Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications

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1. The metabolism of methylglyoxal by the glyoxalase system may be linked to the development of diabetic complications. The glyoxalase system was characterized in blood samples from patients with insulin-dependent diabetes mellitus (n = 43), patients with non-insulin-dependent diabetes mellitus (n = 107) and 21 normal healthy control subjects.

2. The concentrations of glyoxalase metabolites, methylglyoxal, S-D-lactoylglutathione and D-lactate, were increased in diabetic patients, relative to normal control subjects: methylglyoxal [median, range (n) pmol/g], insulin-dependent patients, 470.7, 85.6–1044.3 (42), P<0.001, non-insulin-dependent patients, 286.8, 54.7–2370 (105), P<0.001, control subjects, 79.8, 25.3–892.9 (21); S-D-lactoylglutathione [mean ± SD (n) pmol/10⁶ erythrocytes], combined diabetic patients, 3.37 ± 0.85 (24), control subjects 4.76 ± 1.95 (8) P<0.05; D-lactate [mean ± SD or median, range (n) nmol/g], insulin dependent patients, median 18.3, 5.7–57.4 (42), P<0.001, non-insulin-dependent patients, 20.0 ± 8.9, 2.6–48.4 (105), P<0.001, control subjects 9.7 ± 4.3, 1.8–19.7 (21). The reduced glutathione concentrations in blood samples from the insulin-dependent and non-insulin-dependent diabetic patient groups were not different from the control group values (P>0.05).

3. The activities of glyoxalase enzymes in erythrocytes were increased: glyoxalase I activity [mean ± SD (n) m-units/10⁶ erythrocytes] was increased in diabetic patients, relative to normal control subjects: insulin-dependent patients, 4.35 ± 1.54 (41), P<0.001; non-insulin-dependent patients, 4.61 ± 1.79 (101), P<0.001; control subjects, 3.21 ± 1.81 (21); glyoxalase II activity [mean ± SD (n) m-units/10⁶ erythrocytes] was increased in the non-insulin-dependent diabetic patient group, relative to normal control subjects [non-insulin-dependent diabetic patients, 2.10 ± 0.46 (102); subject controls, 1.83 ± 0.27 (21); P<0.05].

4. In insulin-dependent diabetic patients, the concentration of methylglyoxal correlated positively with the duration of diabetes, and the concentration of D-lactate correlated positively with haemoglobin A₁c and negatively with the reduced glutathione concentration. D-Lactate concentration correlated positively with blood glucose concentration in patients with non-insulin-dependent diabetes mellitus.

5. There was a positive logistic correlation of duration of disease with retinopathy, nephropathy, neuropathy, or any combination thereof. Retinopathy also gave a positive logistic correlation with haemoglobin A₁c concentrations and a negative logistic correlation with D-lactate concentration.

6. When paired for duration of diabetes, patients with retinopathy, neuropathy or nephropathy, or any combination thereof, had significantly higher age, level of haemoglobin A₁c, and glyoxalase I activity than patients with uncomplicated diabetes (P<0.05).

7. We conclude that the glyoxalase system is modified in erythrocytes in both insulin-dependent and non-insulin-dependent diabetic patients and that this modification is related to the development of diabetic complications.

INTRODUCTION

Recent research has demonstrated that the glyoxalase system is activated in human erythrocytes during hyperglycaemia in vitro [1]. The glyoxalase system is also activated in experimental diabetes in vivo [2–4]. It has been suggested that this modification may be linked to the development of diabetic complications [1–5].

The glyoxalase system catalyses the conversion of methylglyoxal to D-lactate via the intermediate S-D-lactoylglutathione. It is comprised of two enzymes, glyoxalase I and glyoxalase II, and a catalytic amount of reduced glutathione [5]. Glyoxalase I (EC 4.4.1.5) catalyses the formation of S-D-lactoylglutathione from the hemithioacetal formed non-enzymically from methylglyoxal and reduced glutathione:

\[
\text{MeCOCHO} + \text{GSH} \rightleftharpoons \text{MeCOCH(OH)} - \text{S} ^{\prime} + \text{SG}
\]

Glyoxalase I \(\rightarrow\) MeCH(OH)CO-SG

Key words: diabetes, diabetic complications, glutathione, glycated haemoglobin, glyoxalase, D-lactate, S-D-lactoylglutathione, methylglyoxal.

