informa
healthcare

High intake of fruit and vegetables is related to low oxidative stress and inflammation in a group of patients with type 2 diabetes

Rikard Åsgård[1], Elisabet Rytter[2], Samar Basu[2], Lilianne Abramsson-Zetterberg[3], Lennart Möller[1] and Bengt Vessby[2]

[1]Department of Biosciences and Nutrition, Karolinska Institutet, Huddinge, Sweden; [2]Clinical Nutrition and Metabolism, Uppsala University, Uppsala, Sweden; [3]National Food Administration, Uppsala, Sweden

Abstract

Background: Patients with type 2 diabetes have increased levels of oxidative stress and inflammation. A high fruit and vegetable intake may be beneficial.
Objective: To study whether fruit and vegetable intake and levels of plasma antioxidants relate to markers of oxidative stress and inflammation in a group of patients with type 2 diabetes. Further, to investigate whether plasma antioxidants are good biomarkers for intake of fruit and vegetables.
Design: Patients with type 2 diabetes were studied. Their dietary intake and levels of plasma antioxidants, and markers of oxidative stress and inflammation were analysed.
Results: Fruit and vegetable intake was inversely related to oxidative stress. Plasma carotenoids were negatively correlated with inflammation. The plasma levels of α-carotene and β-carotene showed strongly positive associations with fruit and vegetable intake.
Conclusions: The results suggest that fruit and vegetable intake may decrease oxidative stress and inflammation in this group of patients. An increased intake of fruit and vegetables can therefore be beneficial for patients with type 2 diabetes, since these patients are documented to have raised oxidative stress and inflammation. The study support the usefulness of plasma α-carotene and β-carotene as biomarkers for fruit and vegetable intake.

Keywords: *antioxidants*; *fruit*; *inflammatory cytokine*; *oxidative stress*; *type 2 diabetes*; *vegetables*

Introduction

It is well known that diet can affect the health status of patients with type 2 diabetes. The beneficial effects of fruit and vegetables are suggested to be related to the presence of antioxidants.

An optimal antioxidative status protects against oxidative stress, which is defined as an imbalance between free radicals and antioxidative defence. Oxidative stress is involved in the development and complications of diabetes (1). Patients with type 2 diabetes have been shown to have reduced antioxidative defence, which negatively correlates with glucose levels and duration of diabetes (2). The metabolic imbalance in patients with type 2 diabetes, with increased plasma reactive oxygen species (ROS) generation and decreased efficiency of inhibitory and scavenger systems (3) that result in oxidative stress, can lead to secondary complications in the disease, such as degeneration of the eyes, kidneys and arteries (4, 5). The enhanced production of ROS seems mainly to be due to hyperglycaemia (6) resulting in stimulation of the polyol pathway, formation of advanced glycosylation endproducts, and subsequent formation of ROS (7). ROS production is also related to hyperinsulinaemia and insulin resistance (1, 8), as well as to inflammation (5).

Specific nutrients, food and obesity have a clear impact on the development of type 2 diabetes and this effect may, in part, be mediated via the inflammatory status (9). A high fruit and vegetable intake has been shown to be associated with a low level of inflammation (10). It has been suggested that development of type 2 diabetes can be reduced by increased intake of antioxidants in the diet (11). Dietary intake of α-carotene, β-carotene and lycopene, as well as plasma β-carotene concentrations, has been shown to have beneficial associations with glucose metabolism in subjects at high risk of type 2 diabetes (12), and glucose metabolism has been associated with oxidative stress (6, 7).

Furthermore, patients with type 2 diabetes have constantly high levels of lipid peroxidation (F_2-isoprostanes) (13), in contrast to the large daily variation in lipid peroxidation in healthy men (14). *In vivo* lipid peroxidation, measured as F_2-isoprostanes, does not seem to be influenced by the fatty acid content of the diet (15, 16), but other dietary components such as antioxidants may affect lipid peroxidation in a positive manner. It is therefore of importance to study dietary effects on both DNA oxidation and lipid peroxidation in patients with type 2 diabetes. Plasma levels of antioxidants as well as markers of oxidative stress and inflammation [C-reactive protein (CRP)] have been described in a healthy population (17), but no previous data are available on a group of patients with type 2 diabetes with stable metabolic control.

The aim of this study was to investigate whether fruit and vegetable intake, dietary antioxidants and levels of plasma antioxidants correlate with oxidative stress and inflammation in patients with type 2 diabetes with stable metabolic control. A further objective was to compare levels of dietary antioxidants and fruit and vegetable intake with plasma antioxidants to see whether plasma levels can be used as biomarkers for dietary intake.

