itself.

Another striking result of the present study was the significantly higher urinary 8-OHdG concentrations in non-diabetic patients than those in diabetic patients after adjustments were made for age, BMI, physical activity level, and energy intake (Table 3). The urinary 8-OHdG concentrations have been described as a predictor of diabetes and its complications in many studies and reported to increase after diabetes onset (20,21). In the study by Dong et al. (20), the urinary 8-OHdG/creatinine ratio was significantly higher in diabetic patients than in non-diabetic patients. Among diabetic patients, the level of 8-OHdG excretion in urine was found to be higher in diabetic patients with proliferative retinopathy than in diabetic patients without retinopathy and diabetic patients with non-proliferative retinopathy. Likewise, Nishikawa et al. (21) demonstrated 2.3-fold higher rates of urinary 8-OHdG excretion in diabetic patients with atherosclerosis compared with that in diabetic patients without atherosclerosis. In contrast to previously reported data, the present study did not reveal any evidence of increased urinary 8-OHdG concentrations in diabetic patients (Table 3) nor were the concentrations higher in formerly diagnosed diabetic patients compared with those in newly diagnosed diabetic patients (Table 3). One possible explanation for such contradictory results might be associated with the drugs used by diabetic patients that have antioxidant properties, such as metformin (22). Since both newly and formerly diagnosed patients with T2DM included in this study commonly use different forms of metformin as a part of their pharmacological therapy (data not shown), the concentrations of oxidized molecules in diabetic patients might have reduced.

Another possible explanation might be related to the balance between the formed and excreted concentrations of oxidized molecules. Urinary 8-OHdG excretion may be a marker of oxidative damage, but it

Table 3. Biochemical markers of participants ($\bar{X}\pm SD$)

	Diabetic patients						Control group			Post-hoc analysis			
	Newly diagnosed			Formerly diagnosed									
Biochemical markers	n	$\bar{X}\pm SD$	Median (Min-Max)	n	$\bar{X}\pm SD$	Median (Min-Max)	n	$\bar{X}\pm SD$	Median (Min-Max)	p	p_1	p_2	p_3
Fasting blood glucose (mg/dL)	27	128.0 ± 43.6	117.0 (75.0-243.0)	26	178.8 ± 56.4	168.0 (94.0 – 332.0)	22	97.0 ± 25.5	93.0 (80.0 – 120.0)	**<0.001**	0.005	0.005	<0.001
HbA1c (%)	28	6.8 ± 1.4	6.3 (4.9-9.9)	29	7.7 ± 1.4	7.9 (4.8 - 11.5)		-	-	**0.019**			
Total cholesterol (mg/dL)	28	172.7 ± 40.3	172.0 (95.0-290.0)	29	193.9 ± 54.8	185.0 (98.0 – 351.0)	23	216.3 ± 32.3	219.0 (149.0 – 289.0)	**0.003**[a]	0.176	**0.002**	0.173
LDL (mg/dL)	30	114.4 ± 31.6	109.5 (51.0-203.0)	29	130.7 ± 45.1	123.0 (77.0 – 248.0)	23	138.2 ± 30.7	134.0 (72.0 – 199.0)	**0.026**	0.699	**0.021**	0.349
VLDL (mg/dL)	29	34.9 ± 17.9	29.0 (17.0-98.0)	29	43.2 ± 25.9	38.0 (10.0 – 124.0)	23	22.3 ± 13.5	19.0 (7.0 – 62.0)	**<0.001**	1.000	**0.004**	**<0.001**
HDL (mg/dL)	28	41.6 ± 9.0	40.0 (19.0-66.0)	29	45.3 ± 11.8	45.0 (28.0 – 85.0)	23	57.0 ± 18.0	52.0 (37.0 – 103.0)	**0.002**	0.650	**0.002**	0.060
Triglyceride (mg/dL)	29	174.1 ± 89.8	145.0 (87.0-492.0)	29	217.4 ± 129.0	195.0 (49.0 – 619.0)	23	114.3 ± 69.3	93.0 (37.0 – 308.0)	**<0.001**	1.000	**0.008**	**<0.001**
8-OHdG/creatinine (ng/mg) †	30	158.5 ±129.5	132.9 (21.6-566.5)	30	129.5 ±86.4	93.7 (46.7 - 358.7)	25	251.0 ±147.3	251.4 (20.7 – 566.5)	**0.002**	1.000	**0.011**	**0.002**

HDL, High-density lipoprotein; LDL, Low-density Lipoprotein; VLDL, Very low –density lipoprotein.

p_1: Newly diagnosed diabetic patients – formerly diagnosed diabetic patients, p_2: Newly diagnosed diabetic patients – controls, p_3: Formerly diagnosed diabetic patients – controls. Analyzed with Kruskal Wallis test, post-hoc analysis performed with Bonferroni test,

a: Analyzed with One-way Anova test. post-hoc analysis performed with Tukey test,

† Analyzed with Two-way Anova test. Square root transformation was used to normalize data, untransformed data is presented (p values for 8-OHdG/creatinine represent the differences after controlling for age, BMI, physical activity level and energy intake).

