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Circulating semicarbazide-sensitive amine oxidase is raised both in Type I (insulin-dependent), in Type II (non-insulin-dependent) diabetes mellitus and even in childhood Type I diabetes at first clinical diagnosis

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Summary Plasma semicarbazide-sensitive amine oxidase is raised in patients with Type I (insulin-dependent) diabetes mellitus. It has been suggested that this enzyme is involved in the development of microvascular damage through its ability to convert amines (e. g. methylamine and aminoacetone) into aldehydes, hydrogen peroxide and ammonia. Plasma semicarbazide-sensitive amine oxidase was found to be equally raised both in patients with Type I diabetes ($n = 73$) and Type II (non-insulin-dependent) diabetes mellitus ($n = 88$) compared with control subjects (621 ± 209 and 619 ± 202 vs 352 ± 102 mU/l, $p < 0.0001$) and to correlate in multiple regression analysis with HbA_{1c}. Since the enzyme could protect the islets from the inhibitory effects of methylamine on insulin secretion, we also tested sera of 100 children, collected consecutively at first diagnosis of Type I diabetes, for semicarbazide-sensitive amine oxidase. The activity was greatly increased compared with serum values of 76 control (siblings) children (757 ± 300 vs 455 ± 138 mU/l, $p < 0.0001$), but not associated with HbA_{1c}. Our study confirms the increase of plasma semicarbazide-sensitive amine oxidase in Type I diabetes and extends this finding to Type II diabetes as well as to childhood Type I at first clinical diagnosis. In the last case increased enzyme activities could serve to protect the islets from inhibitory effects of methylamine but cause damage by generation of hydrogen peroxide, aldehydes and ammonia. In the long run the increased enzyme activities could also contribute to vascular damage by direct cytotoxic action on endothelial cells, including increased oxidative stress and glycosylation of proteins. [Diabetologia (1999) 42: 233–237]

Keywords Type I diabetes, Type II diabetes, semicarbazide-sensitive amine oxidase, methylamine.

In patients with either Type I (insulin-dependent) diabetes mellitus or Type II (non-insulin-dependent) diabetes mellitus, progression of the disease is often accompanied by serious microvascular complications leading to nephropathy, retinopathy and neuropathy. In this context it has been suggested that the semicarbazide-sensitive amine oxidase (SSAO) enzyme plays a part in the development of microvascular damage [1].

Semicarbazide-sensitive amine oxidases are a group of heterogeneous enzymes and are present in various mammalian tissues (especially vascular smooth muscle cells of arteries) and also in plasma [2–9]. They readily convert endogenous methylamine and aminoacetone to the corresponding cytotoxic aldehydes formaldehyde and methylglyoxal, while generating ammonia and hydrogen peroxide [1, 7, 10–13]. The formation of the reaction compounds can cause damage to endothelial cells in three different ways: 1) directly, presumably mostly by direct action of hydrogen peroxide; 2) by reaction of aldehydes with proteins leading to AGE's; and 3) by the formation of oxidative radicals. In Type I diabetes

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Abbreviations: SSAO, Semicarbazide-sensitive amine oxidase; GAD, glutamic acid decarboxylase; CI, confidence interval.

plasma SSAO was increased compared with control subjects; the increase was greater in patients with, than in those without, microvascular complications [14]. No data on plasma SSAO activity in Type II diabetes are as yet available.

The SSAO substrate, methylamine, is an inhibitor of the glucose-stimulated insulin release [15,16]. Plasma SSAO becomes increased gradually in rats made diabetic with streptozotocin or alloxan, but not in those resistant to the diabetogenic action of alloxan [17]. We therefore examined SSAO activity at the onset of diabetic symptoms. For this, blood samples were taken at first diagnosis in children with Type I diabetes where hyperglycaemia presents more abruptly than in Type II diabetic patients.

The aim of our study was to establish whether plasma SSAO is also raised in Type II diabetes and at first clinical manifestation of Type I diabetes in children.

