Eur J Nutr (2010) 49:409–416
DOI 10.1007/s00394-010-0099-6

ORIGINAL CONTRIBUTION

Effects of antioxidants on postprandial oxidative stress and endothelial dysfunction in subjects with impaired glucose tolerance and Type 2 diabetes

S. Neri · S. Calvagno · B. Mauceri · M. Misseri · A. Tsami · C. Vecchio · G. Mastrosimone · A. Di Pino · D. Maiorca · A. Judica · G. Romano · A. Rizzotto · S. S. Signorelli

Received: 11 November 2009 / Accepted: 12 February 2010 / Published online: 7 March 2010

Abstract
Aim To compare changes in the oxidation–reduction balance and endothelial function before and after meal in patients with type 2 diabetes or impaired glucose tolerance and determine the effects of standard antioxidant supplementation.
Methods Forty diabetics and 40 subjects with impaired glucose tolerance were compared with a control group. We assessed before and after a test meal (homogenized milk-shake containing 80 g of saturated fat, amounting to 1,480 kcal), some reactive oxygen species, inflammation markers and flow-mediated vascular dilatation. These parameters were then reassessed after standard antioxidant treatment.
Results After the meal, diabetics, subjects with impaired glucose tolerance and controls had higher levels of oxidant compounds compared to fasting levels. In subjects with diabetes and impaired glucose tolerance (IGT), Vascular Adhesion Molecule-1 and CRP were higher after the meal—diabetic subjects exhibited lower fasting flow-mediated dilatation, which deteriorated significantly after the meal. Antioxidant administration significantly improved the parameters investigated in all subjects.
Conclusions In diabetic subjects, altered glycaemia and lipaemia are closely correlated with markers of systemic oxidative stress. Our results show that the abnormal changes in oxidative-reductive balance parameters are paralleled by similar changes in markers of endothelial dysfunction and inflammation at 4 h after ingestion of a fatty meal. Supplementation with a pool of antioxidants can reduce oxidative stress and inflammation in healthy subjects and, more importantly, in IGT patients. This previous aspect suggests that the timing of antioxidant supplementation has an important role in endothelium protection in healthy and pre-diabetic subjects, and along with prompt antioxidant treatment before irreversible endothelial damage has occurred, may have an important protective role in subjects with IGT—patients who require administration of adequate dietary antioxidants.

Keywords Reactive oxygen species · Oxidative stress · Endothelial damage · Impaired glucose tolerance · Diabetes · Antioxidants

Electronic supplementary material The online version of this article (doi:10.1007/s00394-010-0099-6) contains supplementary material, which is available to authorized users.

S. Neri · S. Calvagno · B. Mauceri · G. Mastrosimone · A. Di Pino · D. Maiorca · A. Judica · G. Romano · S. S. Signorelli
Department of Internal Medicine and Systemic Disease, University of Catania, Catania, Italy

M. Misseri · A. Tsami · C. Vecchio
School of Internal Medicine, University of Catania, Catania, Italy

A. Rizzotto
School of Psychology, Kore University, Enna, Italy

S. Neri (✉)
Medicina Interna Policlinico Ed. 29, II Piano, Via S. Sofia, 86, 95123 Catania, Italy
e-mail: sergio.neri4@tin.it

Introduction

There is increasing evidence that suggests that elevated postprandial glucose levels are independent risk factors for cardiovascular morbidity and mortality. In fact, postprandial plasma glucose levels and glycaemic spikes are

strongly associated with risk of atherosclerosis and other cardiovascular diseases [1–5].

A significant relationship between postprandial lipaemia (PPL) and coronary artery disease has been reported in association with both normal and abnormal glycaemic levels [6]. During fasting, subjects with elevated triglycerides, in combination with normal levels of LDL cholesterol, exhibit endothelial dysfunction resulting in an 'atherogenic' lipid profile and endothelial dysfunction [7, 8].

The relationship between early endothelial damage, glycaemic control, lipid metabolism disorders and oxidative stress has been understood for quite a long time, however, precisely how these factors interact, and particularly the mechanism by which excess blood glucose leads to irreversible endothelial damage, remains unclear [8–18].

In order to compare the changes from fasting and postprandial levels of oxide-reductive balance, we assessed a series of circulating markers for antioxidants and endothelial function before and after a meal in three types of subjects: those with impaired glucose tolerance (IGT), patients with early-stage, untreated type 2 diabetes, and healthy controls.

Our aim was that of verifying a possible endothelial protective role of antioxidants in subjects with IGT, as well as in early-stage diabetics.

Patients and methods

The study cohort (see flowchart) was made up of the following groups:

Forty subjects (20 men and 20 women; mean age 41 ± 4 years; mean body mass index (BMI) 24 ± 3 kg/m^2) with IGT (defined as fasting plasma glucose (FPG) between 110 and 126 mg/dl and plasma glucose levels between 140 and 200 mg/dl at hour 2 after oral glucose challenge (oral glucose tolerance test (OGTT)) [10, 11], subjects were recruited from a cohort of 184 outpatients with IGT (Group IGT) and selected from a computer-generated list in a 1:1 gender-equal ratio using statistical software (SAS, version 6.12, SAS Institute Inc., Cary, NC, USA).