$p<0.05$.

Table 4. Logistic regression models showing the effect of dietary antioxidant capacity and oxidative balance score on urinary 8-OHdG/creatinine ratio

	OR estimates for participants in high urinary 8-OHdG/creatinine tertile*		
	Adjusted OR†	**%95 CI**	**p**
DTAC values			
FRAP-1			
FRAP – 1 (low tertile ≤6.4)	0.613	0.190 – 1.980	0.413
FRAP – 1 (high tertile >6.4)		Ref.	
FRAP-2			
FRAP – 2 (low tertile ≤16.3)	0.614	0.219 – 2.451	0.614
FRAP – 2 (high tertile >16.3)		Ref.	
TRAP			
TRAP (low tertile ≤5.77)	0.319	0.090 – 1.137	0.078
TRAP (high tertile >5.77)		Ref.	
TEAC			
TEAC (low tertile ≤5.45)	0.531	0.152 – 1.855	0.321
TEAC (high tertile >5.45)		Ref.	
H-ORAC			
H-ORAC (low tertile ≤19068.8)	0.992	0.311 – 3.159	0.989
H-ORAC (high tertile >19068.8)		Ref.	
L-ORAC			
L-ORAC (low tertile ≤530.45)	4.203	0.997 – 17.712	0.050
L-ORAC (high tertile >530.45)		Ref.	
Total ORAC			
Total ORAC (low tertile ≤19535.29)	0.915	0.282 – 2.971	0.882
Total ORAC (high tertile >19535.29)		Ref.	
TP			
TP (low tertile ≤1401.53)	1.705	0.536 – 5.420	0.336
TP (high tertile >1401.53)		Ref.	
Oxidative balance score			
(low tertile ≤31)	1.399	0.461 – 4.247	0.553
(high tertile >31)		Ref.	

FRAP-1, Ferric Reducing Antioxidant Power (Carlsen Database); FRAP-2, Ferric Reducing Antioxidant Power (Pellegrini Database); H-ORAC, Hydrophilic Oxygen Radical Absorbing Capacity (USDA); L-ORAC, Lipophilic Oxygen Radical Absorbing Capacity (USDA); TEAC, Trolox Equivalent Antioxidant Capacity (Pellegrini Database); Total ORAC, Total Oxygen Radical Absorbing Capacity (USDA); TP, Total Phenolics (USDA); TRAP, Total Radical Trapping Antioxidant Potential (Pellegrini Database).
**Tertile limit for high 8-OHdG/creatinine is >135.291 ng/mg*
†Adjusted for age, physical activity level, total energy intake, diabetes group (no diabetes, newly diagnosed diabetes, formerly diagnosed diabetes), BMI
$p<0.05$.

may also indicate that the repair mechanisms against oxidative products have the ability to scavenge damaged molecules from the cellular environment (7). The presence of the 8-OHdG molecule in urine is a proof that the 8-OHdG residues on DNA are removed from cells via excision repair systems. As the 8-OHdG concentrations increase in tissues, the number of oxidized molecules in bodily fluids may also increase owing to the activity of DNA repair mechanisms. However, in cases where these compensatory mechanisms do not run efficiently, even though the 8-OHdG molecule is formed, it may not be eliminated from the cells and therefore accumulates in tissues. In this case, the concentration of oxidized molecules in bodily fluids may reduce. In concordant with this hypothesis, it has been stated that T2DM is not only associated with elevated concentrations of oxidative DNA damage but also with increased susceptibility to mutagens and decreased efficacy of DNA repair mechanisms (23). Reduced activity of those mechanisms might be a consequence of hyperglycemia-induced oxidative stress. Repair enzymes can be affected by glycation and may lose their efficiency like any other enzyme structures composed of proteins. Furthermore, diabetes may alter the intracellular distribution of micronutrients, which act as cofactors of antioxidant enzymes (24). Considering that antioxidant enzymes and nutrients play an active role in DNA methylation and base excision repair systems (25), degeneration of repair mechanisms may lead to the accumulation of oxidized molecules in tissues rather than their removal.

