Research Article

Vegetables and PUFA-rich plant oil reduce DNA strand breaks in individuals with type 2 diabetes

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Scope: Type 2 diabetes is a multifactorial disease associated with increased oxidative stress, which may lead to increased DNA damage. The aim of this study was to investigate the effect of a healthy diet on DNA oxidation in diabetics and nondiabetics.

Methods and results: Seventy-six diabetic and 21 nondiabetic individuals participated in this study. All subjects received information about the benefits of a healthy diet, while subjects randomly assigned to the intervention group received additionally 300 g of vegetables and 25 mL PUFA-rich plant oil per day. DNA damage in mononuclear cells (Comet Assay), urinary excretion of 8-oxo-7-hydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo) and glycated hemoglobin (HbA1c) were measured at baseline, after 4, 8 (end of intervention), and 16 weeks. The intervention with vegetables and PUFA-rich oil led to a significant increase in plasma antioxidant concentrations. Diabetic individuals of the intervention group showed a significant reduction in HbA1c and DNA strand breaks. Levels of HbA1c were also improved in diabetics of the information group, but oxidative damage to DNA was not altered. Urinary 8-oxodG and 8-oxoGuo excretion remained unchanged in both groups.

Conclusions: This study provides evidence that a healthy diet rich in antioxidants reduces levels of DNA strand breaks in diabetic individuals.

Keywords:
Diabetes mellitus type 2 / DNA damage / Plant oil / Vegetables

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1 Introduction

Type 2 diabetes mellitus (T2DM) is a complex disease characterized by high blood glucose levels resulting from impaired insulin secretion and insulin action, leading to hyperglycemia and a disturbed carbohydrate, fat and protein metabolism [1]. Oxidative stress, mainly caused by hyperglycemia, hyperlipidemia, and hyperinsulinemia is discussed to be involved in the progression of the disease [2] and contributes to the onset of diabetes associated complications [3] and to DNA oxidation [4]. Consistently, the quality of glycemic control plays a major role in preventing DNA oxidation [5–7], implying that an optimum control of factors leading to increased reactive oxygen species production is crucial to diabetes control.

Diet is a cornerstone for the achievement of optimum blood glucose control and lipid concentrations in subjects with T2DM [8]. Based on the nutritional guidelines for the management of diabetes from the American Diabetes Association [9] saturated fatty acid (SFA) intake should be less than

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Abbreviations: **8-oxodG**, 8-oxo-7-hydro-2′-deoxyguanosine; **8-oxoGuo**, 8-oxo-7,8-dihydroguanosine; **FPG**, formamidopyrimidine DNA glycosylase; **HbA1c**, glycated hemoglobin A1c; **ITDM2**, insulin-treated type 2 diabetes; **NIDDM**, non-insulin dependent type 2 diabetes; **PBMCs**, peripheral blood mononuclear cells; **SFAs**, saturated fatty acids; **T2DM**, type 2 diabetes mellitus

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7% of the daily energy intake, as this type of fat is linked to unfavorable plasma lipid profile [10]. Scientific evidence even suggests that increased SFA intake is, among other factors, responsible for the increase in diabetes prevalence [11]. Furthermore, an inverse association between the risk of T2DM and the replacement of PUFA for SFA [12], or PUFA intake alone [13] was observed.

Sufficient amounts of vegetables are considered as an essential part of a healthy diet, since they are rich in antioxidants, such as vitamin C, vitamin E, carotenoids, flavonoids, and other polyphenolic compounds. These antioxidants are able to scavenge or inactivate reactive oxygen species and are therefore of special importance to subjects with increased oxidative stress. However, the number of studies reporting positive effects of antioxidant supplementation on oxidative DNA damage is almost equal to the number of studies showing no effects (for review see [14–16]). This raises the question if antioxidant-rich natural foods are more effective than supplements with purified antioxidants.

To the best of our knowledge there are no data available either about the impact of a whole food approach or about the beneficial effects of a healthy diet on oxidative damage to DNA in subjects with T2DM. Therefore a dietary intervention trial with focus on high diet quality, realized through the replacement of SFA by PUFA and the supply of vegetables, was performed. The aims of the study were (i) to assess the potential of the intervention to reduce oxidative damage to DNA under consideration of health status (insulin treated (ITDM2), non-insulin dependent (NIDDM) type 2 diabetes or nondiabetic) and (ii) to investigate the impact of poor or good glycemic control on the response to the intervention. The biomarkers used to study oxidative damage to DNA were 8-oxo-7-hydro-2′-deoxyguanosine (8-oxodG) in urine, and the comet assay in peripheral blood mononuclear cells (PBMC).

2 Materials and methods

2.1 Study population

Seventy-six subjects with established T2DM (according to the definition of the American Diabetes Association [17]) treated with oral glucose-lowering agents (mean age 65.2 ± 7.38 years; 18 males and 22 females) and/or insulin (mean age 65.0 ± 7.68 years; 16 males and 20 females) and 21 nondiabetic subjects (mean age: 62.7 ± 6.30 years, 6 males and 15 females) participated in this study. Subjects were recruited from a local diabetic clinic (Diabetes outpatient Clinic, Health Centre South, Vienna, Austria) during their annual health assessment. The nondiabetic subjects were partners of the diabetic subjects.