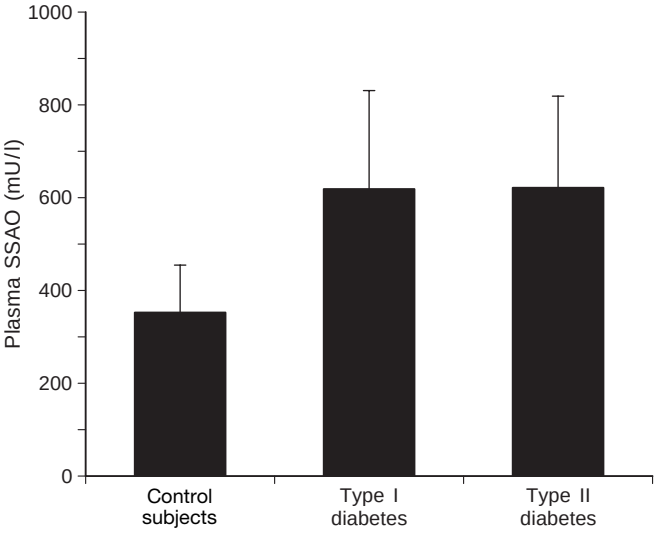

Fig. 1. Plasma SSAO activities (means + SD) in control, patients with Type I, and patients with Type II diabetes

Subjects and methods

Patients and controls. The investigations conformed with the principles outlined in the Declaration of Helsinki. The adults gave informed oral consent, the children over 12 years of age provided signed written consent as did the parents/guardians for those under 12 years of age.

Plasma was obtained from 161 consecutive adult diabetic patients who visited the out-patient clinic of our hospital. Of these patients 73 were classified as having Type I and 88 Type II diabetes (Table 1). The absence or presence of retinopathy (investigated by an experienced ophtalmologist), nephropathy (urinary albumin excretion > 30 mg/24 h), and neuropathy (by biothesiometry) were assessed in each patient. The Quetelet Index is calculated as bodyweight (in kg) divided by the squared height (in m). Patients with Type I diabetes mellitus were younger and had had diabetes for longer than patients with Type II diabetes. Retinopathy occurred more often in Type I and nephropathy and neuropathy more often in Type II diabetes.

Serum was obtained from 100 children (48 males) who had been consecutively diagnosed as having Type I diabetes during a regional prospective Type I diabetes family study. The patients met the World Health Organisation/American Diabetes Association criteria and had islet cell cytoplasmatic antibodies or glutamic acid decarboxylase (GAD) antibodies or both in 82 %. Their age was 9.3 ± 4.4 years (range 0.4–19.5 year). In view of reports of higher SSAO activities in normal children than in adults [18, 19] and the difference between plasma and serum [20], we used 76 serum samples from siblings of the diabetic children (40 males, age 10.9 ± 4.8 years, range 2.3–20.4 years) as control subjects.

Methods. The activity of SSAO was determined as described previously [20]. All plasma and serum was immediately separated and stored at –80 °C until assay. Briefly, plasma or serum, after preincubation with clorgyline (0.9 mmol/l) to inactivate possibly present monoamine oxidase, was incubated for 1 h with the SSAO-substrate benzylamine at 37 °C. The amount of benzaldehyde generated was measured by high-performance liquid chromatography with fluorimetric detection after derivatization with dimedone (5,5-dimethyl-1,3-cyclohexanedione). The SSAO activity is expressed as pmol benzaldehyde formed per ml per min, or mU/l. Normal values of SSAO in adults are 352 ± 102 mU/l in plasma and values in serum are 15 % lower [20].

Glycosylated haemoglobin (HbA_{1c}%) was measured by HPLC within 1 week of first clinical diagnosis in 45 of the 100 diabetic children (normal range 4.0 %–6.2 %). Previously we found a weak association between HbA_{1c} at clinical onset and GAD antibodies [21]. We measured the GAD antibodies within a week of diagnosis in 43 of the 100 Type I diabetic children with a minor modification of a method described previously [22]. Briefly, 2 µl of serum was incubated overnight in a microplate with [35 S]methionine-labelled in vitro translated human recombinant GAD65. Immune complexes were isolated using protein A Sepharose (CL4B Pharmacia, Uppsala, Sweden) and washed using the multiscreen system (Millipore, Bedford, Mass., USA). Precipitated activity was counted in a microbeta-plate-reader (EG&E Wallac, Turku, Finland) and expressed relative to internal reference sera as the GAD index or titre.

Data are reported as means ± SD. Comparison of groups was by Student's unpaired *t*-test, Mann Whitney U-test, and chi-squared analysis. A *p*-value of 0.05 or smaller was considered significant.

Results

Adult Type I and Type II diabetes mellitus. Plasma SSAO was equally raised in both groups of diabetic patients compared with control subjects ($p < 0.0001$; Fig. 1). Plasma SSAO correlated with age in Type I diabetes ($r = 0.38$, $p = 0.0009$) and with duration of diabetes ($r = 0.27$, $p = 0.0124$) and plasma glucose ($r = 0.36$, $p = 0.0007$) in Type II diabetes. When the two diabetes types were subdivided according to the absence or presence of complications (retinopathy, nephropathy and neuropathy), no significant differences in plasma SSAO were found.