Abbreviations: BMI, body mass index; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus.

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Human glyoxalase I has a molecular mass of 46,000 Da and an isoelectric point of 4.9, and is composed of two identical or similar subunits [6]. There are three phenotypes: GLO 1–1, GLO 1–2 and GLO 2–2, representing the homozygous and heterozygous expression of a diallelic gene, GLO1 and GLO2. GLO alleles are inherited autosomally in a co-dominant manner. The GLO locus is on chromosome 6, between the centromere and HLA-DR [7]. The three allozymes in heterozygotes, GLO 1–1, GLO 2–2 and GLO 1–2, have indistinguishable kinetic characteristics [8].

Glyoxalase II (EC 3.1.2.6) catalyses the hydrolysis of S-D-lactoylglutathione to D-lactate, regenerating the reduced glutathione consumed in the glyoxalase I-catalysed reaction:

\[
\text{MeCH(OH)CO} - \text{SG} + \text{H}_2\text{O} \xrightarrow{\text{Glyoxalase II}} \text{MeCH(OH)CO}_2^- + \text{H}^+ + \text{GSH}
\]

Human erythrocyte glyoxalase II is a monomer of molecular mass 29,2 kDa and an isoelectric point of 8.3 [9]. The gene for glyoxalase II, HAGH, is on chromosome 16. There is usually only one phenotype of glyoxalase II [7]. A link between the development of diabetic complications and the glyoxalase system has been suggested at the metabolic and genetic level.

An initial clinical survey revealed that the concentrations of methylglyoxal, S-D-lactoylglutathione and D-lactate were increased in blood samples from patients with insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM), relative to normal control subjects [10, 11], consistent with an increased flux through the glyoxalase pathway during hyperglycaemia associated with clinical diabetes mellitus. Age- and duration-of-disease matched IDDM patients with retinopathy had a significantly higher activity of glyoxalase I and a significantly lower activity of glyoxalase II in erythrocytes than IDDM patients without retinopathy [10]. IDDM patients without complications (retinopathy, neuropathy) had a significantly higher frequency of the glyoxalase phenotype GLO 1–1 than patients with complications [12]. A further survey was required to confirm or deny the modification of the glyoxalase system in blood samples from diabetic patients and the link with the development of diabetic complications.

We have recently developed improved methods for the assay of glyoxalase activities, analysis of glyoxalase I phenotypes and glyoxalase II isoforms, and the assay of the concentrations of methylglyoxal, S-D-lactoylglutathione and D-lactate in human blood samples [11, 13–17]. We now describe a clinical survey of the glyoxalase system in patients with IDDM, patients with NIDDM and control normal, healthy subjects.

**MATERIALS AND METHODS**

**Patients**

The diabetic patients were chosen at random from those having a standardized full clinical review at the Diabetic Day Centre Clinics of St Thomas' Hospital, London, U.K. Forty-three IDDM patients, 107 NIDDM patients and 21 normal healthy control subjects were included in the survey. There were 83 women and 88 men, who were evenly distributed between each group (P > 0.05). The control subjects had a median age of 26 years and an age range of 22–47 years, which described a non-parametric, positively skewed age distribution. IDDM patients had a median age of 32 years and an age range of 18–71 years, which described a non-parametric positively skewed distribution. NIDDM patients had a normal age distribution with a mean ± SD of 58 ± 12 years. The age distributions of IDDM and control groups were not significantly different (Mann–Whitney U-test, P > 0.05), although IDDM patients had a significantly larger variance of age (F-test, P < 0.01). The age distribution of the NIDDM patient group had a significantly greater mean and variance than that of the control group (Mann–Whitney U-test, P < 0.001; F-test, P < 0.001). The study was approved by the Ethics Committee of St Thomas' Hospital. Blood samples were taken randomly from diabetic patients on visiting the clinic and from normal control subjects.