Materials and methods

Subjects
The participants were recruited from Uppsala, Sweden, by advertising in a local newspaper. The inclusion criteria were: type 2 diabetes treated with either diet alone ($n = 20$) or diet and oral hypoglycaemic medication, sulfonylurea or metformin ($n = 34$), glycosylated haemoglobin (HbA_{1c}) $< 10\%$, 40–75 years of age, body mass index (BMI) $< 35 \, kg \, m^{-2}$ and a stable body weight for the past 3 months. However, three subjects with BMI > 35 and $< 37.2 \, kg \, m^{-2}$ were included in the study since all other inclusion criteria were fulfilled. Subjects who took insulin treatment and those who had acute inflammatory diseases, as well as diseases of the liver, kidney and thyroid gland, were excluded. Medication or supplementation that could possibly affect antioxidative, oxidative or inflammatory status, including non-steroidal anti-inflammatory drugs, also meant exclusion. Food supplementation was not allowed during 1 month before the start of the study. Altogether, 54 subjects were included, but the dietary data comprise 28 women and 25 men. Twenty-seven women and 24 men are included in all correlations with inflammatory variables. Four men and four women were smokers. The characteristics of the participants are presented in Table 1.

The subjects gave their written consent to participate in the study, which was approved by the ethical committee of the Medical Faculty at Uppsala University, Uppsala, Sweden (no. 02–502). The present study was a cross-sectional study of patients with type 2 diabetes. Blood and urine samples were drawn in the morning after an overnight fast. Body height, weight and waist circumference were recorded on the same occasion. Subjects received oral and written instructions to refrain from alcohol intake and heavy physical activity on the day before, and on the day of the clinical examination. The data in this paper were from measurements of the patients before treatment in an intervention study. Data from the intervention study with differences between genders, detailed analyses of relations between glucose metabolism and oxidative stress and inflammation, and relations between oxidative stress and inflammation variables, as well as results from the intervention, will be presented elsewhere.

Anthropometric and metabolic measurements
All anthropometric measurements were taken with the subjects wearing light indoor clothes and no shoes, according to standardized routines. The subjects' waist circumferences were assessed, using a non-stretchable tape measure, in a supine position midway between the lowest rib and the iliac crest. Blood glucose concentration was analysed by clinical routine enzymic techniques. HbA_{1c} was analysed by high-performance liquid chromatography (HPLC). Plasma insulin was assayed with an

Table 1. Clinical characteristics of the patient group

	Total	Female	Male	*P*
Subjects				
Number	54	29	25	
Smokers/non-smokers	8/46	4/25	4/21	
Diet/diet and medication[a]	20/34	14/15	6/19	
Characteristics				
Age (years)	62.6 ± 7.0	62.9 ± 6.7	62.2 ± 7.3	ns
	[63.0]	[63.0]	[63.0]	
	(46–75)	(46–75)	(50–72)	
Weight (kg)	82.1 ± 13.9	75.5 ± 12.1	89.6 ± 12.0	***
	[83.1]	[73.8]	[89.9]	
	(54.9–114.5)	(54.9–103.5)	(72.3–114.5)	
Waist circumference (cm)	99.6 ± 11.3	97.2 ± 12.7	102.3 ± 9.0	ns
	[100]	[98.5]	[100.0]	
	(70–127)	(70–127)	(87–125)	
BMI (kg m^{-2})	28.2 ± 3.9	28.3 ± 4.3	28.2 ± 3.4	ns
	[28.1]	[28.2]	[27.8]	
	(19.7–37.2)	(19.7–37.2)	(23.6–35.6)	
HbA$_{1c}$ (%)	6.1 ± 0.8	6.0 ± 0.8	6.1 ± 0.9	ns
	[6.0]	[5.8]	[6.1]	
	(4.7–8.0)	(5.0–7.6)	(4.7–8.0)	
Glucose (mmol l^{-1})	7.8 ± 2.3	7.6 ± 2.0	8.1 ± 2.6	ns
	[7.4]	[7.4]	[7.4]	
	(4.1–14.6)	(4.7–12.1)	(4.1–14.6)	
Insulin (mU l^{-1})	11.5 ± 6.8	11.1 ± 7.0	11.9 ± 6.7	ns
	[8.8]	[8.3]	[9.3]	
	(3.6–28.6)	(3.6–26.2)	(4.9–28.6)	

Data are shown as mean ± SD, [median] and (range).

[a]Subjects were allowed to keep going with their usual treatment of either diet or diet and oral hypoglycaemic medication.

BMI: body mass index; HbA$_{1c}$: glycosylated haemoglobin.

Significant difference: ***$p < 0.001$ between genders; ns: not significant (Wilcoxon two-sample test).

enzymic immunological assay in a Coda Automated EIA Analyser (BioRad Laboratories, CA, USA).

Dietary data

Twenty-eight women and 25 men completed a 3 day dietary survey with a food diary called "Menyboken" 1 week before the other measurements. The subjects were asked to record everything they ate for two weekdays and one weekend day in the precoded food record. Menyboken is originally a precoded 7 day food diary, which has been validated (18) and used in two nationwide dietary surveys in Sweden. The 3 day food diary, used in and regarded as satisfactory for this study, was analysed with computer programs where food groups and macronutrients and micronutrients were estimated. Fruit and vegetable intake included vegetables, root crops, fruits and berries, and also marmalade, jam, stews and preserves made of fruits, berries and root crops.