Forty subjects (20 men and 20 women; mean age 43 ± 3 years; mean BMI 23 ± 2 kg/m^2) with type 2 diabetes mellitus controlled by diet alone (FPG ≥ 126 mg/dl and OGTT at hour 2 ≥ 200 mg/dl), who were selected from a cohort of 258 outpatients with diabetes (Group D).

Forty healthy control volunteers from among the hospital staff (20 men and 20 women; mean age 40 ± 2 years; mean BMI 23 ± 1 kg/m^2), with normal values of FPG and OGTT (Group C).

The characteristics of all subjects at enrolment are shown in Table 1.

Smokers, alcohol abusers (alcohol consumption > 20 g/day), and subjects with microalbuminuria, elevated cholesterol (>6.5 mmol/l), obesity (BMI > 30.1 kg/m^2), chronic kidney diseases (creatinine > 130 μmol/L or creatinine clearance < 1.3 mL/s), liver and lung diseases, or chronic or acute inflammatory diseases, along with patients on chronic drug or radiation therapy were not included in the study. All study subjects were instructed to avoid practicing sports, smoking or taking medications, caffeine, vitamins or antioxidant preparations throughout the week prior to the study. All women were fertile and studied during the follicular phase of their menstrual cycles. The following tests were performed for all patients [2, 19–22]:

Table 1 Characteristics of the study subjects (mean values ± SD)

	Diabetic patients ($n = 40$)	IGT ($n = 40$)	Controls ($n = 40$)
Sex (M + F)	23 + 23	23 + 23	23 + 23
Age (years)	43 ± 3	41 ± 4	41 ± 2
BMI (kg/m^2)	24 ± 2	23 ± 3	23 ± 1
Systolic BP (mmHg; mean 24 h)	131.3 ± 0.5	130.2 ± 0.5	128.2 ± 0.5
Diastolic BP (mmHg; mean 24 h)	76.8 ± 0.2	74.5 ± 0.3	74.3 ± 0.2
Serum creatinine (mg/L)	91 ± 1.6	91 ± 1.8	90 ± 1.5
Fasting cholesterol (mmol/L)	6.1 ± 0.5	5.7 ± 0.6	5.7 ± 0.4
Glycosylated haemoglobin (%)	6.9 ± 0.3	5.2 ± 0.2	4.6 ± 0.2
Intima-media thickness (mm)	1.26 ± 0.02	1.25 ± 0.02	1.22 ± 0.03
Fasting insulin (μgr/mol)	24.5 ± 6.3	22.4 ± 5.04	14.7 ± 7.3

There were no significant differences among the three groups based on Kruskal–Wallis test ($p > 0.05$)

IGT impaired glucose tolerance

1) Electrocardiogram (ECG) according to World Health Organization (WHO);
2) Blood pressure (BP) measurement (from 24-h Holter monitoring) to exclude those with hypertension;
3) Fundoscopy in order exclude subjects with micro-aneurysms, haemorrhage, lipid exudates, cotton wool spots and venous changes;
4) Ultrasound imaging of the proximal and distal walls of the internal carotid artery, common carotid and carotid bifurcation by ultrasound B-mode imaging (Acuson Apogèe CX800 scanner; ATL Industries, USA) with a high-resolution (7.5 MHz linear electronic probe) to exclude subjects with abnormal intimal-medial thickness measurement [21, 22].

All examinations were performed by expert cardiologists and ultrasonographers, using standard acquisition and imaging reading protocols to interpret the ECG, BP and ultrasound imaging data obtained. Levels of glycated haemoglobin (HbA_{1c}) were determined by cation-exchange chromatography and used to monitor glycaemic control in diabetics and subjects with IGT.

Venous and/or arterial blood samples were drawn from each subject before the meal, in order to determine levels of serum LDL, HDL and total cholesterol, triglycerides (TG), lipoprotein subfractionation, HbA_{1c}, glucose, antioxidant status, and markers of inflammation and endothelial function. Samples were drawn via venous and arterial lines, which remained in place until sampling was completed. Analyses were performed immediately after the blood was drawn; baseline brachial artery endothelial function was also measured.

These assessments were then repeated at 2, 4 (during the peak of lipaemic phase) and 8 h after the meal, when abnormalities of lipaemic profile would be expected to be present only in subjects with diabetes. All measurements were repeated again after 8 days of vitamin supplementation.

