Early Increase of Oxidative Stress and Reduced Antioxidant Defenses in Patients With Uncomplicated Type 1 Diabetes

A case for gender difference

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OBJECTIVE — Diabetes increases the risk of coronary heart disease (CHD) to a greater extent in women than in men. We investigated whether type 1 diabetic patients with short duration of disease and without complications have an altered oxidative status and whether there are differences between men and women.

RESEARCH DESIGN AND METHODS — We investigated oxidative status in 29 control subjects and 37 patients with uncomplicated type 1 diabetes with duration of 6 ± 3 years.

RESULTS — Compared with control subjects, type 1 diabetic patients had lower total plasma antioxidant capacity (TRAP) (720.3 ± 579.8 vs. 930.1 ± 84.2 in women, P < 0.001), higher lipid hydroperoxide (ROOH) levels (6.4 ± 2.2 vs. 2.0 ± 0.7 μmol/l in men, P < 0.001; 8.1 ± 1.9 vs. 2.2 ± 0.6 in women, P < 0.001), higher total conjugated diene (CD) levels (0.037 ± 0.005 vs. 0.033 ± 0.002 A.U. in men, P < 0.001), lower 246-nm CD levels (0.0032 ± 0.001 vs. 0.0070 ± 0.0012 A.U. in men, 0.001; 0.0022 ± 0.0011 vs. 0.0072 ± 0.0014 A.U. in women, P < 0.001), and higher 232-nm CD levels (0.0348 ± 0.0041 vs. 0.0257 ± 0.0022 A.U. in men, P < 0.001; 0.0346 ± 0.0031 vs. 0.0246 ± 0.0074 A.U. in women, P < 0.001). Compared with diabetic men, diabetic women had lower TRAP (P < 0.01), higher ROOH levels (P < 0.01), and lower 246-nm CD levels (P < 0.05). Plasma concentration of uric acid was significantly lower in patients with type 1 diabetes than in control subjects (3.3 ± 0.3 vs. 4.3 ± 0.2 mg/dl, P = 0.009) with a significant difference between women and men with type 1 diabetes (2.6 ± 0.3 vs. 3.9 ± 0.3, respectively, P = 0.009).

CONCLUSIONS — Our findings suggest that reduced antioxidant activity and increased oxidative stress occur early after the diagnosis of type 1 diabetes, especially in women, and this might explain, at least in part, the increased susceptibility of diabetic women to cardiovascular complications.

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Abbreviations: ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); CD, conjugated diene; CDH, coronary heart disease; ROOH, hydroperoxide; TPP, triphenylphosphine; TRAP, total plasma antioxidant capacity.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Diabetes is a significant risk factor for macrovascular disease, and diabetic patients have a greater risk of cardiovascular mortality compared with the general population (1,2). Diabetes reduces the gender protection against vascular disease in premenopausal women, and in diabetic women, the coronary heart disease (CHD) mortality rate is significantly higher than in nondiabetic women (3–5). Moreover, several studies show that diabetes increases cardiovascular risk to a greater extent in women than in men (6,7). There is currently great interest in the potential role of increased oxidative stress in the development of diabetic complications (8,9). In patients with diabetes, oxidative stress seems to be caused by both increased production of plasma reactive oxygen species and reduction of antioxidant defenses (10), with a consequent increase in lipid peroxidation (11,12) and oxidized LDLs, which are more atherogenic than native LDLs (13). Some authors observed increased oxidative stress in early stages of type 1 diabetes in children and adolescents (14).

Recently, we demonstrated in patients with well-controlled type 1 diabetes that total plasma antioxidant capacity (TRAP) was significantly lower and that two different markers of lipid peroxidation, such as conjugated dienes (CDs) and lipid hydroperoxides (ROOHs), were significantly augmented (15). The aim of our study was to investigate whether type 1 diabetic patients with short duration of disease and without diabetic complications have altered oxidative status, and whether there is any difference in the parameters of oxidative damage between men and women in order to better understand the increased risk of CHD in the diabetic women.