**Measurement of standard biochemical variables**

Plasma glucose concentration was measured in all patients and healthy subjects using a hexokinase/glucose-6-phosphate dehydrogenase method on a COBAS MIRA PLUS analyser (Hoffmann-La Roche, Welwyn Garden City, Herts, U.K.) by the Department of Clinical Biochemistry, St Thomas' Hospital [18]. The reference range was 3.0–5.3 mmol/l (fasting) and the inter-batch imprecision was 4.4%. HbA1c was measured using a Hi-AUTO HA-8121 (Kyoto Daichichi Kagaku, Co., Ltd, Kyoto, Japan) by the Department of Clinical Biochemistry, St Thomas' Hospital. The method is specific for stable HbA1c, eliminating labile HbA1c by pretreatment with 4 mmol/l tetrapolyphosphate (pH 6, 45°C, 2 min), before separation by cation-exchange h.p.l.c. [19]. The reference range of HbA1c was 3.8–5.5%; the interbatch coefficient of variation was 3.7%.

**Assessment of clinical complications**

**Diagnostic criteria for retinopathy.** In this study patients with retinal micro-aneurysms, soft exudates, small intra-retinal haemorrhages, blot haemorrhages, venous bleeding, neovascularization, retinal traction or detachment, were considered to have retinopathy. The presence of retinopathy was deter-
determined by an experienced observer using direct ophthalmoscopy through dilated pupils using 0.5% (w/v) tropicamide and confirmed using a Canon CR4-45NM Retinal Camera (Clement Clarke International Ltd, London, U.K.) in the majority of cases.

**Diagnostic criteria for neuropathy.** The vibration thresholds were assessed using a biothesiometer (Biomedical Instrument Co., Newbury, OH, U.S.A.) at both medical malleoli and both great toes; neuropathy was considered to be present if the thresholds were more than 2 SDs above the age-adjusted mean [20]. Neuropathy was also diagnosed where there were symptoms compatible with sensorimotor polyneuropathy, autonomic neuropathy or mononeuropathy, absent ankle reflexes or impairment of light touch or pin-prick sensation in the feet.

**Diagnostic criteria for nephropathy.** Nephropathy was considered to be present if there was proteinuria of 1 g/l (or 0.3 g/l on more than one occasion) when measured by Labstix SG Multiple Reagent Strips (Bayer Diagnostic U.K. Ltd, Basingstoke, U.K.) in urine samples in the absence of infection or any evident cause for renal disease other than the diabetes.

Duration of disease was defined in this study as the time that had elapsed between the patient's initial diagnosis of diabetes and their present visit to the clinic. The weight of the patient was given with reference to their height as their body mass index (BMI). BMI was defined as their weight (kg) divided by the square of their height (m).

**Diagnostic criteria for macrovascular disease**

Macrovascular disease was considered to be present if there was a history of myocardial infarction, angina, stroke, intermittent claudication, vascular surgery or amputation for atherosclerotic disease, or one or more absent foot pulses on examination.

**Characterization of the glyoxalase system**

Materials for characterization of the glyoxalase system were purchased or synthesized and purified as described previously [11, 13–17, 21, 22]. Venous blood samples were taken by venepuncture into Vacutainers [Becton Dickenson U.K. Ltd, Cowley, Oxford, U.K.). Two (3 ml each) venous whole blood samples were taken into EDTA tubes [15% (w/v) tripotassium EDTA, 54 μl] and mixed. Three aliquots of whole blood (approximately 1 g) were precipitated with perchloric acid solution (0.6 mol/l, 2 ml) in pre-weighed 4.5 ml polypropylene cryovials. After mixing, the cryovials were weighed and the volume of whole blood was deduced (assuming a blood density of 1.06g/ml). The remaining whole blood sample was divided between two 1.5 ml polypropylene cryovials. All samples were stored in the liquid phase of a liquid nitrogen cryostore until use. One 3 ml venous whole blood sample was drawn into a fluoride/oxalate Vacutainer (4 mg of dipotassium oxalate, 5 mg of sodium fluoride) for use in the glucose assay described below.

The activities of glyoxalase I and glyoxalase II in blood samples were determined spectrophotometrically, as described in [13]. Glyoxalase I phenotypes and glyoxalase II isoforms were analysed by non-denaturing gel electrophoresis [14, 15]. The concentration of methyglyoxal in whole blood samples was determined by derivatization with 1,2-diamino-4,5-dimethoxybenzene and h.p.l.c. of the resulting quinoxaline adduct [15]. The concentration S-D-lactoylglutathione in blood samples was determined by reversed phase h.p.l.c. with spectrophotometric detection as described in [17]. The concentration of D-lactate in blood samples was determined by an endpoint enzymic assay using D-lactate dehydrogenase with fluorimetric detection [11]. The concentration of reduced glutathione in blood samples was determined as non-protein thiol by a fluorimetric method [18]. The concentration of non-protein thiol groups was determined using 5,5'-dithiobis(2-nitrobenzoic acid) and spectrophotometric detection as described in [19].

