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Short Communication

Glycaemic status in relation to oxidative stress and inflammation in well-controlled type 2 diabetes subjects

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The aim of the present observational study was to investigate the relationships between glycaemic status and levels of oxidative stress and inflammation in well-controlled type 2 diabetes subjects. Metabolic variables (weight, BMI, waist circumference (waist), blood glucose, glycated Hb (HbA1c), insulin, blood lipids), biomarkers of oxidative stress (8-iso-PGF2α, malondialdehyde, 8-oxo-7,8-dihydro-2’-deoxyguanosine, formamido pyrimidine glycosylase-sites, frequency of micronucleated erythrocytes, nitrotyrosine) and inflammatory markers (high sensitivity C-reactive protein (hsCRP), IL-6, cyclo-oxygenase-catalyzed PGF2α-metabolite) were measured. Fifty-six patients (thirty women and twenty-six men, age 62·3 (SD 7·0) years, HbA1c 6·1 (SD 0·9) %, BMI 28·3 (SD 3·8) kg/m 2, waist 99·6 (SD 11·1) cm) were included in the study. HbA1c (r 0·29, P = 0·03) and blood glucose (r 0·33, P = 0·01) correlated positively with 8-iso-PGF2α. Positive correlations were also observed between HbA1c and nitrotyrosine (r 0·42, P = 0·01), waist and hsCRP (r 0·37, P = 0·005), hsCRP and IL-6 (r 0·61, P < 0·0001) and between PGF2α-metabolite and 8-iso-PGF2α (r 0·27, P = 0·048). The present study indicates that glycaemic status is associated with oxidative stress even in subjects with well-controlled type 2 diabetes. Furthermore, inflammation was more related to abdominal obesity than to glycaemic control. A large number of biomarkers of oxidative stress and inflammation were investigated, but only a few associations were found between the markers. This could be due to the fact that none of these biomarkers biosynthesises via similar pathways or simultaneously owing to their diverse nature and origin.

Glycaemic control: Oxidative stress: Inflammation: Diabetes mellitus type 2

Diabetes is a disorder associated with an increased risk of developing vascular and other health complications. Oxidative stress and inflammation are the major pathogenetic mechanisms considered to be implicated in these complications(1,2). Subjects with type 2 diabetes have been shown to have increased levels of lipid peroxidation, oxidative damage to DNA and protein oxidation(1), presumably caused by an overproduction of free radicals and a decreased antioxidative defence. Enhanced production of free radicals is related to hyperglycaemia, insulin resistance and hyperinsulinaemia(3). High levels of glucose lead to an increased production of free radicals via different mechanisms such as glucose auto-oxidation and formation of advanced glycation end products(4).

Besides oxidative stress, inflammation is also implicated in the development of complications in type 2 diabetes(2). Cyclo-oxygenase catalyzed PG formation, and subsequently low-grade inflammation is suggested to be an early event in the development of type 2 diabetes that is further linked to oxidative stress(5). Elevated levels of high sensitivity C-reactive protein (hsCRP) and IL-6 are seen in subjects with type 2 diabetes(6), and are also associated with an increased risk for developing the disease in future(7). However, whether inflammation and oxidative stress are related to glycaemic control is still not fully clarified.

The aim of the present observational study was to investigate the relationships between glycaemic control and levels of oxidative stress and inflammation in subjects with well-controlled type 2 diabetes.

Experimental methods

Subjects and study design

Participants were recruited to take part in an intervention study with antioxidant supplementation. Baseline results are described in the present article and results from the intervention study are available from the authors on request.

Abbreviations: fMN-Trf-Ret, frequency of micronucleus transferring-positive reticulocytes; FPG, formamido pyrimidine glycosylase; HbA1c, glycated Hb; hsCRP, high sensitivity C-reactive protein; MDA, malondialdehyde; 8-oxoG, 8-oxo-7,8-dihydro-2’-deoxyguanosine.

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presented elsewhere. Inclusion criteria were age 40–75 years, type 2 diabetes treated with either diet or diet and oral hypoglycaemic medication, glycated Hb (HbA1c) < 10% and BMI < 35 kg/m². Exclusion criteria were insulin-dependent diabetes, known CVD, acute inflammatory, liver, kidney or thyroid diseases as well as medication or supplementation that could affect oxidative or inflammatory status. Subjects gave their written consent to participate in the study. The study was approved by the Ethical Committee of the Medical Faculty at Uppsala University, Sweden.

Blood and urine sample were drawn in the morning after an overnight fast. Body height, weight, waist circumference (waist) and blood pressure were recorded at the same time.

**Laboratory analysis**

Blood glucose concentration was analyzed by an enzymatic technique (HemoCue). HbA1c was analyzed with high performance liquid chromatography. Plasma insulin was assayed with an enzymatic immunological assay (Mercodia, Uppsala, Sweden) in a Konelab 20 Clinical Chemistry Analyzer (Thermo Electron Corporation, Vantaa, Finland) in a Konelab 20 Clinical Chemistry Analyzer (Thermo Electron Corporation). LDL-cholesterol was calculated according to Friedewald(8).