In a multiple regression analysis with (ln) SSAO as a dependent variable and type of diabetes, sex, age,

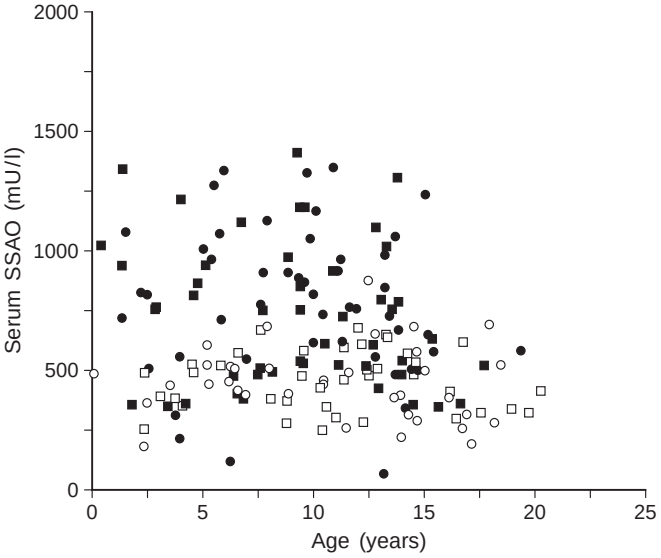

Fig. 2. Serum SSAO activities at first clinical diagnosis compared with age in children ($n = 100$) with Type I diabetes (filled symbols) and control subjects ($n = 76$; open symbols). Squares, males ; Circles, females

Table 1. Characteristics of the adult patients with diabetes mellitus

	Type I diabetes	Type II diabetes	*p*-value
Number	73	87	
Sex (male/female)	36/37	43/44	NS
Age (years)	43 ± 15 (38)	63 ± 11 (62)	< 0.0001
QI (kg/m2)	26 ± 6 (25)	30 ± 5 (29)	< 0.0001
Diabetes duration (years)	20 ± 13 (19)	11 ± 7 (10)	< 0.001
Retinopathy (%)	49	39	0.0276
Background	15	21	
Proliferative	34	18	
Nephropathy (%)	22	58	< 0.0001
Neuropathy (%)	32	58	0.0027
Plasma			
Creatinine (μmol/l)	78 ± 22 (77)	81 ± 24 (75)	NS
Glucose (mmol/l)	8.6 ± 4.6 (7.4)	8.4 ± 3.8 (7.2)	NS
HbA_{1c} (%)	8.2 ± 1.2 (8.2)	8.4 ± 1.4 (8.3)	NS

Data are means ± SD (median)

duration of diabetes, Quetelet Index, HbA_{1c} and presence or absence of the complications retinopathy (background or proliferative), nephropathy and neuropathy as independent variables, only HbA_{1c} ($t = 2.21$, $p = 0.029$, B coefficient 0.042) and Quetelet Index ($t = –2.74$, $p = 0.007$, B coefficient 0.013) were found to be significant, whereas age approached significance ($t = 1.81$, $p = 0.073$, B coefficient 0.0044) in the adult (Type I and Type II) diabetic patients.

Children at first diagnosis of Type I diabetes mellitus and two siblings at risk. In the 100 diabetic children serum SSAO at first diagnosis was much higher than in the control subjects (757 ± 300, confidence interval (CI) 697–817 mU/l vs 455 ± 138, CI 424–487 mU/l, $p < 0.0001$). Regression analysis showed no correlation between serum SSAO and age (Fig. 2). In 45 of the newly diagnosed Type I diabetic children the average HbA_{1c} was 10.6%, median 10.3%. In these children SSAO was not associated with HbA_{1c} ($p > 0.1$) nor with GAD indices ($p > 0.1$). Serum SSAO was also not different between boys and girls.

Two of the control children (76 siblings of the childhood patients) had raised GAD indices as well as islet cell cytoplasmatic antibodies of more than 20 Juvenile Diabetes Foundation units. Their SSAO activities were 450 and 650 mU/l, i.e. in the middle, respectively the high part of the normal SSAO range.