All subjects had to have stable metabolic control (constant medication regarding glucose, lipid, and uric acid metabolism), glycated hemoglobin (HbA_{1c}) concentration <9.5%, serum total cholesterol <300 mg/dL (<7.76 mmol/L), serum triglycerides <500 mg/dL (<5.7 mmol/L) and serum creatinine <2.5 mg/dL (<221 μmol/L). Only subjects with stable body weight, constant dietary habits and physical activity levels for at least 4 weeks before entry to the study were included. Subjects who intended to change dietary habits (e.g. intake of additional supplements), body weight or frequency of physical activity within the study period were not allowed to participate. Exclusion criteria also included smoking, intake of fish oil capsules, and other fatty acid supplements. All medical therapies of subjects were continued unchanged throughout the study.

The study protocol was approved by the Ethical Committee of the City of Vienna (EK09–218-VK_NZ). The inclusion criteria were fulfilled by 151 subjects. One hundred twenty gave their written consent. Out of these, 21 withdrew because of health problems unrelated to the study, digestive discomfort, or scheduling conflicts. Two subjects, reported not having diabetes, were excluded because of increased fasting glucose levels. The study was conducted between January and December 2009.

2.2 Dietary intervention

All participants (diabetics and nondiabetics) received information about the beneficial effects of a healthy diet with special focus on the importance of fat quality and the role of vegetables in a balanced diet. Participants were randomly assigned to the "intervention" or "information only" group. Subjects of the "information only" group received only the above mentioned information, while subjects of the "intervention" group received additionally 300 g of vegetables and 25 mL of plant oil (composition see Table 1) per day. The participants were instructed to use the plant oil as replacement for SFA. A reference "cup" and a booklet with recipes, instructions for replacement of SFA and usage of the plant oil

Table 1. Composition of the intervention oil

	Percentage of total fatty acids
C16:0	7.28 ± 0.09
C18:0	2.06 ± 0.07
C18:1n9c	16.4 ± 0.99
C18:1n7c	0.95 ± 0.03
C18:2n6c	61.8 ± 1.0
C18:3n3	11.5 ± 0.43
	Milligram per 100 g
γ-Tocopherol	33.0 ± 1.82
α-Tocopherol	2.67 ± 0.14
Campesterol	6.2 ± 0.45
Campestanol	1.0 ± 0.00
Stigmasterol	1.0 ± 0.00
Sitosterol	116 ± 5.00
Sitostanol	1.2 ± 0.45
Δ 5-avenasterol	11.6 ± 0.89
Cycloartenol + Δ 7-stigmastenol	38 ± 5.70
Minor sterols	7.8 ± 0.84
Total plant sterols	183 ± 2.10

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Table 2. Intervention vegetables (amount for 14 days)

Amount (g)	Vegetables
225	Strained spinach
400	Leave spinach
300	Green bean
300	Broccoli
300	Brussels sprouts
300	Soybean
300	Pea
300	Carrot[a)]
400	Romaine lettuce with peas
300	Vegetable mix I (broccoli, cauliflower)
300	Vegetable mix II (broccoli, carrot yellow and orange)
300	Vegetable mix III (carrot, potato, kohlrabi, leek, pea)
300	Vegetable mix VI (pea, carrot, corn)
300	Vegetable mix V (carrot, potato, broccoli, green bean, cauliflower)
800	Roasted vegetables I (carrot, broccoli, cauliflower, pole beans, zucchini)[b)]
800	Roasted vegetables II (carrot, Brussels sprouts, kohlrabi, pole beans)[b)]
400	Ready meal: spinach with potatoes[c)]

a) Was given to subjects additionally, in case of any digestive discomfort.
b) Subjects got roasted vegetables I for the first 14 days and roasted vegetables II for the second 14 days.
c) Contains 230 g of spinach.

(oil was not allowed to be heated up, but added to warm foods) was provided to the participants. A variety of frozen vegetables (Table 2) was given to subjects every 2 weeks. Participants could choose the order of consumption of the provided vegetables. A dietary diary had to be completed, and fatty acid profile, vitamin K, γ-tocopherol, and carotenoid concentrations were measured in plasma to monitor compliance.

The intervention period lasted 8 weeks, followed by a period of 8 weeks in which no intervention foods were provided. Blood samples were taken before the intervention, after 4, 8 (end of intervention period), and 16 weeks.

2.3 Anthropometric measurements

Body height (stadiometer: Seca, Modell 214, Hamburg, Germany) was measured at baseline, and waist circumference and weight (digital scale: Seca, Bella 840, Hamburg, Germany) were measured on every blood sampling day. BMI was calculated as kg/m^2.

2.4 Blood sampling and isolation of PBMCs

Venous blood samples were obtained after an overnight fast using heparin tubes (Becton Dickinson, Schwechat, Austria). Blood was centrifuged at 3000 rpm for 10 min to separate plasma from cells. Fasting plasma glucose, HbA1c and insulin were measured immediately by the laboratory of the Health Centre South, Vienna. In order to determine fatty acid and tocopherol concentrations, plasma was frozen in aliquots at −80°C until analysis.