Plasma antioxidant measurements and serum sample analyses

Ethanol was added to serum to precipitate proteins, and then carotenoids, α-carotene, β-carotene, lycopene and lutein were extracted into hexane and evaporated under nitrogen to dryness. The residue was redissolved into ethanol and the carotenoids were detected by HPLC with a diode array detector using a Chromolith Performance column (Merck, Darmstadt, Germany). The mobile phase for the analysis was ACN:DCM:MeOH (72.5:2.5:25). The amounts of α-tocopherol and γ-tocopherol in serum were analysed by HPLC (19) and adjusted for the sum of the cholesterol and the triglyceride concentrations (20). Ascorbic acid concentration in plasma was analysed by a modified fluorometric method, after oxidation using ascorbate oxidase and derivatization with 1-ortho-phenylenediamine (21). The intra-assay coefficient of variation (CV) for the method was ∼4%, the interassay CV was ∼5% and

the detection limit was $\sim 5 \, \mu mol \, l^{-1}$. Serum folate and vitamin B_{12} were analysed at the Clinical Chemistry and Pharmacology Centre for Laboratory Medicine, Uppsala University Hospital, Sweden, by clinical standardized procedures.

Biomarkers for oxidative stress

Venous blood was collected in Vacutainer CPT tubes (Becton Dickinson, Franklin Lakes, NJ, USA). The mononuclear blood cells were separated, washed in RPMI 1640 cell medium (Gibco, Paisley, UK) and slowly frozen in freezing medium consisting of 90% foetal bovine serum (FBS) (Gibco) and 10% dimethylsulfoxide (DMSO) (Merck, Darmstadt, Germany) down to $-80°C$. The lymphocytes and monocytes were thawed at $37°C$ and thereafter washed, on ice, in RPMI 1640 cell medium with 10% FBS. The cells were then kept on ice during the comet assay and the 8-oxo-7,8-dihydroguanine (8-oxodG) analyses, to prevent further oxidations or repair during the analyses. A high-alkaline formamidopyrimidine glycosylase (FPG) version of the comet assay was used (22), with some modifications (23). 8-OxodG was analysed using HPLC/EC/UV and DNA was extracted using a cold work-up procedure (24).

The plasma malondialdehyde (MDA) concentration was measured by HPLC and fluorescence detection (25), by a well-used protocol for this lipid peroxidation biomarker.

Free 8-iso-prostaglandin-$F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) in urine was analysed by a validated radioimmunoassay (26) with a detection limit of about 23 $pmol \, l^{-1}$. The urinary levels of 8-iso-$PGF_{2\alpha}$ were adjusted for creatinine concentration and the intra-assay CV was 14.5% at low and 12.2% at high concentrations. The cross-reactivity of the 8-iso-$PGF_{2\alpha}$ antibody with 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, thromboxane B_2 (TXB_2), 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$ was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%, respectively.

Biomarker for chromosomal damage

The method used ($n = 36$) for the analysis of micronucleated transferrin-positive reticulocytes is the flow-cytometer-based micronucleus assay, where enriched transferrin-positive reticulocytes, the youngest erythrocytes (Trf-Ret), were studied (27). The micronuclei studied are formed in the bone marrow at the last cell division of the erythroblasts.

The detection limit is approximately a difference of 0.2 of the background frequency of micronuclei.

Biomarkers for inflammation

High-sensitivity CRP measurements from plasma samples were performed with latex-enhanced reagent (Dade Behring, Deerfield, IL, USA) with the use of a Behring BN ProSpec analyser (Dade Behring). The intra-assay CV was 1.4% at both 1.23 and 5.49 $mg \, l^{-1}$. Since CRP $>10 \, mg \, l^{-1}$ suggests an ongoing acute inflammation and the intention was to study a group with stable metabolism without extremely high inflammation levels, two patients with a CRP $>10 \, mg \, l^{-1}$ were excluded before analysing the relationships of inflammation with diet and plasma antioxidants.

Interleukin-6 (IL-6) was analysed with an enzyme-linked immunosorbent assay (ELISA) kit (IL-6 HS; R&D Systems, Minneapolis, MN, USA). Samples from plasma and standards were added to a microtitre plate coated with monoclonal antibody against IL-6. After incubation and washing, enzyme substrate solution was added, followed by anti-IL-6 antibody. The colour reaction was proportional to the bound IL-6. The total CV of the method was 7% and the interassay CV was 5%.

Urinary 15-keto-dihydro-$PGF_{2\alpha}$ was analysed with a validated radioimmunoassay developed by Basu (28). The intra-assay CV was 12.2% at low and 14.0% at high concentrations. The cross-reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, PGE_2, 15-keto-13,14-dihydro-PGE_2, 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, TXB_2 and 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, < 0.001, < 0.001, < 0.001 and 0.01%, respectively. The detection limit was about 45 $pmol \, l^{-1}$. Levels were corrected for urinary creatinine.

Statistical analysis

Statistical analyses were performed using the statistical software JMP, version 3.2 (SAS Institute, Cary, NC, USA). All correlation coefficients were calculated as Spearman's rank correlation coefficients. Probability values of <0.01 were considered as significant for the correlation tests. This was to protect against false-positive significances because of the multiple analyses in the study. In the correlation analyses of oxidative stress or inflammation probability, significant values of $p < 0.05$ was used instead of $p < 0.01$ (as for the other tests), after correction with Bonferroni–Holm (29) to catch

significant correlations <0.05 and >0.01 that are not false-positive correlations. Energy-corrected dietary values (per 1000 kJ) were used in all correlation analyses with dietary intake. Many variables did not have a normal distribution (Shapiro–Wilks W test, <0.95). Therefore non-parametric tests were used. The Wilcoxon two-sample test was used to analyse differences between the genders. Probability values of <0.05 were considered significant. Statistical analyses were calculated on $n = 54$ for the whole group, $n = 53$ on dietary data and $n = 51$ for inflammatory data.