Discussion

In the adult diabetic patients SSAO was raised both in Type I and in Type II diabetes; in fact, both groups had equally high plasma SSAO activities. The Type II diabetic group was older than the Type I diabetic group and plasma SSAO could increase a little with age [23]; in this study, however, age did not reach statistical significance in multiple regression analysis. Even compared to an older control group, however, the activity in the Type II diabetic group was clearly raised (619 vs 455 mU/l, $p < 0.0001$) [23]. The mean activity of 621 mU/l in Type I was a little higher than the mean activity we found previously in Type I diabetic patients without complications (486 mU/l) but similar to the activities in those with Type I with complications (581–641 mU/l) [14]. This fits in well with the higher percentage of patients with complications in this than in the earlier study. The correlation between plasma SSAO and HbA_{1c}, found previously in prevalent Type I diabetes is confirmed in the present study. The negative association with the Quetelet Index needs to be explored otherwise [24].

The study of the Type I diabetic children shows that serum SSAO is substantially raised even at first diagnosis: 757 vs 455 mU/l. Half of the patients had serum activities higher than the means + 2SD of the control subjects. When corrected for the mean difference between plasma and serum, plasma SSAO values of the control children would be 535 mU/l, which agrees well with the value of 554 ± 206 (median 534, range 47–1117) mU/l we have found in 105 plasma samples of children (58 boys, median age 6.6, range 0.1–15.1 years) collected with the specific purpose of establishing normal ranges for various plasma variables. The SSAO activities in children are thus higher than in adults, in agreement with earlier reports [18, 19] about plasma monoamine oxidase (now believed to have been SSAO) which described no differences in SSAO with age during adulthood but higher levels in children. Similarly, plasma SSAO activity in Type I diabetic children (891 mU/l, after correction for the

plasma-serum difference) is higher than in diabetic adults. Within the age-range of both the control and diabetic children of our study, no correlation between age and SSAO was found.

The raised blood SSAO activities in patients with diabetes could constitute a mechanism for damage to the vascular endothelium, through conversion of the endogenous substrates methylamine and aminoacetone to cytotoxic formaldehyde, methylglyoxal, hydrogen peroxide and ammonia. Both methylamine, formed by metabolism of, e. g. creatinine and adrenaline, and aminoacetone, formed from catabolism of threonine and glycine, have been suggested to be increased in diabetes [1, 25]. The possibility that plasma SSAO increases, as a result of such damage, by leakage of SSAO from the membranes of the vascular smooth muscle cells of arteries into the plasma, has been discussed before [14]. It is notable that blood concentrations of methylglyoxal, as well as of the enzymes of the glyoxalase system breaking down methylglyoxal, are reported to be increased in diabetes and correlate with diabetic microvascular complications [26]. Besides being cytotoxic, methylglyoxal is also involved in the modification of proteins and in the formation of advanced glycosylation products. Further, the methylglyoxal scavenger aminoguanidine has been proposed as a prophylactic agent for preventive therapy of diabetic complications [27, 28]. That pancreatic islet homogenates catalyse the incorporation of radioactivity from [14 C]methylamine in N,N-dimethylcasein [15] could also possibly be attributed to transformation of methylamine by SSAO into formaldehyde. Methylglyoxal is produced by enzymatic and non-enzymatic elimination of phosphate from dihydroxyacetone phosphate and glyceraldehyde 3-phosphate [29]; whether under (patho)physiological conditions it also stems from oxidation by SSAO from aminoacetone remains to be established. In the latter case, or if formation of formaldehyde from methylamine is substantial or both, treatment with an SSAO inhibitor could prove useful in preventing vascular damage during chronic diabetes mellitus.

The finding that SSAO is already raised at first diagnosis of Type I diabetes in children suggests involvement of pancreatic endothelial cells in the course of insulitis, as the islets receive 10 % of the total pancreatic blood flow [30]. It is conceivable that the enhanced SSAO activity serves to protect the islets from increases in methylamine emerging with the insulitis. The report that methylamine inhibits the glucose-mediated release of insulin [16], raises the possibility that increased concentrations of methylamine might be such a triggering factor for increases in SSAO causing pancreatic islet damage, in particular through the formation of hydrogen peroxide [31]. Duration and degree of hyperglycaemia (HbA_{1c}) in the weeks prior to clinical diagnosis was apparently no such trigger nor were concentrations of GAD antibodies associated with SSAO.

In summary, we have found that plasma SSAO activity is raised both in patients with Type I and patients with Type II diabetes and relates to HbA_{1c}. In children with Type I diabetes, plasma SSAO is considerably raised even at first clinical diagnosis, but without being associated with HbA_{1c} or GAD antibody indices.

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