Serum xylosyltransferase activity in diabetes mellitus patients as a possible marker of reduced proteoglycan biosynthesis

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Objective: Proteoglycan metabolism is altered in diabetic patients. The xylosyltransferases are the initial and rate-limiting enzymes in the biosynthesis of the glycosaminoglycan chains in proteoglycans. Here, we analyzed whether the changed proteoglycan metabolism leads to altered serum xylosyltransferase levels in diabetics.

Research design and methods: Serum xylosyltransferase activity was determined in 100 diabetic patients and 100 blood donors using a novel HPLC electrospray ionization tandem mass spectrometry assay.

Results: Serum xylosyltransferase activities in male and female diabetic patients were significantly decreased compared to the corresponding normoglycemic controls (male patients, mean value 19.3 mU/l, SD 4.44; male controls, 26.6 mU/l, SD 2.79; female patients, 18.9 mU/l, SD 3.14; female controls, 21.8 mU/l, SD 3.74; p<0.0001). No significant differences were detected between patients with type 1 or type 2 diabetes.

Conclusions: Our data show a decreased xylosyltransferase activity in diabetes mellitus, a disease which is accompanied by an altered proteoglycan biosynthesis.

ABBREVIATIONS
XT, xylosyltransferase; XT-I, xylosyltransferase I; XT-II, xylosyltransferase II
Diabetes patients were reported as having an altered proteoglycan metabolism, which results in a decreased content of heparan sulfate proteoglycans in basement membranes (1). Proteoglycans consist of a core protein to which glycosaminoglycan chains are attached. The biosynthesis of the glycosaminoglycan chains is initiated by a xylosylation of the proteoglycan core protein. The xylosyltransferases I and II (XT-I, XT-II, EC 2.4.2.26) catalyze the first and rate-limiting step in the glycosaminoglycan biosynthesis and have been shown to play an important role in proteoglycan homeostasis (for review: see (2)). XT-I and XT-II are Golgi-resident proteins which are being shed from the Golgi membrane and are secreted into the extracellular space attached to large proteoglycans. Consequently, the xylosyltransferase activity present in the blood was proposed to be a biochemical marker for the determination of an enhanced proteoglycan biosynthesis rate (2).

Hyperglycemia has been shown to affect a series of cellular processes including glycosylation (3,4). In diabetic patients the biosynthesis of heparan sulfate proteoglycans was found to be decreased (5,6) and a reduced heparan sulfate glycosaminoglycan content was reported in arteries from diabetic patients (7). Furthermore, the association between diabetes mellitus, heparan sulfate glycosaminoglycans and impaired organ function has been intensively studied in the kidneys, where heparan sulfate proteoglycans are important components of the glomerular basement membrane (8,9).

**RESEARCH DESIGN AND METHODS**

The study cohort comprised 100 unrelated Caucasian diabetic patients (50 males, aged 53 ± 8 years, 50 females, aged 54 ± 7 years) and 100 unrelated age- and sex-matched blood donors with normal blood glucose levels (3.9-5.6 mmol/l). Within the patient cohort, 35 individuals were suffering from type 1 diabetes (17 males, aged 26 ± 12 years; 18 females, aged 29 ± 11 years); 65 patients with type 2 diabetes were included in the study (33 males, aged 56 ± 9 years; 32 females, aged 57 ± 12 years). The definition of type 1 and type 2 diabetes was according to current ADA and WHO recommendations (10). Disease duration was at least 5 years in all patients. The experimental design was approved by the local ethics committee, and all patients gave their informed consent. Blood was drawn after overnight fast and the determination of xylosyltransferase activity was performed as described previously (11). Statistical analysis was performed using the t-test and the Kolmogoroff-Smirnoff-test where appropriate. Normality testing for Gaussian distribution of values was performed using the F-test and multiple linear regression analyses was used to assess the independent role of the serum XT activity and sex, age, HbA1c, duration of diabetes and other serum parameters determined (alanine aminotransferase, aspartate aminotransferase, bilirubin, calcium, cholesterol, cholinesterase, C-reactive protein, creatine kinase, creatinine, gamma-glutamyl transferase, HDL cholesterol, lactate dehydrogenase, LDL cholesterol, potassium, sodium, total protein, triglycerides, urea, uric acid). P values of < 0.05 were considered statistically significant.