**Statistical analysis**

For significance testing, matched-paired data sets were analysed using a paired t-test. Independent data sets were compared using a one- or two-tailed t-test as appropriate. Where the analysis of greater than two data sets was required, analysis of variance was used. For non-parametric data, matched-paired data sets were analysed using the Wilcoxon signed rank test. Independent data sets were analysed using the Mann–Whitney U-test. Where significance testing was required and the data sets were not continuous, the frequency of glyoxalase I GLO 1–1 phenotype, the chi-squared test was used.

Correlation analysis was performed and regression coefficients are given where correlation was significant at the 5% level (two-tailed t-test). Multiple logistical regression was used to identify factors influencing the development of diabetic complications:

\[
\ln (\pi / (1 - \pi)) = \alpha + (\beta x)_n
\]

where \(x\) is an explanatory variable, \(\pi\) is the probability of the binary dependent variable, \(\alpha\) is a constant and \(\beta\) is the logistical regression coefficient.
Table 1. Frequency of occurrence of clinical diabetic complications

<table>
<thead>
<tr>
<th>Clinical complication</th>
<th>Subjects</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy</td>
<td>IDDM</td>
<td>14 (32%)</td>
</tr>
<tr>
<td></td>
<td>NIDDM</td>
<td>26 (25%)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>40 (28%)</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>IDDM</td>
<td>4 (9%)</td>
</tr>
<tr>
<td></td>
<td>NIDDM</td>
<td>9 (9%)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>13 (9%)</td>
</tr>
<tr>
<td>Neuropathy</td>
<td>IDDM</td>
<td>5 (12%)</td>
</tr>
<tr>
<td></td>
<td>NIDDM</td>
<td>17 (17%)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>22 (15%)</td>
</tr>
<tr>
<td>Retinopathy, nephropathy or neuropathy</td>
<td>IDDM</td>
<td>15 (35%)</td>
</tr>
<tr>
<td></td>
<td>NIDDM</td>
<td>34 (34%)</td>
</tr>
<tr>
<td></td>
<td>All</td>
<td>49 (34%)</td>
</tr>
</tbody>
</table>

(Chi-squared). This was solved iteratively using the Statistical Program for Social Scientists.

RESULTS

Characterization of biochemical and clinical features (Tables 1 and 2)

In IDDM patients, there was an increased mean and variance of the blood glucose concentration, relative to normal control subjects. The glucose concentration in samples from NIDDM patients was also increased, relative to normal control subjects. There were also increases in the mean and variance of HbA₁c values in IDDM and NIDDM diabetic groups, relative to control subjects. IDDM and NIDDM patients had an increased BMI, relative to normal control subjects.

Characterization of the glyoxalase system in blood samples from diabetic patients and control subjects (Table 3)

There were significant increases in glyoxalase I activity in both IDDM and NIDDM patient groups, relative to normal control subjects. There was no significant difference between the distribution of glyoxalase I phenotypes in diabetic patient groups and normal control subjects (χ², P > 0.05, data not shown). There was a significant increase in glyoxalase II activity in the NIDDM patient group, relative to normal control subjects. There was no significant difference between the glyoxalase II isoforms in diabetic patient groups and normal control subjects: all samples analysed had two isoform activity bands of approximately equivalent intensity (data not shown).

The concentrations of reduced glutathione in IDDM and NIDDM patient groups were not significantly different from the concentration of reduced glutathione in normal control subjects. The distributions of the concentration of methylglyoxal in whole blood samples from diabetic patient groups and normal control subjects were not normally distributed and positively skewed (Fig. 1). The concentrations of methylglyoxal in IDDM and NIDDM patient groups were significantly increased, relative to normal control subjects. For a combined diabetic patient group, the concentration of S-D-lactoylglutathione was significantly increased in diabetic patients, relative to normal control subjects. The concentration of D-lactate in whole blood samples from IDDM patients was significantly increased, relative to normal control subjects. The distribution of blood D-lactate concentration in samples from IDDM patients was not normally distributed and positively skewed. The blood D-lactate concentration in the NIDDM patient group was significantly increased, relative to normal control subjects.