Each healthy patient took in 1,800 to 2,000 kcal/day, while IGT subjects and diabetics received 1,200–1,800 kcal/day, depending on each individual's weight and caloric requirement for daily activities. This calorie-controlled diet was maintained throughout the study until 10 days prior to the start of the study, when all study subjects were instructed to adhere to a controlled, well-balanced diet with 55% carbohydrate, 0.8 g/kg/day of protein, 20% lipid with <7% saturated fat, 100 mg of cholesterol, 15 g of fibre, avoiding nitrates whenever possible). At 9:00 a.m. (after a 12-h overnight fast) on the day of the tests, each subject was given a standard meal consisting of a homogenised milkshake containing 80 g of saturated fat, amounting to 1,480 kcal. Subjects were then instructed to take a dietary antioxidant supplement consisting of 600 mg/day of NAC (a thiol-containing antioxidant capable of blocking nuclear factor kB activation and which reacts with some oxidants), 50 mg/day of vitamins E (a major membrane-bound antioxidant) and 500 mg/day of C (a major aqueous-phase antioxidant, able to restore oxidised vitamin E).

From enrolment in the study up to conclusion, patients were followed by a psychologist and a dietician, both of whom assessed compliance with the study restrictions, diet and antioxidant therapy. Participants also completed a self-rated questionnaire on compliance and food consumption during a well-defined 24-h period before the study started.

The study was approved by the Ethics Committee of Catania University, and the entire cohort gave informed consent at the time of study enrolment.

Chylomicron-free plasma was analysed to assess the compositional variation in the three major lipoprotein subclasses during postprandial lipaemia (PPL) [23].

Baseline measurements of internal brachial artery diameter, blood flow and changes in brachial artery diameter in response to reactive hyperaemia (flow-mediated dilatation) (FMD) were measured using a high-resolution ultrasonic wall-tracking system (Valdirec Wall-track System, resolution ± 3 μm) [24]. Repeat measurements of brachial artery diameter and blood flow were taken 3 min after sublingual glyceryl trinitrate (GTN; 400 μg). Flow-mediated and GTN-mediated vasodilatation data are presented as the percentage of diameter change from baseline in the brachial artery at fasting and 4 and 8 h after a meal.

The capacity of the endothelium to auto-induce nitric oxide (NO)-dependent vasodilatation [25, 26] was assessed by drawing arterial blood samples from the radial artery 1 h before and 2 h after the meal, thus determining the amount of nitrite and nitrate present as final products of nitric oxide metabolism, through centrifugal ultra-filtration and capillary electrophoresis (Cayman's Nitrite/Nitrate Assay Kit, Cayman Chemical Company, USA; Millipore, Bedford MA, USA) [27].

Plasma malondialdehyde (MDA) concentration was measured using a Jasco (Japan) HPLC preconditioned C_{18} column (washed with water and acetonitrile 85:15 after incubation with thiobarbituric acid) [28]. A spectrocolorimetric assay (LPO-586 method Bioxytech) was used to measure 4-hydroxynonenal (4-HNE) concentration [29]. Erythrocyte glutathione peroxidase (GSH-Px) was measured by spectrophotometric assay (Hitachi model 200-20) [30], which assesses the magnitude of the antioxidant response to oxidative stress. As a marker of systemic oxidative stress, we also measured urinary levels of free 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$; F_2-isoprostanes) [31]. The content of free 8-iso-$PGF_{2\alpha}$ was determined by a validated radioimmunoassay with a specific antibody against free 8-iso-$PGF_{2\alpha}$. Urinary

content of 8-iso-$PGF_{2\alpha}$ is presented after adjustment for creatinine and expressed as nmol/mmol creatinine.

Serum C-reactive protein (CRP) is an acute-phase reactant. This was measured using an ultrasensitive colorimetric competitive enzyme-linked immunosorbent assay [32].

Serum vascular cell adhesion molecule-1 (VCAM-1) may represent an important biomarker for inflammatory processes involving activation or damage to cells. This was measured by ELISA (R&D System Europe, Abington, UK) after centrifugation of whole blood collected in 0.13 M sodium citrate [33].

Statistical analysis

Student's *t*-test and the Tukey test were performed to obtain *p* values. Values of $p < 0.05$ were considered significant. Results are presented as mean ± SD. Two-group comparisons were performed using Wilcoxon's test (paired) or Kruskal- Wallis test (unpaired). We compared the baseline data for each of the three groups with a 2-factor ANOVA. In following, we used a 2-factor nonparametric analysis of covariance after calculating the changes from baseline for each of the variables (ANCOVA)—the two factors were diet and diabetic status. Adjustment for covariates (age, gender, weight) was also performed using this method. As a measure of plasma glucose and the total amounts of lipid and lipoprotein present in plasma during the postprandial period, areas under the curve (AUC) were calculated for plasma concentrations without subtracting baseline values, using Simpson's integration rule. Statistical analysis of data was carried out using SPSS 11.5 (SPSS Production; Chicago, IL, USA).