PBMCs were isolated using Cell Preparation Tubes (Becton Dickinson, Schwechat, Austria). They were separated by centrifugation (3100 rpm, 25 min, room temperature) according to the manufacturer's instructions and washed twice with cold PBS.

2.5 Detection of oxidative damage to DNA in PBMCs

Oxidative damage to DNA and resistance against H_2O_2-induced DNA damage were measured with the single-cell gel electrophoresis assay (Comet Assay) [4]. Briefly, a freshly prepared suspension of PBMCs (approx. 1×10^6 cells/mL in PBS) was mixed with 1% low melting point agarose and put on agarose coated slides (1% normal melting agarose). Four slides were prepared from each cell suspension: "lysis," "buffer," "FPG" (also known as formamidopyrimidine DNA glycosylase), and "H_2O_2". All slides were put in lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris base plus 1% Triton X-100 added before use, pH 10) for at least 1 h. Only "H_2O_2" slides were put in 100 μM H_2O_2 solution for 5 min at 4°C before lysis. After lysis, the "buffer" and "FPG" slides were washed three times with enzyme buffer (40 mM HEPES, 0.1 M KCl, 0.5 mM EDTA, 0.2 mg/mL BSA, pH 8) and incubated with 50 μL of enzyme buffer ("buffer" slide) or FPG solution ("FPG" slide) for 30 min at 37°C in a moist box. Slides were put in an electrophoresis tank (CSL-COM40, Biozym, Austria) containing electrophoresis solution (0.3 M NaOH, 1 mM EDTA). After 20 min of unwinding and 30 min of electrophoresis (25 V, 300 mA at 4°C, pH $>$ 13) slides were washed with PBS and dried at room temperature.

Slides were stained with ethidium bromide (20 μg/mL) and the amount of DNA damage was quantified using a fluorescence microscope coupled with an imaging analysis system (Komet 5.5, Kinetinc Imaging, Liverpool, UK). For each sample, two replicate gels were analyzed and the mean %DNA in tail of 50 comets per gel was calculated. Slides were blinded before microscopic analysis. The net amount of FPG-sensitive sites was determined by calculating the difference between the obtained DNA damage after FPG and buffer treatment.

2.6 Detection of oxidative damage to DNA and RNA in urine

Urine samples were collected on the day of blood samplings, aliquoted and stored at –20°C until analysis. 8-oxodG and 8-oxo-7,8-dihydroguanosine (8-oxoGuo) were measured at the Laboratory of Clinical Pharmacology, Rigshospitalet, in Copenhagen, using a validated method for ultraperformance LC and MS/MS [18]. 8-oxodG and 8-oxoGuo were normalized against urinary creatinine concentration determined by the Jaffe reaction.

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2.7 Biochemical analyses

HbA1c was analyzed in whole blood by HPLC (Automated Glycohemoglobin Analyzer HLC-723G8, Tosoh, Tokyo, Japan). Fasting plasma glucose was determined enzymatically by the hexokinase method (Aeroset, Abbott Diagnostics, IL, USA) and plasma insulin concentrations were measured on an Immulite 2000 immunochemistry system using reagents and calibrator obtained from the instrument supplier (Siemens Medical Solutions Diagnostics, Flanders, USA). The principle of the method is a solid-phase, tow-site chemiluminescent immunometric assay. Homeostasis model assessment of insulin resistance was calculated as the product of fasting plasma glucose (mmol/L) and insulin (μU/mL) concentrations, divided by 22.5 as originally described by Matthews et al. [19].

2.8 Measurement of compliance markers

The fatty acid profile in plasma was determined by a GC equipped with a flame ionization detector [20]. Identification of fatty acids was based on the comparison of the samples' retention times to those of a 37 Component FAME Mix standard (Supelco, Bellefonte, USA). TotalChrom Workstation 6.3.0, PE Nelson, Perkin Elmer was used for peak integration.

Plasma concentrations of vitamin K, α- and γ-tocopherol, retinol, lutein, β-cryptoxanthin, lycopene, α-, and β-carotene were determined by RP-HPLC [21]. Each vitamin was quantitated on the basis of peak area using a calibration curve generated from standard solutions. To ensure quality control a control plasma sample was run throughout the study. CVs for all fatty acids and vitamins were $<$10%.

2.9 Oil analyses

The fatty acid pattern and tocopherol concentrations of the plant oil were determined with the same method as described above. Sterol content of the plant oil was analyzed by capillary GC at the Department of Food and Environmental Sciences, Helsinki, Finland [22]. The concentration of the given sterols or stanols is the sum of their free and esterified forms. The samples were measured in triplicate and the sterol values obtained were accepted if RSD $<$5%.

2.10 Statistical analyses

Statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL, USA). Normal distribution within the data set was tested by the Kolmogorov–Smirnov test. Independent samples *t*-test (for parametric data) or Mann–Whitney U-test (for nonparametric data) was conducted to assess differences between two groups. Multiple group comparisons were performed with one-way ANOVA followed by Bonferroni post hoc test. If the assumptions of normality and homogeneity of variance were violated, Kruskal–Wallis *H*-test was used followed by Dunnett-T3 as post hoc test.