Results

Characteristics

This study investigated a group of 54 patients with type 2 diabetes with stable metabolic control. The distribution between men and women was similar and no significant differences between the genders

could be seen (Table 1), except for weight, which was to be expected.

Dietary intake

The results of the 3 day food diary are presented in Table 2. The mean energy intake (8.6 MJ day^{-1}) as well as energy intake from proteins and carbohydrates were in line with the Nordic nutrition recommendations (30), while fat intake was slightly above 30 energy per cent ($E\%$). The average intake of fruit and vegetables (501.8 g day^{-1}) followed the recommendations, as did the intake of various micronutrients, except for vitamin E, folate and selenium for men, which were lower than the recommended daily intakes. The antioxidant vitamin intake was in accordance with the recommendations. No gender differences could be seen in the dietary intake when the data had been corrected for energy intake, except for vitamin B$_{12}$, which was higher for women. Dietary intake of the antioxidants β-carotene, vitamin E, α-tocopherol, vitamin

Table 2. Dietary intake in the population of patients

Dietary intake (per day)	Total	Female	Male	p
Energy (kJ)	8488 ± 2148	8273 ± 2369	8729 ± 1891	ns
	(5226–13 722)	(5226–13 722)	(5758–12 490)	
Proteins (E%)	17.4 ± 2.30	17.6 ± 2.06	17.2 ± 2.57	ns
	(12.5–22.5)	(13.6–21.8)	(12.5–22.5)	
Fats (E%)	31.4 ± 5.74	32.1 ± 5.51	30.5 ± 5.99	ns
	(18.8–44.6)	(20.6–44.0)	(18.8–44.6)	
Carbohydrates (E%)	48.1 ± 7.00	47.8 ± 5.93	48.5 ± 8.15	ns
	(32.8–67.2)	(35.7–60.5)	(32.8–67.2)	
β-Carotene (µg)	3687 ± 2592	4180 ± 3037	3135 ± 1890	ns
	(369–16 276)	(973.0–16 276)	(369.2–6860)	
Vitamin E (α-TE)	9.67 ± 7.06	10.4 ± 9.49	8.81 ± 2.29	ns
	(4.28–53.5)	(4.28–53.5)	(5.43–12.2)	
α-Tocopherol (mg)	9.17 ± 6.98	9.95 ± 9.38	8.29 ± 2.24	ns
	(4.14–52.8)	(4.14–52.8)	(5.26–11.58)	
Vitamin C (mg)	131.1 ± 77.9	138.1 ± 83.9	123.1 ± 71.3	ns
	(43.0–376.4)	(43.0–376.4)	(43.5–307.5)	
Vitamin B$_{12}$ (µg)	7.03 ± 3.62	7.90 ± 4.39	6.05 ± 2.20	*
	(1.95–21.5)	(2.45–21.5)	(1.95–11.9)	
Zinc (mg)	12.2 ± 3.11	11.9 ± 3.23	12.5 ± 3.00	ns
	(6.14–18.8)	(6.14–18.1)	(7.43–18.8)	
Folate (µg)	296.2 ± 92.0	307.7 ± 112.6	283.3 ± 61.2	ns
	(160.4–601.5)	(160.4–601.5)	(160.9–401.6)	
Selenium (µg)	44.1 ± 12.2	42.7 ± 10.6	45.6 ± 14.0	ns
	(22.0–68.2)	(27.2–64.9)	(22.0–68.2)	
Fruit and vegetables (g)	501.8 ± 322.9	497.0 ± 257.6	507.2 ± 388.9	ns
	(91.7–2054)	(180.0–1095)	(91.7–2054)	

Data are shown as mean ± SD (range).

TE: tocopherol equivalents.

Significant difference: *$p < 0.05$ between genders; ns: not significant (Wilcoxon two-sample test).

C and folate was significantly related to the intake of fruit and vegetables (not shown).

Antioxidant levels in plasma

Plasma levels of α-carotene, β-carotene, lycopene, lutein, α-tocopherol, γ-tocopherol and ascorbate are shown in Table 3. Folate (15.1 ± 5.03 nmol l^{-1}) and vitamin B_{12} (324.6 ± 113.5 pmol l^{-1}) were also measured in plasma (not shown), but are not considered antioxidants and were therefore not further analysed in this study. Women had higher levels of plasma antioxidants except for lycopene (not shown). The plasma values of α-carotene and β-carotene showed strongly positive correlations with fruit and vegetable intake (Table 4). Levels of β-carotene in plasma were positively correlated with dietary β-carotene intake (Table 4). Plasma levels of α-carotene correlated positively with dietary β-carotene, and plasma levels of both α-carotene and β-carotene correlated with vitamin C. Further, plasma α-tocopherol was positively correlated with vitamin C (Table 4). In contrast to the other antioxidants, plasma levels of γ-tocopherol had negative correlations with dietary β-carotene, vitamin C, and fruit and vegetable intake (Table 4). Associations between plasma antioxidants were also found, but are not presented here. All antioxidants measured in diet and plasma are listed in Tables 2 and 3, but no further significant correlations between them other than those mentioned in Table 4 and above were found.