RESEARCH DESIGN AND METHODS — We studied 37 patients with type 1 diabetes of short duration (19...
men, 18 women) who were attending the Diabetic Outpatient Clinic of the Catholic University School of Medicine, and 29 control subjects (15 men, 14 women) who were comparable for age, gender, smoking habit, diet, and physical activity. Patients were included in the study only if their duration of disease was <10 years and if they had no history of diabetic complications, such as nephropathy, neuropathy, hypertension, cardiac ischemic disease, and peripheral vasculopathy, according to the criteria of the EURODIAB IDDM Complications Study (16), as reported in detail in a previous study (15). Further exclusion criteria included administration of drugs other than insulin (such as antihypertensive agents, aspirin, or vitamin supplements), history of smoking, pregnancy, and current illness (such as hepatic, cardiac, or renal disease). Neither diabetic nor control women were on estroprogestin therapy. The characteristics of the study population are shown in Table 1.

### Table 1—Baseline characteristics of diabetic and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Type 1 diabetes (women)</th>
<th>Type 1 diabetes (men)</th>
<th>Overall type 1 diabetes</th>
<th>Control subjects (women)</th>
<th>Control subjects (men)</th>
<th>Overall control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.3 ± 8.4</td>
<td>31.6 ± 9.3</td>
<td>30 ± 8.2</td>
<td>31.3 ± 8.4</td>
<td>31.6 ± 9.3</td>
<td>31.5 ± 8.2</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>6.8 ± 2.7</td>
<td>5.6 ± 2.9</td>
<td>6 ± 3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.3 ± 0.8</td>
<td>7.4 ± 1.3</td>
<td>7.4 ± 1.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 3.2</td>
<td>24.0 ± 2.0</td>
<td>23.6 ± 2.8</td>
<td>23.3 ± 4.1</td>
<td>25.6 ± 1.7</td>
<td>23.7 ± 3.4</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>195 ± 38.8</td>
<td>189 ± 37</td>
<td>193 ± 34</td>
<td>189 ± 39.4</td>
<td>191.6 ± 33.9</td>
<td>190.3 ± 35.6</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>87.2 ± 30.6</td>
<td>83.9 ± 34.4</td>
<td>86 ± 33.1</td>
<td>80.4 ± 33.5</td>
<td>91.5 ± 31.1</td>
<td>85.9 ± 31.9</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>57.1 ± 9.5</td>
<td>57.6 ± 8.3</td>
<td>57 ± 9</td>
<td>55.2 ± 8.9</td>
<td>57.6 ± 6.6</td>
<td>56.6 ± 7.3</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>116 ± 32</td>
<td>120 ± 26</td>
<td>119 ± 29</td>
<td>118 ± 29</td>
<td>123 ± 34</td>
<td>121 ± 32</td>
</tr>
<tr>
<td>Uric acid (mg/dl)†</td>
<td>2.6 ± 0.3†</td>
<td>3.9 ± 0.3</td>
<td>3.3 ± 0.3‡</td>
<td>4.0 ± 0.2</td>
<td>4.5 ± 0.2</td>
<td>4.3 ± 0.2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>116 ± 12</td>
<td>118 ± 11</td>
<td>117 ± 11</td>
<td>117 ± 13</td>
<td>116 ± 10</td>
<td>116 ± 11</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>74 ± 13</td>
<td>75 ± 10</td>
<td>74 ± 12</td>
<td>72 ± 11</td>
<td>76 ± 12</td>
<td>74 ± 11</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.01 for men with type 1 diabetes versus control men and women with type 1 diabetes versus control women; †P < 0.009 for women with type 1 diabetes versus men with type 1 diabetes; ‡P < 0.009 for type 1 diabetes versus control subjects.

### Table 2—Antioxidant status and lipoperoxide levels in the normal men and normal women, men with type 1 diabetes, and women with type 1 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Total CD</th>
<th>246-nm CD</th>
<th>232-nm CD</th>
<th>TRAP</th>
<th>ROOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal men</td>
<td>0.033 ± 0.002</td>
<td>0.0070 ± 0.0012</td>
<td>0.0257 ± 0.0022</td>
<td>972.5 ± 97.7</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td>Normal women</td>
<td>0.032 ± 0.003</td>
<td>0.0072 ± 0.0014</td>
<td>0.0246 ± 0.0074</td>
<td>930.1 ± 84.2</td>
<td>2.2 ± 0.6</td>
</tr>
<tr>
<td>Diabetic men</td>
<td>0.037 ± 0.003*</td>
<td>0.0032 ± 0.0010*</td>
<td>0.0348 ± 0.0014*</td>
<td>720.3 ± 111.2*</td>
<td>6.4 ± 2.2*</td>
</tr>
<tr>
<td>Diabetic women</td>
<td>0.036 ± 0.003</td>
<td>0.0022 ± 0.0011††</td>
<td>0.0346 ± 0.003‡</td>
<td>579.8 ± 95.4‡§</td>
<td>8.1 ± 1.9‡§</td>
</tr>
<tr>
<td>ANOVA F value (df3,62)</td>
<td>11.46</td>
<td></td>
<td>81.81</td>
<td>25.78</td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD. Analyses of variance and Scheffé’s F test were used for multiple comparisons. *P < 0.001 versus normal men; †P < 0.05 versus type 1 diabetic men; ‡P < 0.001 versus normal women; §P < 0.01 versus type 1 diabetic men; ||P < 0.001.
tively (21,22). These minima were quantified in arbitrary units as $d^2A/d\lambda^2$. The CD ratio measurement at 246- and 232-nm minima of the derivative spectra, which can provide an indirect evaluation of plasma antioxidant capacity, was then calculated (19,20). Intra- and interassay coefficients of variation for this method were 8.2 and 11.4%, respectively.