The effect of the intervention was assessed by (i) comparing the changes after 4 and 8 weeks between the two treatment (information or intervention) groups (univariate ANOVA with treatment as fixed factor) and by (ii) comparing baseline values with values after 4 and 8 weeks of intervention (univariate ANOVA). Time point of blood sampling and squared time point of blood sampling were used as covariates. The effect of the intervention was assessed in the total study group and in the subgroups with different health status (ITDM2, NIDDM, nondiabetic). Data of the subgroups are shown, if significant changes were found. Statistical tests were carried out on log-transformed data (e.g. log strand breaks week 8 – log strand breaks week 0). Furthermore, the impact of the intervention was analyzed in participants with poor and good glycemic control (HbA1c below or above 7%) based on the above-mentioned procedure. Pearson correlation and Spearman rank correlation were used to evaluate the association between variables. Results were considered significant at $P < 0.05$.

3 Results

3.1 Baseline characteristics of the study population

In total 97 subjects completed the study. There were no significant differences between subjects of the intervention and information group at baseline (Table 3), apart from γ-tocopherol, which was significantly higher ($P = 0.026$), and vitamin K, which trended to be higher ($P = 0.059$) in subjects of the intervention group.

Baseline concentrations of fasting plasma glucose, HbA1c and BMI were significantly lower in nondiabetic subjects compared to both ITDM2 and NIDDM subjects (Supporting Information Table 1). Diabetes duration and HbA1c levels differed significantly between ITDM2 and NIDDM subjects. Significant differences in levels of 8-oxoGuo were observed between nondiabetic and NIDDM subjects. Furthermore, subjects with HbA1c concentrations even at 6.5% had significantly higher global RNA oxidation compared to subjects with HbA1c $<$6.5% (8-oxoGuo: 3.69 $\pm$ 1.51 vs. 3.04 $\pm$ 0.85 nmol/mmol creatinine, respectively; $P = 0.011$). Further analyses about the impact of glycemic control on oxidative damage to DNA revealed higher levels of FPG-sensitive sites in subjects in the highest tertile of fasting plasma glucose ($>$8.6 mmol/L), compared to the lowest tertile ($<$6.39 mmol/L; FPG-sensitive sites in the highest and lowest tertile: 2.85 $\pm$ 1.99 vs. 4.47 $\pm$ 2.95% DNA in tail; $P = 0.031$).

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Table 3. Baseline characteristics of participants in the information and intervention group

	Intervention	Information	*P*-value[a)]
Number (ITDM2/NIDDM/nondiabetic)	66 (25/29/12)	31 (11/11/9)	
BMI (kg/m^2)	32.5 ± 5.79	32.2 ± 7.03	0.934
Fasting plasma glucose (mmol/L)	8.13 ± 2.37	7.41 ± 2.07	0.261
HbA1c (%)	7.26 ± 1.08	7.01 ± 1.05	0.711
Strand breaks (% DNA in tail)	5.74 ± 2.32	4.63 ± 0.98	0.595
Resistance to H_2O_2 (% DNA in tail)	23.6 ± 10.7	21.2 ± 7.08	0.400
FPG-sensitive sites (% DNA in tail)	3.62 ± 2.31	3.84 ± 2.93	0.202
8-oxoGuo (nmol/mmol creatinine)	3.66 ± 1.44	3.17 ± 1.17	0.615
8-oxodG (nmol/mmol creatinine)	2.18 ± 0.79	1.87 ± 0.78	0.838
Lutein (μmol/L)	0.22 ± 0.17	0.19 ± 0.09	0.292
α-Carotene (μmol/L)	0.09 ± 0.04	0.09 ± 0.04	0.898
b-Carotene (μmol/L)	0.25 ± 0.25	0.25 ± 0.20	0.746
γ-Tocopherol (μmol/L)	1.60 ± 0.68	2.04 ± 1.09	0.026
Vitamin K (nmol/L)	0.74 ± 0.59	1.05 ± 0.88	0.059

ITDM2, insulin-treated type 2 diabetes mellitus; NIDDM, noninsulin dependent type 2 diabetes mellitus; HbA1c, glycated haemoglobin. Data are presented as means ± SD.
a) *P*-values for difference between subjects of information and intervention group adjusted for time point and squared time point of blood sampling.

3.2 Dietary compliance

To asses dietary compliance the fatty acid profile and levels of γ-tocopherol (for the oil intake) and carotenoids and vitamin K (for the vegetable intake) were measured. In subjects of the intervention group plasma levels of linoleic and linolenic acid, γ-tocopherol, lutein, α-carotene, β-carotene, and vitamin K were significantly increased after 4 and 8 weeks of intervention compared with baseline (Table 4). Analysis of the groups with different health status confirmed the

Table 4. Changes in compliance parameters after 4 and 8 weeks in the intervention and information group