**Biomarkers of oxidative stress**

Comet assay and 8-oxo-7,8-dihydro-2′-deoxyguanosine. A high-alkaline formamido pyrimidine glycosylase (FPG) version of the comet assay(9) was used with some modifications. For the 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) analyses, DNA was isolated using a cold work-up procedure(10) with small modifications, followed by enzymatic hydrolysis(11). The amount of 8-oxodG per undamaged 2′-deoxyguanosine was analysed using on-line electrochemical and uv detection after separation of the nucleosides with HPLC.

**Frequency of micronucleated erythrocytes**

A flow cytometry-based micronucleus assay in very young erythrocytes from humans, transferrin-positive reticulocytes(12), was used. In this micronucleus assay, measuring the frequency of micronucleus-transferrin-positive reticulocytes (fMN-Trf-Ret), the very young erythrocytes were separated from the mature before analysis. The detection limit was approximately a difference of 0.2 of the background fMN-Trf-Ret. The background fMN-Trf-Ret was approximately 1‰.

**Malondialdehyde**

Plasma malondialdehyde (MDA) concentration was measured by HPCL and fluorescence detection as earlier described(13).

**F₂-isoprostanes**

Free 8-iso-PGF₂ₐₐ, a major F₂-isoprostane in urine, was analysed by a validated RIA developed by Basu(14). Levels were adjusted for urinary creatinine concentration.

**Nitrotyrosine**

Nitrotyrosine was assayed in plasma using a commercially available enzymatic immunological assay (Bioxxytech, OxisResearch, Portland, OR, USA).

**Biomarkers of inflammation**

High-sensitivity C-reactive protein. High-sensitivity C-reactive protein measurement was performed in plasma by a latex-enhanced reagent (Dade Behring, Deerfield, IL, USA) with the use of a Behring BN ProSpec analyzer (Dade Behring).

**Interleukin-6**

IL-6 was analysed in plasma by a high-sensitivity ELISA kit (IL-6 HS, R&D Systems, Minneapolis, MN, USA). Samples and standards were pipetted in a microtitre plate coated with monoclonal antibody against IL-6. After incubation and washing enzyme substrate solution was pipetted and followed by anti-IL-6 antibody. The colour reaction was proportional to the bound IL-6.

**Prostaglandin F₂α-metabolite**

Urinary 15-keto-dihydro-PGF₂α, a major metabolite of primary PGF₂α, was analysed, by a validated RIA developed by Basu(15). Levels were corrected for urinary creatinine concentration.

**Statistical analysis**

The statistical software JMP version 3.2 (SAS Institute, Cary, NC, USA) was used. Probability values < 0.05 were regarded as statistically significant. The unpaired t test or the Wilcoxon two-sample test was used to analyse sex differences. The correlation coefficients (Pearson’s or Spearman’s coefficients) were calculated when analysing correlations.

**Results**

**Clinical characteristic**

Fifty-six participants (thirty women and twenty-six men, forty-eight non-smokers and eight smokers) were included in the study. Significant differences between sexes were found for weight (men > women), cholesterol, HDL- and LDL-cholesterol (women > men), 8-iso-PGF₂α and nitrotyrosine (women > men) and IL-6 (men > women). Smokers had a higher IL-6 level compared with non-smokers and almost every biomarker for oxidative stress and inflammation tended to be higher in smokers. Twenty-one persons were treated with diet only, thirty-five with the addition of anti-diabetic medication (sulfonylureas or other insulin-stimulating compounds or/and biguanides). Baseline characteristics are presented as means and standard deviations; age 62.3 (SD 7.0)
years; BMI 28.3 (SD 3.8) kg/m²; weight 81.9 (SD 13.8) kg; 
waist 99.6 (SD 11.1) cm; systolic blood pressure 143 (SD 14) 
nmHg; diastolic blood pressure 78 (SD 9) mmHg; HbA1c 
6.1 (SD 0.9)%; fasting blood glucose 7.8 (SD 2.3) mmol/l; insulin 
69.3 (SD 40.2) pmol/l; TAG 17 (SD 9.7) mmol/l; LDL 
cholesterol 2.9 (SD 0.9) mmol/l; HDL-cholesterol 1.1 (SD 
0.2) mmol/l; cholesterol 4.7 (SD 1.0) mmol/l; 8-oxodG/10⁶ 
dG 0.96 (SD 0.45); FPG-sites 30.2 (SD 15.1) % tail; MN 
Trf-Ret 0.90 (SD 0.50) ‰; MDA 0.68 (SD 0.08) mol/mmol 
creatinine; nitrotyrosine 245 (SD 401) nmol/l; hsCRP 3.1 (SD 
2.2) ng/l; 15-keto-dihydro-PGF2α 0.24 (SD 0.10) mol/mmol 
creatinine.