**RESULTS**

Xylosyltransferase activities in male patients with diabetes (n=50) were significantly reduced in comparison to the control group (p<0.0001). The mean value and 90% range were 19.3 mU/l (SD 4.44) and 13.2-26.6 mU/l in men with diabetes and 26.6 mU/l (SD 2.79) and 18.1-29.1 mU/l in males without diabetes (n=50), respectively (Figure 1). XT activities in serum specimens from
women suffering from diabetes were also significantly decreased compared to female controls (p<0.0001). In female diabetes patients (n=50) the mean value and 90% range were 18.9 mU/l (SD 3.14) and 14.6-24.4 mU/l (Figure 1). In the corresponding group of women without diabetes mellitus (n=50) the mean XT activities were calculated as 21.8 mU/l (SD 3.74) and the 90% range as 15.3-29.2 mU/l. The serum XT activities did not differ between patients with type 1 (mean value 19.9 mU/l, SD 3.60) or type 2 diabetes (mean value 18.7 mU/l, SD 3.91). The observed differences of serum XT activities remained significant after adjustment for sex, age, HbA1c, duration of diabetes and other serum parameters as described above. Furthermore, no significant correlation of the serum XT activities and these parameters was observed.

CONCLUSIONS
In the diabetic state a reduced biosynthesis of proteoglycans has been described (5,6,12). A decrease in the glycosaminoglycan content has been reported for multiple tissues including arteries (7), glomerular basement membranes or the endothelium (12). The association between hyperglycemia, glycosaminoglycan concentration and impaired organ function has been well studied in the kidneys (1). These alterations point to those enzymes involved in the biosynthesis of the glycosaminoglycan chains and both their regulation and enzymatic activity as potential modifiers of this process.

XT-I and XT-II catalyze the initial and rate-limiting transfer of xylose to selected serine residues of the proteoglycan core protein (2). Both enzymes are shed from the Golgi membrane and are being released into the extracellular space attached to large proteoglycans (2,13). Therefore, the xylosyltransferase activity in body fluids reflects the actual proteoglycan biosynthesis rate. While the biological role of xylosyltransferase secretion is not understood, the quantification of xylosyltransferase activity in the peripheral blood and other body fluids could be successfully validated as a marker of the actual proteoglycan biosynthesis rate (2).

In the present study we show for the first time a reduced xylosyltransferase activity in diabetes mellitus, a disease where a reduced proteoglycan biosynthesis rate has been demonstrated (7,8,12). This low xylosyltransferase activity is proposed to result from a decreased enzyme biosynthesis or a lowered release from the Golgi apparatus, but also an increase of enzyme turnover or an elimination of the enzyme from the blood stream have to be taken into account. Furthermore, there is a significant overlap in the xylosyltransferase activities of diabetics and normal subjects pointing to multiple factors affecting the individual serum xylosyltransferase activity. The xylosyltransferase activity determined in peripheral blood is supposed to be a mixture of XT-I and XT-II enzyme activity as both enzymes are released into the extracellular space and share highly similar acceptor specificity. To date, neither immunologic nor enzyme activity assays are available which are suitable for discriminating between the two xylosyltransferase isoforms. The future development of XT-I and XT-II specific assays will help to elucidate whether diagnostic advancement is achieved by a selected determination of the xylosyltransferase isoforms.

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REFERENCES
Figure legends

Figure 1
Serum xylosyltransferase activities in male and female patients with diabetes mellitus and age- and sex-matched controls.
The black bar represents the mean value. The xylosyltransferase activities of the male and the female diabetics were significantly reduced compared to the corresponding control cohort (p<0.0001).