	Baseline	Change after 4 weeks	Change after 8 weeks	*P* after 4 weeks[a)]	*P* after 8 weeks[b)]
Lutein (μmol/l)					
Information	0.19±0.09	+0±0.06	+0±0.06	0.509	0.466
Intervention	0.22±0.17	+0.14±0.12[c)]	+0.13±0.12[d)]	<0.001	<0.001
α-Carotene (μmol/l)					
Information	0.09±0.04	+0.01±0.03	+0.01±0.06	0.484	0.165
Intervention	0.09±0.04	+0.07±0.05[c)]	+0.08±0.06[d)]	<0.001	<0.001
β-Carotene (μmol/l)					
Information	0.25±0.2	+0.03±0.11	+0.07±0.17	0.273	0.007
Intervention	0.25±0.25	+0.12±0.12[c)]	+0.13±0.16[d)]	<0.001	<0.001
γ-Tocopherol (μmol/l)					
Information	2.04±1.09	+0.12±0.8	−0.03±0.89	0.186	0.929
Intervention	1.6±0.68	+0.69±0.56[c)]	+0.59±0.68[d)]	<0.001	<0.001
Vitamin K (nmol/l)					
Information	1.05±0.88	−0.31±0.78	−0.16±0.75	0.206	0.559
Intervention	0.74±0.59	+1.08±1.56[c)]	+1.13±1.58[d)]	<0.001	<0.001
Linoleic acid (%)[e)]					
Information	24.86±3.77	−0.54±1.91	−0.14±2.39	0.169	0.795
Intervention	24.85±4.1	+3.72±3.23[c)]	+3.51±3.09[d)]	<0.001	<0.001
α-Linolenic acid (%)[e)]					
Information	0.51±0.19	+0±0.22	−0.01±0.15	0.902	0.939
Intervention	0.5±0.18	+0.3±0.26[c)]	+0.31±0.3[d)]	<0.001	<0.001

Data are presented as means ± SD.
a) *P*-values for differences between baseline and week 4.
b) *P*-values for differences between baseline and week 8.
c) Significant difference between subjects of information and intervention group after 4 weeks.
d) Significant difference between subjects of information and intervention group after 8 weeks.
e) Reported as percentage of total fatty acids.
P-values were calculated with univariate ANOVA after adjustment for time point and squared time point of blood sampling.

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significant increase in all vegetable (Supporting Information Fig. 1) and oil compliance parameters (Supporting Information Fig. 2) after 4 and 8 weeks in ITDM2, NIDDM, and nondiabetic subjects. Interestingly, a significantly weaker increase in plasma α- and β-carotene was observed after 8 weeks of intervention in diabetic individuals (α-carotene: ITDM2: +0.04 ± 0.06 μmol/L; NIDDM: +0.06 ± 0.07 μmol/L; β-carotene: ITDM2: +0.05 ± 0.13 μmol/L; NIDDM: +0.1 ± 0.12 μmol/L) compared to nondiabetic subjects (α-carotene: +0.09 ± 0.09 μmol/L, β-carotene: +0.24 ± 0.21 μmol/L).

None of the compounds changed significantly in the information group, apart from β-carotene, which showed a slight but significant increase after 8 weeks. Analysis of the groups with different health status revealed significant changes in β-carotene only in healthy individuals (Supporting Information Fig. 1). The changes in all the compliance markers were significantly higher in the intervention group, after 4 and 8 weeks, compared with the information group (Table 4).

Since subjects were instructed to consume their habitual diet after the intervention period, most of the compliance markers returned to their baseline levels at week 16. Only linolenic acid, α-, and β-carotene remained significantly higher than at baseline. However, there were no significant differences in compliance markers between intervention and information group at week 16 (data not shown).

3.3 Glycemic control

Significant reductions in HbA1c levels were observed after 4 and 8 weeks of oil and vegetable consumption in ITDM2 and NIDDM subjects. HbA1c levels remained constant in nondiabetic individuals. In the information group, HbA1c levels were not significantly changed after 4 weeks, but significantly improved in ITDM2 and NIDDM subjects after 8 weeks (Supporting Information Fig. 3A–C). Consequently, changes in HbA1c levels were not significantly different between the two treatment groups (Table 5).

After return to the usual diet (week 16), subjects of the intervention group still had lower HbA1c levels than at baseline (week 0: 7.3 ± 1.1%, week 16: 7.1 ± 1.1%; $P = 0.065$). The reduction was significant in ITDM2 subjects (week 0: 8.1 ± 0.9%, week 16: 7.9 ± 0.9%; $P = 0.031$) but not in NIDDM and nondiabetic individuals (data not shown).

3.4 Levels of oxidative damage to DNA in PBMCs (Comet Assay)

Strand breaks, H_2O_2 sensitivity, and FPG-sensitive sites, were measured at baseline, and after 4 and 8 weeks of intervention (Table 5). Daily vegetable and oil consumption reduced DNA strand breaks by 13.8% after 4 (P <0.001) and by 17.1% after 8 weeks ($P < 0.001$) of intervention compared to baseline values. In the subgroups of the intervention group with different health status, the reduction of DNA strand breaks was significant in ITDM2 and NIDDM subjects, but not in nondiabetic individuals. No effects on levels of DNA strand breaks were observed in the information group (Fig. 1, Supporting Information Fig. 3D–F).