Relationships between glycaemic control and indicators 
of oxidative stress and inflammation

Urinary 8-iso-PGF2α was positively correlated to fasting 
blood glucose (r 0.33, P=0.01) as well as HbA1c (r 0.29, 
P=0.03). In addition, there was a positive correlation between 
HbA1c and nitrotyrosine (r 0.43, P=0.01). A negative 
correlation was seen between HbA1c and MDA (r −0.32, 
P=0.017). Waist (r 0.37, P=0.005), BMI (r 0.32, P=0.016) 
and weight (r 0.36, P=0.006) were correlated positively with 
hsCRP. Correlations were found between 8-iso-PGF2α and 
MDA (r −0.33, P=0.012), hsCRP and IL-6 (r 0.61, 
P<0.0001) and 8-iso-PGF2α and 15-keto-dihydro-PGF2α 
(r 0.27, P=0.048).

Excluding smokers (n 8) from the correlation analyses 
decreased power of the study but did not change main results. 
No change in main results of correlation analyses was found 
when studying the subjects treated with diet only or treated 
with diet plus diabetic medication. An exception was a 
positive association between FPG-sites and blood glucose 
(r 0.43, P=0.012), hsCRP and IL-6 (r 0.43, P=0.012) as well as HbA1c 
(r 0.32, P=0.016). T o our knowledge, the present study is the first one investigating 
the fMN-Trf-Ret in subjects with type 2 diabetes. Healthy 
subjects have earlier been examined(22) with the same 
method and showed a similar level of frequency as in the pre-

Discussion

The study showed positive associations between glycaemic 
control (blood glucose and HbA1c) and urinary 8-iso-PGF2α 
and HbA1c and nitrotyrosine, demonstrating a significant biologi-
cal relationship between glycaemic control and oxidative 
stress. It was also found that abdominal obesity and low-
grade inflammation (hsCRP) were closely related to each 
other. Despite the inclusion of a considerable number of bio-
markers of oxidative stress and inflammation, only a few 
associations were found among these markers.

To the best of our knowledge, no other study investigating 
subjects with type 2 diabetes has reported so many biomarkers 
of oxidative stress and inflammation simultaneously. Totally 
six different biomarkers for oxidative stress and three for 
inflammation were measured in this patient group in order 
to clarify the relationships between glycaemic control, oxy-

Glycaemic control related to oxidative stress

The direct relationship between glycaemic control and oxy-

showed elsewhere. One report described a highly significant 
correlation between blood glucose and urinary 8-iso-PGF2α, 
and a reduction of 8-iso-PGF2α associated with improved 
glycaemic control(17). However, there are also investigations 
not showing such association(5). These differences could be 
explained by various levels of glycaemic control in the patient 
groups. The negative correlation between HbA1c and MDA 
found in the present study was opposite to findings by Altomare 
et al., who observed a positive correlation in patients with type 2 diabetes(17). MDA is generally 
considered as a less specific marker of oxidative stress than 
the most reliable indicator of oxidative stress, 8-iso-
PGF2α(18).

As far as we know, the correlation shown between HbA1c 
and nitrotyrosine has not been shown elsewhere. However, 
the lack of such correlation has been reported previously by 
Ceriello et al.(19), who at the same time observed a direct 
correlation between plasma glucose and nitrotyrosine.

In the present study, we found a lack of associations 
between glycaemic control and oxidative stress as measured 
by 8-oxodG and FPG-sites. Hinokio et al.(20) reported a posi-
tive correlation between glycaemic control and 8-oxodG (in 
urine and blood mononuclear cells) in diabetic subjects, 
maybe due to the higher level of HbA1c compared with the 
present study. The positive relationship between glucose and 
FPG-sites found in the present study in subjects treated with 
diet only has also been seen by other investigators(21). 

Glycaemic control and inflammation

No correlations were found between markers of glycaemic 
control and inflammation in the present study supported 
by Pickup et al.(23) but contradictory to observations by 
Ford(24). The association found between diabetes and CRP dis-
appeared when adjusted for BMI and waist, indicating that 
inflammation is more related to obesity than features for dia-
betes(5).

The well-known relationship between obesity and low-

Limitations of study

A limitation of the present study was the absence of a healthy 
reference population. Comparisons regarding oxidative stress 
and inflammation therefore had to be made with other inves-
tigations but with care since study conditions and method-
ology could differ in many aspects, especially grade of
glycaemic control and obesity. Furthermore, the present study did not measure the glucose tolerance or record the diabetes duration, parameters that could have impact on the investigated relationships between metabolic disorders, oxidative stress and inflammation in subjects with diabetes.

In conclusion, the present study indicated that glycaemic status was associated with oxidative stress even in well-controlled diabetes subjects. Furthermore, inflammation was more related to abdominal obesity than to glycaemic control. The relatively small study group with a well-controlled type 2 diabetes and moderate obesity, giving a narrow range of HbA1c, blood glucose, waist and BMI, increased the probability that existing relationships may not have been detected. In spite of these limitations, interesting relationships were found.

A large number of biomarkers of oxidative stress and inflammation were investigated, but only a few associations were found between the markers. This could be due to the fact that none of these biomarkers biosynthesizes via similar pathways or simultaneously owing to their diverse nature and origin.

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