Results

In all groups, blood samples drawn at 2, 4 and 8 h after the standardised meal revealed no significant postprandial changes in total cholesterol compared with pre-prandial determinations. Postprandial plasma glucose and triglyceride (Table 2) showed higher than baseline values in all groups; postprandial levels were significantly higher in diabetics than in the IGT group, and postprandial levels in the IGT group were higher than those in the controls. Moreover, there was significant TG enrichment of all proteins in IGT and D Groups, at 4 and 8 h after the meal—in healthy subjects (Group C), VLDL was enriched with TG at 2 and 4 h after the meal. The amount of HDL cholesterol at 4 h after the meal was significantly higher in the control group than in Groups IGT and D. In addition, the AUC for TG and cholesterol content of major lipoproteins in all groups at baseline was also similar at 4 h after the meal, while the AUC for postprandial lipaemia for plasma TG (mmol/L) was significantly higher in Group IGT (196 ± 104) when compared to Group D (184 ± 102) and controls (65.4 ± 11.6). Antioxidant supplementation did not alter any of the lipid variables.

Baseline brachial artery diameters (mm) were 3.49 ± 0.23, 3.41 ± 0.34 and 3.38 ± 0.42 in the control, IGT, and

Table 2 Plasma glucose, triglycerides and HDL serum levels after the standardized meal in three groups of patients

	IGT ($n = 40$)	Diabetic patients ($n = 40$)	Controls ($n = 40$)	Main effects of prandial state and diabetic status
Plasma glucose (mg/ml)				
Fasting	112 ± 29	163 ± 25	88 ± 3.6	PP > F*
Postprandial (at 2nd hour)	132 ± 5	278 ± 11	95 ± 6	D > IGT > C†
Plasma triglycerides (mmol/ml)				
Fasting	1.4 ± 0.3	2.3 ± 0.2	1.3 ± 0.2	PP > F*
Postprandial (at 4th hour)	4.3 ± 1.0	7.5 ± 2.1	1.98 ± 0.3	D > IGT > C†
Postprandial (at 8th hour)				
Plasma HDL (mmol/ml)				
Fasting	1.18 ± 0.3	1.07 ± 0.2	1.36 ± 0.2	PP > F*
Postprandial (at 4th hour)	1.16 ± 0.3	1.07 ± 0.2	1.37 ± 0.3	D < IGT < C†
Postprandial (at 8th hour)	1.25 ± 0.2	1.06 ± 0.3	1.36 ± 0.1	

All values are expressed as mean ± SD

IGT, impaired glucose tolerance; *D*, diabetic patients; *C*, controls; *PP*, postprandial levels; *F*, fasting levels

* Statistical significant difference between PP and F levels in each group: $p < 0.05$ (*Tukey* test)

† Statistical significant difference between D and IGT patients, and between IGT patients and C: $p < 0.05$ (*Tukey* test)

There was no significant interaction between diabetic status and prandial state (F vs. PP: $p > 0.05$)

diabetic groups, respectively. In response to a fatty meal there was a consistent significant decrease in FMD at 4 h at baseline in IGT as well as diabetic subjects. Furthermore, diabetic subjects showed lower fasting FMD (Table 3), which deteriorated significantly after the meal. On the other hand, in healthy controls, the reduction in FMD associated with PPL was inversely correlated with fasting HDL-C levels ($r = -0.84$, $p = 0.001$) and was not correlated with any other measured variable. When multiple regression analysis was performed to assess the associations between FMD and other measured variables, the change in FMD during PPL was found to be the dependent variable with respect to all measured factors. Changes in antioxidant status, the AUC for plasma TG and the AUC for plasma glucose were found to be significant determinants ($p = 0.002$) of the observed reduction in FMD. Using fasting FMD as the dependent variable, age, fasting TG, HbA_{1c} and fasting LDL-C levels ($p < 0.0001$) were the most significant determinants.

In the diabetic group, this post-meal reduction was most strongly correlated to the magnitude of postprandial hypertriglyceridaemia ($p < 0.001$), fasting plasma TG levels ($p < 0.05$) and TG enrichment of both VLDL ($p < 0.001$) and LDL ($p < 0.001$).

All groups exhibited a significant postprandial increase in oxidative stress, significantly correlated with the extent of VLDL-TG enrichment and the AUC for VLDL-TG content. Antioxidant supplementation restored oxidative stress in controls and significantly lowered it in the IGT group (Table 3). Fasting values of antioxidants and NO were significantly lower in diabetics than in IGT patients and were also lower in the IGT group than in controls.

There was no significant interaction between antioxidant supplementation, diabetic status and the prandial state (Table 3).

At baseline, CRP and VCAM-1 levels were comparable in the three groups (Table 3). After the meal there was a significant difference between baseline and postprandial levels, with higher values found after meal (Table 3) and in the diabetic group when compared to IGT and controls. At baseline F2-isoprostane levels were higher in diabetic subjects than IGT and healthy subjects – when compared to healthy subjects, IGT showed higher values. After the meal, these levels were higher in IGT and diabetic subjects. After antioxidant supplementation, healthy and IGT subjects presented comparable values before and after the meal—diabetic subjects presented significant higher values. In an analysis of results after antioxidant supplementation, F_2-isoprostane levels were positively correlated with intake of antioxidants in the healthy group ($r = 0.21$, $p = 0.007$, $n = 40$) and the IGT group ($r = 0.28$, $p = 0.001$, $n = 40$), however this was not the case in the diabetic group.