Table 3. Levels of antioxidants in the patients' plasma

Antioxidants	Total
α-Carotene (mg l^{-1})	0.11 ± 0.15
	(0.01–0.98)
β-Carotene (mg l^{-1})	0.28 ± 0.38
	(0.04–2.58)
Lycopene (mg l^{-1})	0.16 ± 0.08
	(0.01–0.37)
Lutein (mg l^{-1})	0.12 ± 0.05
	(0.04–0.28)
α-Tocopherol (μg ml^{-1})	2.03 ± 0.32
	(1.46–3.48)
γ-Tocopherol (μg ml^{-1})	0.10 ± 0.04
	(0.03–0.22)
Ascorbate (μM)	34.3 ± 12.2
	(13.2–62.8)

Data are shown as mean $\pm$ SD (range).

Table 4. Dietary intake, corrected for energy intake, correlated with antioxidants in plasma

Food variable/MJ	Plasma antioxidant	r	p
Fruit and vegetable intake	α-Tocopherol	0.3191	0.0198*
	γ-Tocopherol	−0.4339	0.0012**
	α-Carotene	0.3969	0.0033**
	β-Carotene	0.4382	0.0010***
	Lycopene	0.2867	0.0374*
	Ascorbate	0.2707	0.0500*
β-Carotene	γ-Tocopherol	−0.5119	< 0.0001***
	α-Carotene	0.6419	< 0.0001***
	β-Carotene	0.6578	< 0.0001***
	Lutein	0.3453	0.0113*
	Lycopene	0.3265	0.0170*
α-Tocopherol	α-Tocopherol	0.2826	0.0403*
	α-Carotene	0.3063	0.0257*
	Lycopene	0.3188	0.0200*
Vitamin E	α-Tocopherol	0.3069	0.0254*
	α-Carotene	0.3145	0.0218*
	Lycopene	0.3206	0.0193*
Vitamin C	α-Tocopherol	0.3795	0.0051**
	γ-Tocopherol	−0.3634	0.0075**
	α-Carotene	0.3586	0.0084**
	β-Carotene	0.4052	0.0026**
	Lutein	0.3145	0.0218*
	Ascorbate	0.3428	0.0120*
Selenium	Lutein	0.3033	0.0272*

Significant differences: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ (considered not significant) (Spearman rank correlation coefficient).

Correlations between dietary intake or plasma antioxidants and oxidative stress or inflammation

The levels of oxidative stress, chromosomal damage and inflammatory biomarkers are shown in Table 5. These levels were correlated with dietary intake and plasma antioxidants. The analyses show that fruit and vegetable intake were negatively correlated with DNA oxidation (comet assay) and lipid peroxidation (8-iso-PGF$_{2\alpha}$), whereas dietary vitamin C was inversely correlated with lipid peroxidation (8-iso-PGF$_{2\alpha}$) (Table 6). Correlations between plasma antioxidants and oxidative stress and inflammation were also analysed. As shown in Table 6, plasma γ-tocopherol was negatively correlated with lipid peroxidation (MDA) and ascorbate with DNA oxidation (8-oxodG). All measured plasma carotenoids were negatively correlated with inflammation (IL-6), while γ-tocopherol was positively correlated with inflammation (CRP) (Table 6). The correlations between dietary or plasma antioxidants and oxidative stress or inflammatory biomarkers

Table 5. Oxidative stress, chromosomal damage and inflammation variables

Variable	Total
Oxidative stress variables	
8-OxodG/10^6 dG	0.97 ± 0.45
	(0.20–2.97)
Comet assay, FPG sites (% tail)	30.2 ± 15.1
	(6.85–65.6)
Malondialdehyde (mmol l^{-1})	0.68 ± 0.08
	(0.52–0.85)
8-Iso-PGF$_{2\alpha}$ (nmol mmol^{-1} creatinine)	0.19 ± 0.09
	(0.06–0.56)
Chromosomal damage variable	
Micronucleated Trf reticulocytes (‰)[a]	0.92 ± 0.50
	(0.21–2.28)
Inflammation variables	
CRP (mg l^{-1})	3.15 ± 4.3
	(0.16–27.6)
CRP (mg l^{-1}), only values <10 included[b]	2.50 ± 2.46
	(0.16–9.53)
IL-6 (ng l^{-1})	2.50 ± 2.20
	(0.40–11.20)
15-Keto-dihydro-PGF$_{2\alpha}$ (nmol mmol^{-1} creatinine)	0.24 ± 0.10
	(0.08–0.68)

Data are shown as mean $\pm$SD (range).

[a] 36 subjects; [b] 51 subjects.