**ROOHs**

As reported elsewhere (15), plasma ROOH content was determined with the FOX 2 assay for lipids (23,24). ROOH content in plasma samples was determined as a function of the mean absorbance difference of samples with and without elimination of ROOH by triphenylphosphine (TPP). Intra- and interassay coefficients of variation for this method were 6.5 and 8.3%, respectively.

**Statistical analysis**

Statistical calculations were performed using StatView software (version 4.01; SAS Institute, Cary, NC). Values are given as means ± SD. Comparisons between diabetic and control groups and within individual groups were made using ANOVA and Scheffe’s F test for multiple comparisons. Simple linear regression analysis was used to detect relationships among TRAP values, 246-nm CD, ROOH, and plasma HbA1c as the independent variable.

**RESULTS**

As shown in Table 2, compared with control subjects, type 1 diabetic patients had significantly lower TRAP levels, significantly higher ROOH levels, increased total CD levels (only in men), significantly lower 246-nm CD levels, and significantly augmented 232-nm CD levels. In type 1 diabetic patients of both sexes, the CD isomer ratio was directly correlated with TRAP ($R = 0.468$, $P < 0.02$) and inversely correlated with ROOH ($R = -0.486$, $P = 0.003$) (Fig. 1). No significant differences were found between patients with type 1 diabetes and control subjects in the concentration of total, HDL, and LDL cholesterol and triglycerides; in age; and in BMI.

As shown in Table 2, compared with diabetic men, women with type 1 diabetes had significantly higher ROOH levels, significantly lower TRAP levels, and significantly lower 246-nm CD levels, but there were no significant differences in total and 232-nm CD levels. No significant differences were found between men and women with type 1 diabetes in concentration of total, HDL, and LDL cholesterol and triglycerides, age, BMI, and insulin dosage.

As shown in Table 1, uric acid levels were significantly lower in type 1 diabetic patients than in control subjects, both in men with type 1 diabetes compared with male control subjects and in women with type 1 diabetes compared with female control subjects. Moreover, there was a significant difference between women and men with type 1 diabetes.

As in type 1 diabetic patients, uric acid levels were directly correlated with TRAP ($R = 0.484$, $P = 0.02$) and inversely correlated with ROOH ($R = -0.449$, $P = 0.03$).

In a simple linear regression analysis between HbA1c as the independent variable and TRAP, ROOH, and 246-nm CD, we did not find any significant relationship in type 1 diabetic patients.

**CONCLUSIONS**

In this study, we investigated whether oxidative stress occurs early during the course of disease in type 1 diabetic patients without diabetic complications, and whether any difference exists between diabetic men and women in this regard.

Compared with control subjects, our type 1 diabetic patients had significantly lower TRAP and 246-nm CD levels and significantly higher ROOH, 232-nm CD, and total CD levels, at least in men (Table 2). Therefore, diabetic patients have an early impairment of plasma antioxidant capacity and increased oxidative stress, even in the early stages of the disease. These data are in agreement with those of other authors (14), although they studied...
the oxidative stress by evaluating the enzymatic activity of superoxide dismutase and glutathione peroxidase in children and adolescents.