Discussion

Postprandial glucose and glycaemia spikes have been shown to be strongly associated with cardiovascular risk and atherosclerosis [1–4]. PPL occurs in the period during absorption when the capacity for triglyceride metabolism is exceeded. It is characterised by increased plasma levels of TG. This results in TG-enriched very low-density lipoprotein (VLDL), a propensity for small, dense LDL and low HDL levels. Endothelial function is diminished after a fatty meal in healthy subjects, independent of total or LDL cholesterol levels. Remnant lipoprotein particles are associated with impaired endothelium-dependent coronary vasodilatation [34, 35]. Furthermore, remnant lipoproteins also induce pro-atherothrombogenic changes in endothelial cells both by increases in adhesion molecule and tissue factor expression and by a redox-sensitive mechanism (blunted by HDL-C).

In this study, we have highlighted an increase in the levels of reactive species of oxygen and a reduction in antioxidant GPx and NO availability during the postprandial phase—this situation can lead to endothelial dysfunction through a number of mechanisms [13–15, 37]. Moreover, we have observed that baseline NO concentrations are significantly lower in IGT and diabetic patients when compared to the control group. We believe that this indicates reduced endothelial capacity to regulate vascular tone.

We have also noted that antioxidant supplementation is able to improve endothelial function only in healthy and IGT subjects—in IGT patients, the antioxidant effect seems to blunt, but not abolish, the deterioration of postprandial endothelial function. Depressed levels of antioxidants have also been found in diabetic patients with increased lipid peroxidation and hypertriglyceridaemia [13–18, 36, 37]. Following our treatment, postprandial endothelial function was most strongly correlated with a reduction in oxidative stress after antioxidant therapy. In the diabetic group, however, there was still a decrease in PP endothelial function despite antioxidant therapy, indicating that lipoprotein interactions with the endothelium continue to blunt some endothelial function. In compliance with previous data [24–26], the present study demonstrates fasting endothelial dysfunction in subjects with type 2 diabetes mellitus, which is correlated with glucose, plasma TG, and total and LDL cholesterol and inversely correlated with HDL-C. The deterioration in FMD in the diabetic group most strongly correlates with the TG enrichment of both VLDL and LDL. These observations suggest that in diabetics the atherogenic insult that occurs after a fatty meal may be caused by TG-rich VLDL and LDL and may involve endothelial dysfunction and oxidative stress.

Levels of sVCAM-1 and CRP were significantly higher in the IGT and diabetic groups when compared to healthy

Table 3 Fasting, fed and fed-changes from fasting mean serum levels of oxidant and anti-oxidant bio-markers, before and after diet supplementation