8-OxodG: 8-oxo-7,8-dihydroguanine; FPG: formamidopyrimidine glycosylase; 8-iso-PGF$_{2\alpha}$: 8-iso-prostaglandin-F$_{2\alpha}$; Trf reticulocytes: transferrin-positive reticulocytes; CRP: C-reactive protein; IL-6: interleukin-6; PGF$_{2\alpha}$: prostaglandin F$_{2\alpha}$.

presented in Table 6 were the only ones found to be significant when testing all variables in Tables 2, 3 and 5.

Discussion

Type 2 diabetes is a disease in which elevated oxidative stress and inflammation has been demonstrated (2, 3, 5, 13), and where dietary factors are possible interactors. This study on 54 patients with type 2 diabetes with stable metabolic control investigated whether fruit and vegetable intake, dietary antioxidants or plasma antioxidants were correlated with markers of oxidative stress and/or inflammation. To the authors' knowledge, no such study has been performed previously on this particular group of patients.

Fruit and vegetable intake was negatively correlated with DNA oxidation (comet assay) and lipid peroxidation (8-iso-PGF$_{2\alpha}$), suggesting that these foods reduce oxidative stress in patients with type 2 diabetes (Table 6). The factors in fruit and vegetables responsible for this effect are not known in detail, but intake of antioxidants such as α-carotene,

β-carotene and lycopene, as well as plasma β-carotene concentrations, had beneficial associations with glucose metabolism in subjects at high risk of type 2 diabetes in a previous study (12). In the present study, several correlations between antioxidants and reduced levels of oxidative stress and inflammation were found. Thus, dietary vitamin C had a negative correlation with lipid peroxidation (8-iso-PGF$_{2\alpha}$) and plasma ascorbate with DNA oxidation (8-oxodG), while all measured plasma carotenoids were negatively correlated with inflammation, measured as IL-6 (Table 6).

Plasma values of α-carotene and β-carotene were strongly correlated with fruit and vegetable intake (Table 4). They can therefore be considered as biomarkers for fruit and vegetable intake. Carotenoids in plasma have previously been shown to be good predictors of dietary intake of fruit and vegetables measured by food questionnaires (31). The positive correlations between several antioxidants in the diet and antioxidants levels in plasma in the present study thus support that the dietary measurements were reliable (Table 4). The statistically strongest correlation between antioxidant levels in diet and in plasma was for β-carotene ($p < 0.0001$). Other studies have also found a positive relationship between plasma levels and the intake of β-carotene in elderly subjects (32, 33).

The micronuclei assay does not measure oxidative stress directly, but measures chromosomal damage formed during replication that, among other factors, can be caused by oxidative stress on DNA. No significant correlations were found with the micronuclei assay.

The levels of DNA oxidation in this group of patients with type 2 diabetes were similar to those of a healthy population as measured with 8-oxodG, but elevated when measured with the comet assay (34, 35). This may be because the comet assay measures a broad range of oxidative DNA damage and is, in that sense, more sensitive than 8-oxodG, which only analyses one specific DNA damage. It could also be due to the high adventitious oxidation that is known to occur during sample preparation when analysing 8-oxodG with HPLC, which could explain the lack of difference between healthy subjects and patients. Since patients with type 2 diabetes have been reported to have high DNA oxidation (3) and lipid peroxidation (2, 13), and both these parameters had inverse relationships with antioxidants in diet and plasma, it is highly

Table 6. Dietary intake, corrected for energy intake, or plasma antioxidant levels correlated with oxidative stress or inflammation (CRP <10)

	Oxidative stress or inflammation	r	p
Food variable/MJ	*Oxidative stress*		
Fruit and vegetables	8-OxodG/10^6 dG	−0.0940	0.5116
Fruit and vegetables	Comet assay, FPG sites (% tail)	−0.2724	0.0485*
Fruit and vegetables	Malondialdehyde (mmol l^{-1})	0.2240	0.1104
Fruit and vegetables	8Table Iso-PGF$_{2\alpha}$ (nmol mmol^{-1} creatinine)	−0.2774	0.0443*
Vitamin C	8-Iso-PGF$_{2\alpha}$ (nmol mmol^{-1} creatinine)	−0.3318	0.0152*
Plasma antioxidants	*Oxidative stress*		
γ-Tocopherol	Malondialdehyde (mmol l^{-1})	−0.2869	0.0373*
Ascorbate	8-oxodG/10^6 dG	−0.2905	0.0367*
Plasma antioxidants	*Inflammation (CRP <10)*		
α-Carotene	IL-6	−0.4101	0.0025**
β-Carotene	IL-6	−0.3614	0.0085**
Lutein	IL-6	−0.2960	0.0331*
Lycopene	IL-6	−0.2795	0.0448*
α-Tocopherol	IL-6	−0.1344	0.3421
γ-Tocopherol	IL-6	−0.1916	0.1736
γ-Tocopherol	CRP <10 (mg l^{-1})	−0.3145	0.0232*

8-OxodG: 8-oxo-7,8-dihydroguanine; FPG: formamidopyrimidine glycosylase; 8-iso-PGF$_{2\alpha}$: 8-iso-prostaglandin-F$_{2\alpha}$; CRP: C-reactive protein; IL-6: interleukin-6.
Significant differences: **p <0.01, *p <0.05; ns: not significant (Spearman rank correlation coefficient with Bonferroni–Holm correction).

likely to be important for this patient group to have a diet rich in fruit and vegetables.