The decrease in 246-nm CD and the increase in 232-nm CD, expression of a decreased CD isomer ratio, are indicative of a large depletion of antioxidants in type 1 diabetic patients, because in physiological conditions, oxidative reactions are directed toward the formation of cis-trans CDs. In type 1 diabetes, the hydrogen-donating ability of plasma, as measured by TRAP, is decreased because of the consumption of antioxidants and because the kinetic equilibrium of the oxidation process is shifted toward trans-trans products, with an increase of the 232-nm CD. Interestingly, in our patients, the CD isomer ratio was positively correlated with TRAP and inversely correlated with ROOH, as shown in Fig. 1.

Our reported increase in plasma lipid ROOHs and CDs was not due to an increase in the content of circulating lipids, because there were no significant differences in the plasma lipid levels between normal and diabetic subjects.

We did not find any relationship between metabolic control, as evaluated by HbA1c, antioxidant status, and markers of oxidative stress. This can be explained by the fact that HbA1c is a “mean” evaluation of glycemic control and reflects, only in part, glycemic fluctuations, such as postprandial hyperglycemia or dysglycemia states, which may induce a pro-oxidative status and may play a significant role in the pathogenesis of diabetic complications (10,11,25). In fact, a recent prospective study by Berg et al. (26) showed that intensive insulin treatment can reduce the increased ROOH levels in type 1 diabetic patients. Moreover, recent epidemiological studies (27) suggest that HbA1c is related to total mortality in the general population, determining an increased risk even for values >5%.

Data from epidemiological surveys have shown a significant decrease of uric acid levels after the onset of type 1 diabetes (28). Uric acid has been proposed to play a pivotal role in the antioxidant defense systems in humans (29–31). The intriguing question of a role for uric acid in the free radical–scavenging processes in diabetes has received little attention. Elevated glucose levels seem to be the principal mechanism, because hyperglycemia has, at least in part, an osmotic diuretic effect, leading to an excessive excretion of uric acid (32). Some authors reported that even in type 1 diabetic patients with good metabolic control, plasma uric acid levels are not completely normalized (33), possibly because of the glomerular hyperfiltration that characterizes the first years of type 1 diabetes, and can contribute to the hyperuricosuria and hyperuricemia found in type 1 diabetic patients (34).

In our study, we report not only decreased levels of uric acid in type 1 diabetic patients compared with control subjects but also, for the first time, a more evident decrease in uric acid levels in diabetic women compared with diabetic men. This fact, together with the positive relationship found between uric acid levels and antioxidant capacity, as shown in Fig. 1, confirms the importance of uric acid for the antioxidant defenses (35).

Many studies have shown that the protective effect of female gender against cardiovascular risk is lost in the presence of diabetes (3) and that the increased relative risk for CHD in diabetic women is independent from the conventional risk factors (3–4). Moreover, it has been reported recently that in diabetic women, coronary artery calcification, a measure of atherosclerotic plaque burden, is greatly increased and the gender difference is lost (36). Because little of this can be explained by known coronary risk factors, the observed increase in lipid ROOH along with the decrease in TRAP and uric acid levels in diabetic women compared with diabetic men could play an important role in the development of the atherosclerotic plaque and may represent one of the pathophysiological explanations for the higher cardiovascular risk of diabetic versus nondiabetic women (3–6,37).

Many studies support the role of estrogen in the primary and secondary prevention of CHD among women (38,39), particularly in normalizing blood lipids or inducing endothelium-dependent vasodilation stimulating nitric oxide synthetase (40–42). It has recently been demonstrated in premenopausal diabetic women that there is a decreased estrogen-induced endothelium-dependent vasodilation (43), which can explain the increased risk of cardiovascular mortality in diabetic women. It could be possible that the augmented oxidative stress in diabetic women induced by hyperglycemic events, not detectable by HbA1c values, could impair the estrogen protective effect on lipoprotein oxidizability (44,45) and/or on endothelin-dependent vasodilation (46).

In conclusion, our data suggest that type 1 diabetic patients with a short duration of disease and good metabolic control show an early imbalance in their antioxidant capacity and augmented levels of lipid ROOH and CD, even in the absence of complications. Reduced uric acid levels seem to contribute to this phenomenon, especially in diabetic women. Diabetic women show, independently from other factors, a decreased antioxidant capacity and an increased rate of lipoperoxidation compared with diabetic men. The severe alteration of the oxidative pattern in diabetic women may offer one possible pathogenetic explanation for the higher incidence of cardiovascular complications observed in diabetic versus nondiabetic women.

Further studies are needed to investigate a possible effect of hyperglycemia and oxidative stress on the hormonal pattern and/or activity in women with type 1 diabetes.

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