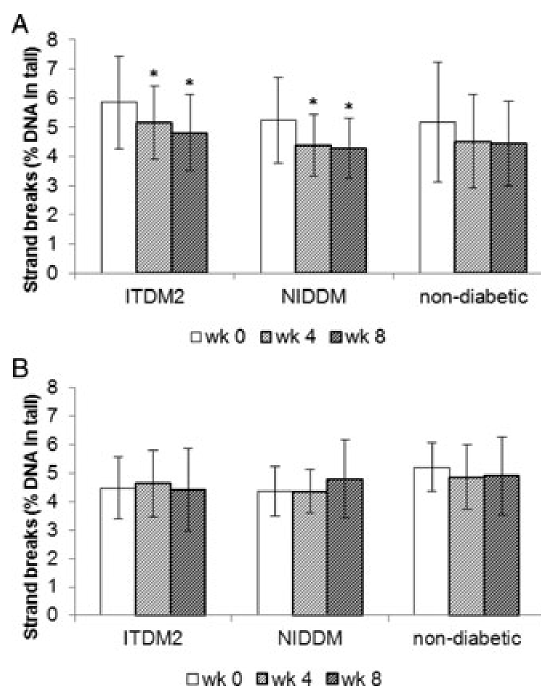

Figure 1. Changes in DNA strand breaks assessed by comet assay in (**A**) subjects of the intervention and (**B**) information group with different health status (insulin treated (ITDM2), non-insulin dependent (NIDDM) subjects with type 2 diabetes and nondiabetic individuals) from baseline (wk 0) to week 4 (wk 4) and week 8 (wk 8). Values are means ± SD. *Significantly different from baseline value (wk 0) of the corresponding group.

FPG-sensitive sites were significantly reduced (−13.2%, $P = 0.037$) in the intervention group after 4 weeks of intervention, whereas after 8 weeks, levels were not significantly different from baseline (−3.31%; Table 5). The reduction of FPG-sensitive sites after 4 weeks was in fact only significant in NIDDM subjects (week 0: 3.46 ± 2.56% DNA in tail, week 4: 2.47 ± 1.68% DNA in tail; $P = 0.048$).

The reductions in DNA strand breaks, FPG-, and H_2O_2-sensitive sites after 4 and 8 weeks were not significantly different between information and intervention group.

After return to the usual diet, levels of FPG- and H_2O_2-sensitive sites were not significantly different from baseline (data not shown). Levels of strand breaks increased at week 16, but were still significantly lower than at baseline (week 0: 5.4 ± 1.6% DNA in tail; week 16: 4.7 ± 1.3% DNA in tail).

3.5 Urinary excretion of 8-oxodG and 8-oxoGuo

Levels of 8-oxodG and 8-oxoGuo, markers for DNA and RNA oxidation, respectively, were not affected by the dietary

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Table 5. Changes in HbA1c, levels of DNA strand breaks, FPG-sensitive sites, H_2O_2-sensitivity and urinary excretion of 8-oxodG and 8-oxoGuo after 4 and 8 weeks in the intervention and information group

	Baseline	Change after 4 weeks	Change after 8 weeks	*P* after 4 weeks[a)]	*P* after 8 weeks[b)]
HbA1c (%)					
Information	7.01±1.05	−0.07±0.22	−0.16±0.25	0.118	0.002
Intervention	7.26±1.08	−0.17±0.34	−0.19±0.47	<0.001	0.002
Strand breaks (% DNA in tail)					
Information	4.63±0.98	−0.03±1.16	+0.07±1.61	0.785	0.953
Intervention	5.45±2.32	−0.73±1.5	−0.93±1.74	<0.001	<0.001
FPG-sensitive sites (% DNA in tail)					
Information	3.84±2.93	−0.26±2.91	−0.39±3.83	0.364	0.794
Intervention	3.62±2.31	−0.54±2.63	−0.15±3.56	0.037	0.558
H_2O_2-sensitive sites (% DNA in tail)					
Information	21.15±7.08	−0.28±5.6	+0.7±4.29	0.804	0.244
Intervention	23.55±10.66	−0.2±7.46	−1.56±7.65	0.225	0.795
8-oxodG (nmol/mmol creatinine)					
Information	1.87±0.78	+0.02±0.31	+0.02±0.39	0.297	0.528
Intervention	2.18±0.79	−0.01±0.53	−0.05±0.66	0.432	0.482
8-oxoGuo (nmol/mmol creatinine)					
Information	3.17±1.17	−0.01±0.86	+0.02±1.34	0.529	0.659
Intervention	3.66±1.44	+0.18±0.99	+0.2±1.43	0.293	0.155

Data are presented as means ± SD.
a) *P*-value for difference between baseline and week 4.
b) *P*-value for differences between baseline and week 8.
P-values were calculated with univariate ANOVA after adjustment for time point and squared time point of blood sampling.

intervention. There was no difference in 8-oxodG and 8-oxoGuo excretion after 4, 8, and 16 weeks between the two treatment groups.

3.6 Response to the dietary intervention based on preintervention glycemic control

To test the hypothesis whether the response to the dietary intervention is related to the quality of glycemic control, subjects were divided into groups with HbA1c below or above 7%, which is the HbA1c target level recommended by the American Diabetes Association [23].