Both IL-6 and CRP are common biomarkers for inflammation. An increased level of CRP, even within the normal range, is considered a risk factor for cardiovascular disease and for the development of atherosclerosis, and a high fruit and vegetable intake has been associated with low-grade inflammation (36). In the present study, IL-6 was negatively correlated with all carotenoids in plasma, which indicates that these antioxidants can have a positive effect on the inflammatory process. In a previous study, however, long-term supplementation with α-tocopherol and ascorbate showed no anti-inflammatory effects in healthy men (37). Based on the findings in this study it seems to be the carotenoids in fruit and vegetables that are associated with anti-inflammatory effects, since IL-6 was negatively correlated with all carotenoids in plasma, whereas there was no association with the other antioxidants analysed. The same association was seen in healthy subjects, where CRP was negatively associated with carotenoid-rich fruit and vegetables (38), while in another study vitamin C and fruit intake showed similar associations with CRP (39).

In contrast to the other antioxidants, plasma γ-tocopherol had a positive correlation with inflammation (CRP). A high intake of α-tocopherol is thought to decrease the plasma level of γ-tocopherol, owing to competition between α- and γ-tocopherol for the tocopherol-binding protein (10). The fact that γ-tocopherol correlated, negatively with lipid peroxidation (MDA) and fruit and vegetable intake, and positively with inflammation (CRP), may therefore be related to this antagonistic effect of α-tocopherol.

Many of the dietary antioxidants were significantly related to the intake of fruit and vegetables (not shown), which was expected since they are present in fruit and vegetables. In take of foods rich in dietary antioxidants, mainly fruit and vegetables, should be encouraged in patients with type 2 diabetes (40). The present study group had an intake of fruit and vegetables that followed the dietary recommendations for the Nordic population (30).

In conclusion, high intakes of fruit and vegetables in patients with type 2 diabetes may decrease oxidative stress and inflammation. Oxidative stress seems to be related mainly to ascorbate, while inflammation may be particularly affected by carotenoids. Plasma values of α-carotene and β-carotene were strongly correlated with fruit and vegetable intake, and can therefore be considered good biomarkers for the intake of these foods.

References

1. Mercuri F, Quagliaro L, Ceriello A. Oxidative stress evaluation in diabetes. Diabetes Technol Ther 2000; 2: 589–600.
2. Colak E, Majkic-Singh N, Stankovic S, Sreckovic-Dimitrijevic V, Djordjevic PB, Lalic K, Lalic N. Parameters of antioxidative defense in type 2 diabetic patients with cardiovascular complications. Ann Med 2005; 37: 613–20.
3. Maxwell SR, Thomason H, Sandler D, Leguen C, Baxter MA, Barnett AH, et al. Antioxidant status in patients with uncomplicated insulin-dependent and non-insulin-dependent diabetes mellitus. Eur J Clin Invest 1997; 27: 484–90.
4. Ruhe RC, McDonald RB. Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. J Am Coll Nutr 2001;20:363–9; Discussion 381–3.
5. Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991; 40: 405–12.
6. Ceriello A. Acute hyperglycaemia and oxidative stress generation. Diabet Med 1997; 14: 45–9.
7. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition 2001; 17: 888–95.
8. Paolisso G, Giugliano D. Oxidative stress and insulin action: is there a relationship? Diabetologia 1996; 39: 357–63.
9. Browning LM, Jebb SA. Nutritional influences on inflammation and type 2 diabetes risk. Diabetes Technol Ther 2006; 8: 45–54.
10. Azzi A, Stocker A. Vitamin E: non-antioxidant roles. Prog Lipid Res 2000; 39: 231–55.
11. Montonen J, Knekt P, Jarvinen R, Reunanen A. Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 2004; 27: 362–6.
12. Ylönen K, Alfthan G, Groop L, Saloranta C, Aro A, Virtanen SM. Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. Am J Clin Nutr 2003; 77: 1434–41.
13. Helmersson J, Vessby B, Larsson A, Basu S. Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative stress in an elderly population. Circulation 2004; 109: 1729–34.
14. Helmersson J, Basu S. F2-isoprostane excretion rate and diurnal variation in human urine. Prostaglandins Leukot Essent Fatty Acids 1999; 61: 203–5.
15. Richelle M, Turini ME, Guidoux R, Tavazzi I, Metairon S, Fay LB. Urinary isoprostane excretion is not confounded by the lipid content of the diet. FEBS Lett 1999; 459: 259–62.
16. Gopaul NK, Halliwell B, Anggard EE. Measurement of plasma F_2-isoprostanes as an index of lipid peroxidation does not appear to be confounded by diet. Free Radic Res 2000; 33: 115–27.
17. Block G, Dietrich M, Norkus E, Jensen C, Benowitz NL, Packer L, et al. Intraindividual variability of plasma antioxidants, markers of oxidative stress, C-reactive protein, cotinine, and other biomarkers. Epidemiology 2006; 17: 404–12.
18. Becker W, Lennernas M, Gustafsson I-B, Haraldsdottir J, Nydahl M, Vessby B, Ytterfors A. Precoded food records compared with weighed food records for measuring dietary habits in a population of Swedish adults. Scand J Nutr 1998; 42: 145–9.
19. Öhrvall M, Tengblad S, Vessby B. Lower tocopherol serum levels in subjects with abdominal adiposity. J Intern Med 1993; 234: 53–60.
20. Thurnham DI, Davies JA, Crump BJ, Situnayake RD, Davis M. The use of different lipids to express serum tocopherol:lipid ratios for the measurement of vitamin E status. Ann Clin Biochem 1986; 23: 514–20.
21. Vuilleumier JP, Keck E. Fluorometric assay of vitamin C in biological materials using a centrifugal analyser with fluorescence attachment. J Micronutr Anal 1989; 5: 25–34.
22. ESCODD; Gedik CM, Collins A. Establishing the background level of base oxidation in human lymphocyte DNA: results of an interlaboratory validation study. FASEB J 2005;19:82–4.
23. Åsgård R, Hedman M, Sjöström M, Möller L. Long term reduction of DNA oxidation after a life style intervention. 2007; Submitted.
24. Nagy E, Johansson C, Zeisig M, Moller L. Oxidative stress and DNA damage caused by the urban air pollutant 3-NBA and its isomer 2-NBA in human lung cells analyzed with three independent methods. J Chromatogr B Analyt Technol Biomed Life Sci 2005; 827: 94–103.
25. Öhrvall M, Tengblad S, Ekstrand B, Siegbahn A, Vessby B. Malondialdehyde concentration in plasma is inversely correlated to the proportion of linoleic acid in serum lipoprotein lipids. Atherosclerosis 1994; 108: 103–10.
26. Basu S. Radioimmunoassay of 8-iso-prostaglandin $F_{2\alpha}$: an index for oxidative injury via free radical catalysed lipid peroxidation. Prostaglandins Leukot Essent Fatty Acids 1998; 58: 319–25.
27. Abramsson-Zetterberg L, Durling LJ, Yang-Wallentin F, Rytter E, Vessby B. The impact of folate status and folic acid supplementation on the micronucleus