Variable	Pre supplementation			Post supplementation			Statistical difference
	Controls ($n = 40$)	IGT ($n = 40$)	Diabetes ($n = 40$)	Controls ($n = 40$)	IGT ($n = 40$)	Diabetes ($n = 40$)	
MDA (micro mol/dl)							
Fasting	0.89 ± 0.02	1.18 ± 0.001	1.30 ± 0.01	0.90 ± 0.03	0.95 ± 0.02	1.20 ± 0.01	Post < Pre; D > IGT > C*
Fed	0.93 ± 0.03	1.75 ± 0.04	1.91 ± 0.30	0.97 ± 0.05	1.25 ± 0.03	1.90 ± 0.41	Post < Pre; D > IGT > C†
Fed-change from fasting	+0.04 ± 0.02	+0.57 ± 0.04	+0.61 ± 0.36	+0.07 ± 0.04	+0.30 ± 0.03	+0.70 ± 0.47	
4-HNE (micro mol/l)							
Fasting	1.25 ± 0.14	2.01 ± 0.03	2.04 ± 0.02	1.21 ± 0.01	1.29 ± 0.02	2.05 ± 0.01	Post < Pre; D > IGT > C*
Fed	1.35 ± 0.14	2.78 ± 0.15	2.73 ± 0.16	1.28 ± 0.03	1.38 ± 0.05	2.58 ± 0.15	Post < Pre; D > IGT > C†
Fed-change from fasting	+0.10 ± 0.15	+0.77 ± 0.17	+0.69 ± 0.17	+0.07 ± 0.02	+0.09 ± 0.04	+0.53 ± 0.18	
LDL-Ox (nmol/ml)							
Fasting	5.47 ± 0.10	7.80 ± 0.03	12.11 ± 0.01	4.90 ± 0.10	6.20 ± 0.10	12.10 ± 0.01	Post < Pre; D > IGT > C*
Fed	6.21 ± 0.22	8.76 ± 0.14	12.92 ± 0.02	5.90 ± 0.28	6.80 ± 1.20	12.92 ± 0.07	Post < Pre; D > IGT > C†
Fed-change from fasting	+0.74 ± 0.24	+0.96 ± 0.14	+0.81 ± 0.02	+1.00 ± 0.29	+0.60 ± 1.10	+0.82 ± 0.04	
GSH-Px (IU/ml)							
Fasting	6.08 ± 0.01	5.06 ± 0.03	5.04 ± 0.01	6.10 ± 0.02	6.03 ± 0.01	5.07 ± 0.03	Post > Pre; D < IGT < C*
Fed	7.06 ± 0.05	5.74 ± 0.04	5.73 ± 0.04	7.04 ± 0.07	6.99 ± 0.06	5.76 ± 0.05	Post > Pre; D < IGT < C†
Fed-change from fasting	+0.98 ± 0.07	+0.68 ± 0.04	+0.69 ± 0.03	+0.94 ± 0.07	+0.96 ± 0.05	+0.69 ± 0.04	
NO (micro mol/dl)							
Fasting	30.00 ± 0.01	31.00 ± 0.20	20.00 ± 0.2	30.02 ± 0.01	30.04 ± 0.02	20.00 ± 0.10	Post > Pre; D < IGT < C*
Fed	30.04 ± 0.02	31.58 ± 0.25	20.50 ± 0.31	30.03 ± 0.20	30.07 ± 0.03	20.90 ± 0.12	Post > Pre; D < IGT < C†
Fed-change from fasting	+0.04 ± 0.02	+0.58 ± 0.25	+0.50 ± 0.32	+0.01 ± 0.20	+0.03 ± 0.02	+0.90 ± 0.11	
VCAM-1 (ng/ml)							
Fasting	688 ± 65.0	652 ± 65.0	684 ± 65.0	634 ± 55.0	672 ± 55.0	688 ± 55.0	Post < Pre; D > IGT > C*
Fed	798 ± 60.5	898 ± 61.1	988 ± 60.5	688 ± 65.0	702 ± 71.0	994 ± 71.5	Post < Pre; D > IGT > C†
Fed-change from fasting	+110 ± 60.5	+246 ± 61.0	+304 ± 60.5	+54 ± 65.0	+30 ± 70.0	+306 ± 72.0	
CRP (ng/ml)							
Fasting	1.48 ± 0.60	1.52 ± 0.40	1.55 ± 0.40	1.47 ± 0.50	1.56 ± 0.50	1.54 ± 0.30	Post < Pre; D > IGT > C*
Fed	2.98 ± 0.70	3.30 ± 0.50	3.56 ± 0.60	2.99 ± 0.40	3.14 ± 0.30	3.52 ± 0.40	Post > Pre; D > IGT > C†
Fed-change from fasting	+1.50 ± 0.50	+1.78 ± 0.40	+2.01 ± 0.50	+1.52 ± 0.60	+1.58 ± 0.40	+1.98 ± 0.20	
F2-IsoP (nmol/mmol/creatinine)							
Fasting	0.32 ± 0.20	0.50 ± 0.10	0.98 ± 0.20	0.32 ± 0.10	0.37 ± 0.10	0.96 ± 0.20	Post < Pre; D > IGT > C*
Fed	0.35 ± 0.10	0.61 ± 0.20	1.12 ± 0.10	0.32 ± 0.10	0.38 ± 0.20	1.02 ± 0.10	Post > Pre; D > IGT > C†
Fed-change from fasting	+0.03 ± 0.10	+0.11 ± 0.10	+0.14 ± 0.20	–	+0.01 ± 0.10	+0.06 ± 0.20	

Values are: mean ± standard deviation (SD)

MDS, malondialdehyde; *4-HNE*, 4-hydroxynone; *LDL-Ox*, oxidated low density lipoprotein; *GSH-Px*, erythrocyte glutathione peroxidase; *NO*, nitric oxide; *VCAM-1*, vascular adhesion molecule 1; *CRP*, C reactive protein; *Pre*, pre supplementation; *Post*, post supplementation; *IGT*, impaired glucose tolerance; *D*, diabetic patients; *C*, controls

*$p < 0.001$; † $p < 0.05$

subjects. Antioxidant supplementation induced a significant improvement in redox balance parameters, CRP and VCAM-1 in IGT and healthy subjects, but not in diabetic subjects. Monocytes and polymorphonuclear cells release more superoxide anions when exposed to plasma from hypertriglyceridaemic patients [41]. This is believed to alter the thrombogenic properties of the endothelium, favouring oxidation of circulating LDL, reducing NO bioavailability and, in diabetic patients, accelerating the formation of advanced glycation end products (AGEs). In our study, we have given an arbitrary combination of antioxidants with different mechanisms (clearly doses that were inferior to the toxic doses) to induce a synergistic effect leading to the reduction of oxidant levels [36–42]. We suggest that the timing of antioxidant treatment in relation to the duration of plasma glucose homeostasis is crucial. In other words, prompt antioxidant treatment before irreversible endothelial damage has occurred may have an important protective role in subjects with IGT—patients where it is particularly important to include adequate dietary antioxidants, such as those from fruits and vegetables.