Association between glyoxalase and clinical parameters (Table 4)

In IDDM patients, the concentration of methylglyoxal correlated positively with the duration of diabetes, with a mean increase in methylglyoxal concentration of approximately 9 pmol year⁻¹ g⁻¹. The concentration of reduced glutathione correlated positively with duration of diabetes and age, and negatively with HbA₁c. The concentration of D-lactate correlated positively with HbA₁c and negatively with reduced glutathione concentration. HbA₁c correlated positively with blood glucose concentration.

In NIDDM patients, the activity of glyoxalase II correlated positively with patient age. The concentration of reduced glutathione correlated negatively with glucose concentration, where the mean decrease in reduced glutathione was 1.53 pmol/10⁶ erythrocytes per mmol/l increase in glucose concentration. D-Lactate concentration and HbA₁c correlated positively with glucose concentration.

Association of biochemical and clinical parameters with the development of diabetic complications

For retinopathy, nephropathy, neuropathy, or any combination thereof, the duration of diabetes gave a significant positive logistic correlation coefficient in each of the patient groups, except nephropathy in IDDM patients and neuropathy in NIDDM patients, which had no significant correlations (Table 5). The presence of retinopathy also gave a positive logistic correlation with the concentration of HbA₁c, and a negative logistic correlation with D-lactate concentration. For retinopathy and the group of patients with any of the three complications, there was also a positive logistic correlation with patient age. For neuropathy in IDDM
patients, there was a negative logistic correlation with BMI.

For macrovascular disease, there were significant logistic correlations with patient age in both NIDDM and combined diabetic patient groups, and a significant correlation with duration of diabetes in the combined diabetic patient group.

When diabetic patients pairs with and without complications were matched for duration of disease, patients with retinopathy, neuropathy or nephropathy, or any combination thereof, had a significantly higher age, per cent of HbA1c and activity of glyoxalase I than patients with uncomplicated disease (P<0.05, Table 6). There was no significant change in glyoxalase I phenotypes for any of the diabetic patient groups with complications (data not shown).

DISCUSSION

Modification of the glyoxalase system in clinical diabetes mellitus

In blood samples from clinical diabetic patients, there were significant increases in the median or mean concentrations of methylglyoxal, S-D-lactoylglutathione and D-lactate, relative to those in normal control subjects. The median concentration
of methylglyoxal in whole blood samples for IDDM patients was increased approximately 6-fold, and in NIDDM patients approximately 2–3-fold, relative to normal control subjects. A previous report [10] gave a mean concentration of methylglyoxal in whole blood of 1.4 nmol/ml in 17 control subjects and 3.6 nmol/ml in 32 diabetic patients. The disparity is attributed to the assay technique employed, adapted from the method of Ohmori et al. [24], now known to suffer interference from glyceraldehyde 3-phosphate and dihydroxyacetone phosphate [16, 25].

No significant changes were found in the concentration of reduced glutathione in whole blood samples from IDDM and NIDDM patient groups, relative to normal control subjects. Previous reports have shown either no change [26, 27] or decreases [28-30] in the concentration of reduced glutathione associated with diabetes. In these studies, the methods of sample storage for later assay of reduced glutathione concentration were not stated. We have shown that reduced glutathione was unstable to oxidation in samples stored at 4°C and −20°C. There may be differential instability of reduced glutathione in blood samples from diabetic patients and normal control subjects: the reduced glutathione concentrations in both control and diabetic groups previously reported were lower than the values reported herein. From this study, there is expected to be no significant change in the availability of reduced glutathione for the metabolism of methylglyoxal in diabetic patients, relative to normal control subjects.

The mean concentrations of S-D-lactoylglutathione in erythrocytes of diabetic patients and normal control subjects were 54 nmol/ml and 41 nmol/ml, respectively, which was similar to those in the previous report [10].

The increases in glyoxalase metabolite concentrations in blood samples from diabetic patients are consistent with the effect of hyperglycaemia on the erythrocyte glyoxalase system [1]. Decreased excretion of methylglyoxal and D-lactate may also contribute to this, although this has not been studied. There may be increased formation of methylglyoxal from triose phosphates from increased metabolic flux through the Embden–Meyerhof pathway and from sorbitol and fructose metabolism via the polyol pathway [31], and increased formation of methylglyoxal from the metabolism of acetone [32]. The concentration of methylglyoxal increased with duration of diabetes in IDDM patients at a rate of approximately 10% of the mean control/year. This was only found in IDDM patients and may reflect the induction of enzymes catalysing the formation of methylglyoxal, for example from acetoacetate.