frequency in human erythrocytes. Mutat Res 2006; 603: 33–40.

28. Basu S. Radioimmunoassay of 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$: an index for inflammation via cyclooxygenase catalysed lipid peroxidation. Prostaglandins Leukot Essent Fatty Acids 1998; 58: 347–52.

29. Holm S. A simple sequentially rejective multiple test procedure. Scand J Statist 1979; 6: 65–70.

30. NORD. Nordic Council of Ministers C. Nordic Nutrition Recommendations 2004. Integrating nutrition and physical activity. 4th edn. NORD 2004;13.

31. Al-Delaimy WK, Ferrari P, Slimani N, Pala V, Johansson I, Riboli E, et al. Plasma carotenoids as biomarkers of intake of fruits and vegetables: individual-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). Eur J Clin Nutr 2005; 59: 1387–96.

32. Heseker H, Schneider R. Requirement and supply of vitamin C, E and beta-carotene for elderly men and women. Eur J Clin Nutr 1994; 48: 118–27.

33. Bates CJ, Prentice A, Cole TJ, van der Pols JC, Doyle W, Finch S, et al. Micronutrients: highlights and research challenges from the 1994–5 National Diet and Nutrition Survey of people aged 65 years and over. Br J Nutr 1999; 82: 7–15.

34. ESCODD. Comparative analysis of baseline 8-oxo-7,8-dihydroguanine in mammalian cell DNA, by different methods in different laboratories: an approach to consensus. Carcinogenesis 2002;23:2129–33.

35. Hofer T, Karlsson H, Möller L. DNA oxidative damage and strand breaks in young healthy individuals: a gender difference and the role of life style factors. Free Radic Res 2006; 40: 707–14.

36. Gao X, Bermudez OI, Tucker KL. Plasma C-reactive protein and homocysteine concentrations are related to frequent fruit and vegetable intake in Hispanic and non-Hispanic white elders. J Nutr 2004; 134: 913–8.

37. Bruunsgaard H, Poulsen HE, Pedersen BK, Nyyssonen K, Kaikkonen J, Salonen JT. Long-term combined supplementations with alpha-tocopherol and vitamin C have no detectable anti-inflammatory effects in healthy men. J Nutr 2003; 133: 1170–3.

38. Watzl, Bernard, Kulling SE, Möseneder J, Barth SW, Achim B. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. Am J Clin Nutr 2005;82:1052–8.

39. Wannamethee SG, Lowe GDO, Rumley A, Bruckdorfer KR, Whincup PH. Associations of vitamin C status, fruit and vegetable intakes, and markers of inflammation and hemostasis. Am J Clin Nutr 2006; 83: 567–74.

40. Mann JI, De Leeuw I, Hermansen K, Karamanos B, Karlström B, Vessby B. Evidence-based nutritional approaches to the treatment and prevention of diabetes mellitus. Nutr Metab Cardiovasc Dis 2004; 14: 373–94.

Lennart Möller
Karolinska Institutet
Department of Biosciences and Nutrition
SE-141 57 Huddinge
Sweden
Tel: +46 8 608 91 89
Fax: +46 8 774 68 33
E-mail: lennart.moller@biosci.ki.se