In fact, our study highlights an improvement of antioxidant levels in IGT after antioxidant treatment. This suggests that antioxidants may further protect and attenuate endothelial dysfunction and atherogenesis in healthy subjects and particularly in IGT subjects. Moreover, this study demonstrates that, in diabetic patients, altered glycaemia and lipaemia are closely correlated with markers of systemic oxidative stress, findings that strongly agree with results from previous studies. Furthermore, our results show that the abnormal changes in oxidative-reductive balance parameters are paralleled by similar changes in markers of endothelial dysfunction and inflammation at 4 h after ingestion of a fatty meal.

In addition, we demonstrated that supplementation with a pool of antioxidants reduces oxidative stress and inflammation in healthy subjects and, more importantly, in IGT patients.

Limitations of the study

1) The study made use of the random sampling method, with extensive collection of similar anthropometric, clinical and dietary data. However, it is a cross-sectional study. Additionally, it is possible that the associations and significant differences observed were in part explained by other underlying risk factors not controlled for in this study, including lifestyle or socioeconomic factors.
2) We chose synergistic compounds with well-known antioxidant capacity, but by no means do we wish to suggest that this specific treatment should be the recommended antioxidant regimen in therapeutic trials.

Acknowledgments This study was partially supported by a grant (60%) from the Italian Ministry of University. There was no industrial financial support for the study; there are no real or potential conflicts of interest.

Conflict of interest statement None.

References

1. Lautt WW (2007) Postprandial insulin resistance as an early predictor of cardiovascular risk. Ther Clin Risk Manag 3:761–770
2. Ginsberg HN (2000) Insulin resistance and cardiovascular disease. J Clin Invest 106:453–458
3. Ceriello A, Colagiuri S (2008) International Diabetes Federation guideline for management of postmeal glucose: a review of recommendations. Diabet Med 25:1151–1156
4. The Diabetes Control and Complications Trial Research Group (1995) The relationship of glycemic exposure to the risk of development and progression of retinopathy in the diabetes control and complication trial. Diabetes 44:968–983
5. Bonora E, Muggeo M (2001) Postprandial blood glucose as a risk factor for cardiovascular disease in type 2 diabetes: the epidemiological evidence. Diabetologia 44:2107–2114
6. Anderson RA, Evans ML, Ellis GR, Graham J, Morris K, Jackson SM (2001) The relationships between post-prandial lipaemia, endothelial function and oxidative stress in healthy individuals and patients with type 2 diabetes. Atherosclerosis 154:475–483
7. Doi H, Kugiyama K, Oka H, Sugiyama S, Ogata N, Koide S et al. (2000) Remnant lipoproteins induce pro atherothrombogenic molecules in endothelial cells through a redox-sensitive mechanism. Circulation 102:670–676
8. Wright E Jr, Scism-Bacon JL, Glass LC (2006) Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. Int J Clin Pract 60:308–314
9. Ceriello A (2003) Coagulation activation in diabetes mellitus. The possible role of hyperglycaemia in the pathogenesis of diabetic complications. Diabetologia 46:9–16
10. Ceriello A, Esposito K, Picoli L, Ihnat LA, Thorpe JE, Testa R et al (2008) Oscillating glucose is more deleterious to endothelial function and oxidative stressthan mean glucose in normal and Type 2 diabetic patients. Diabetes 57:1349–1354
11. Wannmethee SG, Lowe GDO, Shager AG et al (2004) Insulin resistance, haemostatic and inflammatory markers and coronary heart disease risk factors in Type 2 diabetic men with and without coronary heart disease. Diabetologia 47:1557–15565
12. Evans JL, Goldfine ID, Maddux BA, Grodsky GM (2002) Oxidative stress and stress-activated signalling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev 23:599–622
13. Wen Y, Skidmore JC, Porter-Turner MM, Rea CA, Khokher MA, Singh B (2002) Relationship of glycation, antioxidant status and oxidative stress to vascular endothelial damage in diabetes. Diab Obes Metab 4:305–308
14. Hasainan B, Mooradian AD (2002) Antioxidant vitamins and their influence in diabetes mellitus. Curr Diab Rep 2:448–456
15. Lee DH, Falsom AR, Harnack L, Halliwell B, Jacobs DR Jr (2004) Does supplemental vitamin C increase cardiovascular disease risk in women with diabetes? Am J Clin Nutr 80:1194–2000