Finally, individual variabilities such as genetic susceptibility might also be associated with lower 8-OHdG/creatinine concentrations in diabetic patients. The 8-oxoguanine DNA glycosylase (OGG1) gene encodes the enzyme responsible for excision of 8-oxoguanine base formed by exposure to reactive oxygen derivatives, and some specific polymorphisms in this gene may reduce the activity of antioxidant repair mechanisms. In a study by Vodicka et al. (26), it was revealed that OGG1 Ser326Cys polymorphism significantly reduced DNA oxidative damage repair capacity. In another study conducted by Gonul et al. (27), this gene polymorphism was found to occur more frequently in diabetic patients than in non-diabetic patients. This may also stand as an evidence for lower urinary 8-OHdG concentrations in diabetic patients; however, gene polymorphisms are not within the scope of the present study.

In this study, the effect of DTAC on urinary 8-OHdG concentrations was also evaluated. Based on the outcomes, no significant associations were found between the urinary 8-OHdG/creatinine ratio and DTAC, which were estimated using different methods after being controlled for age, physical activity level, energy intake, diabetes status, and BMI (Table 4). These results were similar to those of a study conducted in Japan (28). There are several possible explanations why DTAC does not have a positive effect on DNA damage. First, the effect of DTAC on plasma total antioxidant capacity may not be as high as expected. The non-enzymatic antioxidant capacity in the circulatory system can be influenced by various endogenous and exogenous parameters. Secondly, the activity of antioxidant foods and beverages may vary in the body. In a systemic meta-analysis study, it was revealed that plasma non-enzymatic antioxidant capacity increased with the consumption of foods rich in antioxidants but not with the beverages rich in antioxidants (29). Compared with other antioxidants such as ascorbic acid, polyphenols, which are mostly found in beverages such as green and black tea, have been shown to be absorbed in lower amounts and have lower plasma concentrations in humans (30). Still, the antioxidant capacity of tea and coffee was significantly high in databases composed by Carlsen et al. and Pellegrini et al. (12,14). Since these databases do not include any information on bioavailability, the total antioxidant capacity of a diet may not have a measurable effect on plasma antioxidant concentrations. Some studies considered the ORAC assay to be a more preferable method because of its biological relevance to in vivo antioxidant efficacy (31). However, the ORAC method did not have any significant effect on 8-OHdG concentrations, either (Table 4).

To the best of our knowledge, we were the first to compare the difference in 8-OHdG/creatinine concentrations among the newly diagnosed diabetic patients, formerly diagnosed diabetic patients, and non-diabetic patients and to evaluate the effect of DTAC on 8-OHdG/creatinine concentrations while controlling for covariates. However, this study has not been free

of limitations. First, we determined oxidative damage based on the 8-OHdG biomarker in urine. It would have been more accurate if we had evaluated 8-OHdG concentrations in both urine and tissue samples. In this way, we could discuss both the concentration of 8-OHdG in tissues and the excreted amounts in urine and eliminate any possible dysfunctions of excision repair system. Second, diabetic patients were assessed in two different groups (formerly and newly diagnosed), according to their age of diabetes onset, with the intention of discussing the long-term effects of diabetes on oxidative mechanisms. It would have been more valuable if the effect of complications could have also been evaluated. Hence, this part may be considered to be a new topic to focus upon in future studies. Finally, the sample size used in this study is small; moreover, to achieve more valid results and provide far-reaching recommendations, a larger sample size will best serve the purpose.

In conclusion, our data suggest that there are significant differences in DTAC and urinary 8-OHdG/creatinine concentrations among the formerly diagnosed diabetic patients, newly diagnosed patients, and non-diabetic individuals. Furthermore, dietary antioxidant intake did not have a significant effect on urinary 8-OHdG/creatinine ratio. The findings in this study support that urinary 8-OHdG/creatinine concentration may not always reflect the current oxidative status of the body. Because of that, analyzing solely 8-OHdG excretion, may not be a specific and sensitive method for evaluating oxidative status of the body. Together with urinary excretion, analysis of tissue samples may be considered as a more reliable method. Additionally, consistent with the previous literature, DTAC values in diabetic groups was lower than those in the control group. This may be considered as a risk factor for disease pathogenesis. Although an optimal dietary intake level for DTAC to avoid diabetes has not yet been set, an adequate and balanced diet consisting of foods and beverages that are rich in antioxidants can be adopted by both healthy and diabetic individuals.

Declarations of Interest: No potential conflict of interest relevant to this article was reported by the authors.

Funding information: This study did not receive any specific grant from funding agency in the public, commercial or not-for-profit sectors.

Acknowledgments: Ö. Çetiner, N. Rakicioglu, T. Yalçin, SN. Sendur and M. Bayraktar equally contributed to the conception and design of the research; Ö. Çetiner contributed to the acquisition and analysis of the data; Ö. Çetiner and N. Rakicioglu contributed to the interpretation of the data and drafted the manuscript. All authors critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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