Although there was no evidence for decreased reduced glutathione levels indicative of oxidative stress in diabetic patients, the negative correlation of reduced glutathione concentration with HbA1c in IDDM patients and glucose concentration in NIDDM patients is consistent with oxidative effects induced by the oxidative degradation of glycated proteins and glucose, respectively [33–35].

The concentration of D-lactate correlated with HbA1c levels in IDDM patients and with glucose concentration in NIDDM patients, reflecting the importance of glucose and glycaemic control in D-lactate formation. The negative correlation of D-lactate with reduced glutathione in IDDM patients may reflect the accumulation of S-D-lactoylglutathione during periods of high metabolic flux through the glyoxalase pathway.

Glyoxalase activities in blood samples from IDDM and NIDDM patient groups were increased, and the activity of glyoxalase II was increased, with patient age in the NIDDM group. The regulatory mechanisms for the modification of glyoxalase activities are unknown. Glyoxalase activities did not correlate with glucose concentration or HbA1c; therefore non-enzymic glycation of glyoxalases probably does not mediate the increases in glyoxalase activities. Chronic exposure to increased systemic methylglyoxal concentration in diabetic patients may lead to induction of increased glyoxalase synthesis in erythrocyte precursors.

**Glyoxalase system and the development of chronic clinical complications associated with diabetes mellitus**

Logistical regression showed, as expected, that duration of disease was a risk factor for the development of diabetic complications. HbA1c was a risk factor from logistical regression only for IDDM patients with retinopathy. However, HbA1c was also significantly increased in duration-of-disease-matched diabetic patients with any combination of retinopathy, nephropathy and neuropathy, con-
Table 4. Correlation coefficients for biochemical measurements and clinical features for correlations significant at the 5% level. Independent variables are given along the top of the table, and dependent variables are given down the side of the table. Values are means ± SEM.

<table>
<thead>
<tr>
<th>IDDM patients</th>
<th>Duration of disease (years)</th>
<th>Age (years)</th>
<th>Glucose concn. (mmol/l)</th>
<th>HbA₁c (%)</th>
<th>Reduced glutathione concn. (pmol/10⁹ erythrocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylglyoxal concn. (pmol/g of blood)</td>
<td>8.81 ± 3.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced glutathione concn. (pmol/10⁹ erythrocytes)</td>
<td>1.24 ± 0.514</td>
<td>1.67 ± 0.428</td>
<td>-7.53 ± 3.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Lactate concn. (nmol/g of blood)</td>
<td></td>
<td></td>
<td>2.25 ± 0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td></td>
<td></td>
<td>-9.71 ± 2.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td></td>
<td>0.39 ± 0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NIDDM patients</th>
<th>Glyoxalase II activity (µ-units/10⁹ erythrocytes)</th>
<th>Reduced glutathione concn. (pmol/10⁹ erythrocytes)</th>
<th>D-Lactate concn. (nmol/g of blood)</th>
<th>HbA₁c (%)</th>
<th>BMI (kg/m²)</th>
<th>Duration of disease (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.7 ± 3.7</td>
<td>-1.53 ± 0.59</td>
<td>0.31 ± 0.13</td>
<td>0.24 ± 0.03</td>
<td>-0.11 ± 0.05</td>
<td>0.13 ± 0.05</td>
</tr>
</tbody>
</table>

Table 5. Logistic regression of the development of diabetic complications. Data were fitted to the logistical equation
\[ \ln\left(\frac{n}{1-n}\right) = \alpha + (\beta x) \]. Units: duration, years; age, years; HbA₁c, %; D-lactate, nmol/g of blood.