16. Upritchard JE, Sutherland WHF, Mann JI (2002) Effect of supplementation with tomato juice, vitamin E, and vitamin C on LDL oxidation and products of inflammatory activity in type 2 diabetes. Diabetes Care 23:733–738
17. Salonen JT, Nyyssonenk K, Salonen R, Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study (2000) A randomised study of the effects of vitamin E and C on 3-years progression of carotid atherosclerosis. J Int Med 284:377–386
18. Neri S, Signorelli SS, Torrisi B, Pulvirenti D, Mauceri B, Abate G et al (2005) Effects of antioxidant supplementation on postprandial oxidative stress and endothelial dysfunction: a single blind 15-day clinical trial in patient with untreated type 2 diabetes, subjects with impaired glucose tolerance, and healthy controls. Clin Ther 27:1764–1773
19. World Health Organization Study Group (1985) Diabetes mellitus. Technical report. Series 727. Geneva, WHO, pp 1–113
20. Rose GA, Blackburn H (1968) Cardiovascular survey methods. WHO, Geneva
21. Salonen R, Haapanen A, Salonen JT (1991) Measurement of intima-media thickness of the common carotid artery with high resolution B-mode ultrasonography: inter and intra-observer variability. Ultrasound Med Biol 17:225–230
22. Agwall S, Fagenberg B, Wendelberg I, Urbanavicin V, Vilkstrand J (1995) Carotid artery wall intima-media thickness associated with insulin-mediated glucose disposal in men at high and low coronary risk. Stroke 26:956–960
23. Graham JM, Higgins JA, Gillott T, Taylor T, Wilkinson J, Ford T et al (1996) A novel method for the rapid separation of lipoproteins using self generating gradients of iodixanol. Atherosclerosis 124:124–135
24. Ramsey M, Goodfellow J, Jones C, Luddington L, Lewis M, Henderson AH (1995) Endothelial control of arterial distensibility is impaired in chronic heart failure. Circulation 92:3212–3219
25. Inoue T, Sanibadi AR, Matsunaga R, Hosgi K, Yagushi I, Morooka S (1998) Impaired endothelium-dependent acetylcholine-induced coronary artery relaxation in patients with high serum remnant lipoprotein particles. Atherosclerosis 139:363–367
26. Plotnick GD, Correti MC, Vogel RA (1997) Effect of antioxidant vitamins on the transient impairment of endothelium-dependent brachial artery vasoactivity following a single high-fat meal. J Am Med Assoc 278:1682–1686
27. Ueda T, Maekawa T, Sadamitsu D, Oshita S, Ogino K, Nakamura K (1995) The determination of nitrite and nitrate in human blood plasma by capillary zone electrophoresis. Electrophoresis 16:1002–1004
28. Yagi K (1982) Assay for serum lipid peroxide level and its chemical significance. In: Yagi K (ed) Lipid peroxides in biology and medicine. Academic Press, New York, pp 223–242
29. Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynonenal. Methods Enzymol 18:407
30. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization on erythrocyte glutathione peroxidase. J Lab Clin Med 70:158
31. Basu S (2004) Isoprostanes: novel bioactive products of lipid peroxidation. Free Radic Res 38:105–122
32. Macy E, Hayes T, Tracy R (1997) Variability in the measurement of C-reactive protein in healthy subjects: implication for reference interval and epidemiological applications. Clin Chem 43:52–60
33. Fiotti N, Giansante C, Ponte E, Del Bello C, Calabrese S, Zacchi T et al (1999) Atherosclerosis and inflammation. Patterns of cytokine regulation in patients with peripheral arterial disease. Atherosclerosis 145:51–60
34. Grieve DJ, Avella MA, Elliot J, Botham KM (1998) The influence of chylomicron remnants on endothelial function in the isolated perfused rat aorta. Atherosclerosis 139:273–281
35. Vogel RA, Corretti MC, Plotnik GD (1997) Effect of a single high-fat meal. J Am Med Assoc 278:1682–1686
36. Whiteside CI (2005) Cellular mechanism and treatment of diabetes vascular complications converge on reactive oxygen species. Curr Hypertens Resp 5:148–154
37. Huang HY, Appel LJ, Croft KD, Miller ER 3rd, Mori TA, Puddey IB (2002) Effects of vitamin C and vitamin E on in vivo lipid peroxidation: results of a randomized controlled trial. Am J Clin Nutr 76(3):549–555
38. Sampson MJ, Davies IR, Brown JC, Ivory K, Hughes DA (2002) Monocyte and neutrophil adhesion molecule expression in type 2 diabetes and control patients. Arter Thromb Vasc 22:1187–1193
39. Ceriello A (2000) The post prandial state and cardiovascular disease: relevance to diabetes mellitus. Diabetes Metab Res Rev 16:125–132
40. Halliwell B (1995) How characterize an antioxidant: an update. In: Rice-Evans C, Halliwell B, Lunt GG (eds) Free radicals and oxidative stress: environment, drugs and food additives. Portland Press, London, pp 73–102
41. Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. Nature 414:813–820
42. Esposito K, Maiorino MI, Ciotola M, Di Palo C, Scognamiglio P, Gicchino M et al (2009) Effects of a Mediterranean-style diet on the need for antihyperglycemic drug therapy in patients with newly diagnosed type 2 diabetes: a randomized trial. Ann Intern Med 151:306