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/m², 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 biosynthesize 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 elsewhere.

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-oxoD, 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 hy- 
glycaemic medication, glycated Hb (HbA1c) < 10% and BMI < 35 kg/m². Exclusion criteria were insulin-dependent 
diabetes, known CVD, acute inflammatory, liver, kidney or/ 
and 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 circum- 
ference (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 perfor- 
ance liquid chromatography. Plasma insulin was assayed 
with an enzymatic immunological assay (Mercodia, Uppsala, 
Sweden) in a Coda Automated EIA Analyzer (Bio-Rad Labora-
tories, Hercules, CA, USA). Serum cholesterol, HDL- chole-
ssterol and TAG concentrations were analysed by enzymatic 
colorimetric methods (Thermo Electron Corporation, Vantaa, 
Finland) in a Konelab 20 Clinical Chemistry Analyzer 
(Thermo Electron Corporation). LDL- cholesterol was calcu-
lated 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 reticulo-
cytes(12), was used. In this micronucleus assay, measuring 
the frequency of micronucleus-transferrin-positive reticulo-
cytes (fMN-Trf-Ret), the very young erythrocytes were sepa-
rated 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 approxi-
mately 1‰.

**Malondialdehyde**

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

**F₂-isoprostanes**

Free 8-iso-PGF₂α, a major F₂-isoprostane in urine, was ana-
lysed 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, Oxis-
Research, 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 wash-
ing 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 pri-
mary 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 cor-
relation coefficients (Pearson’s or Spearman’s coefficients) 
were calculated when analysing correlations.

**Results**

**Clinical characteristic**

Fifty-six participants (thirty women and twenty-six men, 
fourty-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 trea-
ted with diet only, thirty-five with the addition of anti-diabetic 
medication (sulfonylureas or other insulin-stimulating com-
ounds or/and biguanides). Baseline characteristics are pre-
sentated 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) mmHg; 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 1·7 (SD 0·9) 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-oxo-dG 0·96 (SD 0·45); FPG-sites 30·2 (SD 15·1) % tail; fMN-Trf-Ret 0·90 (SD 0·50) ‰; MDA 0·68 (SD 0·08) ng/ml; 8-oxodG/10⁶ dG 0·32 (SD 0·10) nmol/mmol creatinine.

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 positive 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). To 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 present study.

Glycaemic control and inflammation

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

The well-known relationship between obesity and low-grade inflammation was also observed in the present study. A highly significant correlation between hsCRP and obesity was found, which also has been shown by others in subjects with type 2 diabetes (24). A strong association between IL-6 and CRP was also observed in the present study. The positive correlation between the cyclo-oxygenase-mediated inflammatory marker PGF2α-metabolite and the oxidative stress biomarker 8-iso-PGF2α found in the present study has also been observed by others (5), showing a link between free radical generation and inflammatory response.

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 investigations but with care since study conditions and methodology 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 subject 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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