In the intervention group 22 of 25 ITDM2, 12 of 29 NIDDM and none of the 12 nondiabetic subjects had HbA1c levels >7%. The effect of the dietary intervention on DNA strand breaks was significant after 4 and 8 weeks of intervention in both subgroups (HbA1c below or above 7%). The effect was stronger in subjects with HbA1c > 7%; however the changes were not significantly different from subjects with HbA1c levels <7% (Table 6). Changes in H_2O_2 sensitivity after 4 and 8 weeks of intervention were significantly different between the HbA1c subgroups due to the reduction in H_2O_2 sensitivity in subjects with HbA1c >7% and the slight increase in subjects with HbA1c ≤7%. FPG-sensitive sites were significantly reduced after 4 but not 8 weeks of intervention in subjects with HbA1c >7%. Changes in FPG-sensitive sites and urinary excretion of 8-oxodG and 8-oxoGuo were not significantly different between subjects above or below the recommended HbA1c level.

Analysis of the groups with different health status revealed significant reductions of DNA strand breaks in NIDDM (in both, HbA1c above ($n = 12$) and below 7% ($n = 17$)) and ITDM2 subjects (HbA1c above 7% ($n = 22$); Supporting Information Fig. 4). Furthermore, significant reductions in FPG-sensitive sites in ITDM2 subjects with HbA1c >7% after 8 weeks (week 0: 4.42 ± 2.22% DNA in tail, week 8: 3.17 ± 2.62% DNA in tail) and in 8-oxodG levels in NIDDM subjects with HbA1c ≤7% (week 0: 2.48 ± 0.93 nmol/mmol creatinine, week 4: 2.16 ± 0.98 nmol/mmol creatinine, week 8: 2.05 ± 0.89 nmol/mmol creatinine; $P = 0.015$, $P = 0.010$, respectively) were observed.

4 Discussion

T2DM is a rapidly increasing epidemic affecting people worldwide [24]. Dietary habits are among other factors intensively discussed to be responsible for the onset and the progression of the disease. A disturbed redox status, which is linked to T2DM itself and diet quality, leads to oxidative stress and can trigger oxidative DNA damage. Therefore, the aim

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Table 6. Changes in levels of DNA strand breaks, FPG-sensitive sites, H_2O_2-sensitivity and urinary excretion of 8-oxodG and 8-oxoGuo after 4 and 8 weeks of intervention with vegetables and plant oil in subjects with poor ($n = 34$) and good glycemic control ($n = 32$)

	Baseline	Change after 4 weeks	Change after 8 weeks	*P* after 4 weeks[a)]	*P* after 8 weeks[b)]
Strand breaks (% DNA in tail)					
HbA1c ≤7%	5.27±1.66	−0.59±1.35	−0.86±1.43	0.019	0.002
HbA1c >7%	6.18±2.76	−1.47±2.92	−1.57±3.03	<0.001	<0.001
FPG-sensitive sites (% DNA in tail)					
HbA1c ≤7%	3.49±2.42	−0.43±2.97	+0.28±3.74	0.453	0.906
HbA1c >7%	3.75±2.23	−0.65±2.34	−0.63±3.36	0.037	0.373
H_2O_2-sensitive sites (% DNA in tail)					
HbA1c ≤7%	21.94±10.41	+1.76±6.36	+0.56±7.57	0.035	0.256
HbA1c >7%	25.07±10.83	−2.04±8.03[c)]	−3.56±7.27[d)]	0.526	0.024
8-oxodG (nmol/mmol creatinine)					
HbA1c ≤7%	2.3±0.84	−0.13±0.46	−0.13±0.7	0.118	0.200
HbA1c >7%	2.05±0.73	+0.11±0.57	+0.02±0.61	0.839	0.771
8-oxoGuo (nmol/mmol creatinine)					
HbA1c ≤7%	3.44±1.02	+0.18±0.99	+0.27±0.97	0.333	0.195
HbA1c >7%	3.86±1.75	+0.17±1.02	+0.13±1.81	0.633	0.408

Data are presented as means ± SD.
a) *P*-values for differences between baseline and 4 week.
b) *P*-value for difference between baseline and 8 week.
c) Significantly different from subjects with HbA1c ≤7% after 4 weeks.
d) Significantly different from subjects with HbA1c ≤7% after 8 weeks.
P-values were calculated with univariate ANOVA after adjustment for time point and squared time point of blood sampling.

of the present intervention study was to investigate whether a healthy diet, with focus on vegetable supply and fat quality (replacing SFA by PUFA), is able to reduce oxidative damage to DNA in subjects with T2DM. Furthermore baseline values of oxidative damage to DNA were compared between nondiabetic individuals and subjects with T2DM.

In the present study, DNA damage in lymphocytes did not differ between diabetic and nondiabetic subjects. Studies, which have investigated this issue, have reported conflicting results [25–29], which can be explained by the complexity of diabetes, the big interindividual variations, leading to incomparability of study groups.

Also body weight, especially obesity, is discussed as a confounding factor regarding oxidative damage to DNA [30]. However within this trial no associations between BMI and oxidative damage to DNA were observed, either in diabetic or in nondiabetic subjects. A positive association between BMI and DNA strand breaks was described in individuals with type I diabetes [31] and in a study population consisting of healthy subjects, patients with impaired glucose regulation, and newly diagnosed diabetes [29]. On the contrary, no association between DNA strand breaks [32], or levels of 8-oxodG and BMI were reported in healthy individuals [33] and within a case–control study consisting of healthy individuals and breast cancer patients [34].