<table>
<thead>
<tr>
<th>Diabetic clinical complication</th>
<th>Constant (α)</th>
<th>Explanatory measurement</th>
<th>Regression coefficient (β)</th>
<th>Probability (β = 0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinopathy</td>
<td></td>
<td>Duration</td>
<td>0.435</td>
<td>0.035</td>
</tr>
<tr>
<td>IDDM</td>
<td>-19.64</td>
<td>Duration</td>
<td>4.009</td>
<td>0.032</td>
</tr>
<tr>
<td>NIDDM</td>
<td>-2.082</td>
<td>Duration</td>
<td>-0.363</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-3.781</td>
<td>Duration</td>
<td>0.183</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nephropathy</td>
<td></td>
<td>Duration</td>
<td>0.123</td>
<td>0.021</td>
</tr>
<tr>
<td>IDDM</td>
<td>-5.220</td>
<td>Duration</td>
<td>0.145</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-8.122</td>
<td>Duration</td>
<td>0.134</td>
<td>0.034</td>
</tr>
<tr>
<td>NIDDM</td>
<td>-5.220</td>
<td>Duration</td>
<td>0.145</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-8.122</td>
<td>Duration</td>
<td>0.134</td>
<td>0.034</td>
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<tr>
<td>Neurotropathy</td>
<td></td>
<td>Duration</td>
<td>-0.728</td>
<td>0.050</td>
</tr>
<tr>
<td>IDDM</td>
<td>12.289</td>
<td>Duration</td>
<td>0.059</td>
<td>0.017</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-3.970</td>
<td>Duration</td>
<td>0.059</td>
<td>0.017</td>
</tr>
<tr>
<td>Retinopathy and/or nephropathy and/or neuropathy</td>
<td></td>
<td>Duration</td>
<td>0.164</td>
<td>0.005</td>
</tr>
<tr>
<td>IDDM</td>
<td>-6.755</td>
<td>Duration</td>
<td>0.235</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NIDDM</td>
<td>-1.513</td>
<td>Duration</td>
<td>0.152</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-2.959</td>
<td>Duration</td>
<td>0.037</td>
<td>0.018</td>
</tr>
<tr>
<td>Macrvascular disease</td>
<td></td>
<td>Age</td>
<td>0.171</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NIDDM</td>
<td>-11.717</td>
<td>Age</td>
<td>0.180</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes</td>
<td>-13.382</td>
<td>Age</td>
<td>0.079</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Firming that HbA₁c is a risk factor for the development of complications. D-Lactate was also a risk factor for retinopathy from logistical regression, but with a negative logistic regression coefficient. Individuals with a low blood D-lactate concentration may metabolize methylglyoxal relatively slowly in tissues and blood cells.

Duration-of-diabetes-matched complicated and uncomplicated patient pairs revealed that patients with any or any combination of retinopathy, nephropathy or neuropathy had significantly higher mean age and glyoxalase I activity. The increased glyoxalase I activity in patients with complications may indicate the induction of increased activity in response to chronic exposure to high methylglyoxal concentration. Future studies on the risk factors for the development of diabetic complications should consider glyoxalase characteristics for inclusion in the logistical model.

Patient age and duration of disease were risk
factors for macrovascular disease. For duration-of-disease-matched patients with and without macrovascular disease, there was no significant increase in HbA1c and glyoxalase I, as found for patients paired for duration of disease with and without retinopathy, neuropathy and nephropathy, suggesting that the increase in HbA1c and glyoxalase I activity in patients with complications was not due to underlying macrovascular disease.

Pathophysiological consequences of increased systemic concentrations of methylglyoxal

The glyoxalase system provides the major route for detoxification of methylglyoxal. It is the major factor for prevention of methylglyoxal toxicity: irreversible modification of protein [36, 37], modification of DNA and RNA [38] and inhibition of cell growth [39]. Methylglyoxal is also a substrate for aldose reductase: it is reduced to hydroxyacetone. Both methylglyoxal and hydroxyacetone bind and modify proteins to produce apparently the same fluorescent product [40]; hydroxyacetone may also accumulate to high concentrations in diabetes [32]. Protein modification by methylglyoxal and hydroxyacetone in diabetes may contribute to the formation of advanced glycation endproducts [41], leading to deterioration in protein structure and function [34], and receptor-mediated uptake by monocytes and macrophages [42]. Aldose reductase inhibitors and aminoguanidine have been proposed as agents for the preventative therapy of diabetic complications [43, 44], and both serve to decrease methylglyoxal concentration in diabetes [4, 45].

The increased concentrations of methylglyoxal found in blood samples from diabetic patients suggest that pathogenetic reactions mediated by methylglyoxal (and hydroxyacetone) are enhanced in diabetes mellitus. The chronic exposure to high methylglyoxal concentrations appears to be linked to the development of diabetic complications.

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REFERENCES