The difference in FPG-sensitive sites between ITDM2, NIDDM, and healthy subjects did not reach statistical significance ($P = 0.068$). An association between fasting plasma glucose and oxidized purines, already described in other studies [5, 27, 28, 31], could be confirmed within this trial by the increase in FPG-sensitive sites with increasing fasting plasma glucose tertile. This result supports the suggestion that FPG-sensitive sites specifically reflect damage resulting from hyperglycemia [31].

8-oxoGuo, a biomarker for global RNA oxidation, was recently suggested as a clinical biomarker for the assessment of mortality risk in newly diagnosed T2DM patients [35]. Within our trial, global RNA oxidation levels were significantly lower in nondiabetic subjects compared to ITDM2 and NIDDM subjects. Furthermore, glycemic control can be suggested as one determinant of global RNA oxidation, since subjects with HbA1c concentrations even at 6.5% had significantly higher 8-oxoGuo levels compared to subjects with HbA1c <6.5%.

A very novel finding of the present study was the reduction in strand breaks, measured by Comet Assay, in T2DM subjects after the intervention with vegetables and plant oil, while levels were not significantly changed in nondiabetic individuals. To the best of our knowledge, no data about the impact of vegetables on DNA damage in T2DM subjects are published. Supplementation trials with vitamin E [36] or flavonols [37] are consistent with our findings and showed a reduction in strand breaks in T2DM subjects. Intervention studies in healthy individuals with a vegetable/fruit concentrate [38]; 600 g of fruit and vegetables [39]; 200 g of

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cooked minced carrots [40]; a tomato-based drink [41]; 2, 5, or 8 servings/day of vegetables and fruit [42]; 300 g of Brussels sprouts [43]; or 225 g of spinach [44] however reported no effect on DNA strand breaks. The discrepancy in effects between diabetic and nondiabetic individuals in the present study might be due to significantly lower baseline levels of α- and β-carotene in ITDM2 and NIDDM subjects compared to nondiabetics, indicating that subjects with low antioxidant status benefit more from the intervention. We also observed a significantly weaker increase in plasma α- and β-carotene after 8 weeks of intervention in diabetic individuals compared to nondiabetic subjects, which might reflect the use of the known antioxidants as radical scavengers. Similar observations were made in intervention studies with watercress [45] or with 300–400 g of vegetables and fruit [46] in smokers, who showed a significantly lower plasma response in antioxidants after the intervention compared to nonsmokers.

Moller et al. also suggested that the oxidative burden at baseline has a major impact on the effect of dietary interventions [47]. Within this trial subjects of the information group had significantly higher plasma γ-tocopherol levels at baseline compared to the intervention group. Furthermore, levels of DNA strand breaks were lower, although not significantly, in the information group compared to the intervention group. One cannot rule out the possibility that these factors influence the outcome of the dietary intervention.

Oxidative burden is increased in T2DM patients, especially in those with poor glycemic control via autoxidation of glucose, the activation of protein kinase C, the increased production of advanced glycation end products and the increase in polyol and hexosamine pathway activity [3, 48]. Within this trial the impact of glycemic control on the outcome of the intervention with vegetables and plant oil could be confirmed via the significant reduction in FPG- and H_2O_2-sensitive sites in subjects (ITDM2 and NIDDM) with HbA1c >7% after 4 and 8 weeks, respectively. The reduction in FPG-sensitive sites was also significant after 8 weeks in ITDM2 but not in IDDM patients with HbA1c >7%. The mechanisms, responsible for the reduction in oxidative damage to DNA after vegetable and oil consumption are so far unknown. The reduction may be related to the improvement in antioxidant status [49] and the decreased HbA1c concentration, indicating improved glycemic control. However, our data further suggest that an improvement in glycemic control alone, as seen in the information group, is not sufficient to reduce oxidative damage to DNA.

Another possible mechanism against oxidative damage to DNA is cellular repair [50]. 8-oxodG and 8-oxoGuo are excreted into the urine as a result of DNA repair [18, 51], and are important biomarkers of oxidative DNA and RNA damage [18]. However, 8-oxodG and 8-oxoGuo remained constant during the intervention. Similar findings were reported by Freese et al. [52], who found no effect of a diet rich in fruit and vegetables (total amount 1059 g/10 MJ) combined with PUFA (11% of energy) on 8-oxodG levels in healthy subjects.

At week 16 (return to usual diet), levels of strand breaks increased compared to week 8 (end of intervention) but were still significantly lower compared to baseline. After week 8, intervention foods were no longer provided and oil bottles were recollected. Interestingly, HbA1c and dietary antioxidants were still improved at week 16 compared to baseline, suggesting that subjects of the intervention group kept their healthier diet, at least in part. This was also confirmed by food frequency questionnaires, which were collected at baseline and week 16, showing a significantly higher consumption of vegetables at week 16 compared to baseline (data not shown).

In summary, a healthy diet, rich in vegetables and with a considerable amount of PUFA, replacing SFA for 8 weeks, decreases DNA strand breaks. This positive effect might be related to the reduction in HbA1c and the improvement in antioxidant status, strengthening the idea that diet quality is important in treatment of T2DM. However, the present results also suggest that providing information to subjects with T2DM about the beneficial effects of a healthy diet once, particularly pointing out the role of PUFA and differently colored vegetables, improves glycemic control, but does not alter antioxidant status or oxidative damage to DNA.

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