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Insulin-Regulated Increase of Soluble Vascular Adhesion Protein-1 in Diabetes

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Vascular adhesion protein-1 (VAP-1) is one of the molecules on the endothelial cell membrane, which may guide inflammatory cells into atherosclerotic lesions. This dual function molecule may also contribute to the pathogenesis of atherosclerosis and other vasculopathies via its enzymatic activity that oxidizes primary amines to produce their corresponding aldehydes, hydrogen peroxide, and ammonium. Because VAP-1 also exists in a soluble form, we analyzed its potential usefulness as a biomarker to monitor and predict the extent of ongoing atherosclerotic processes. Soluble VAP-1 (sVAP-1) levels were determined from the sera of 136 Finnish men with established coronary heart disease and in 275 controls using sandwich enzyme immunoassays and correlated to multiple risk factors for coronary events. Intriguingly, sVAP-1 showed a statistically significant correlation with diabetes in both cohorts. We then collected patients with type 1 diabetes and observed that sVAP-1 levels were highly elevated when the patients were metabolically compromised. On normalization of their blood glucose and ketone body levels by exogenous insulin, their sVAP-1 concentration rapidly decreased to control levels. Intravenous glucose tolerance and hyperinsulinemic clamp tests further showed that elevation of blood glucose per se did not increase sVAP-1 levels, but rather, sVAP-1 was inversely correlated with circulating insulin concentrations. In conclusion insulin appears to regulate shedding or clearance of VAP-1, and an increase in sVAP-1 because of absolute or relative insulin deficiency may be directly involved in the pathogenesis of diabetic angiopathy. ***(Am J Pathol 2002, 161:2255–2262)***

Leukocyte extravasation from the blood into tissues plays a crucial role in the pathogenesis of various chronic inflammatory diseases such as atherosclerosis and type 1 diabetes.[1–5] Vascular adhesion protein-1 (VAP-1) is unique among the endothelial adhesion molecules recruiting leukocytes into tissue because it is a dual-function molecule.[6,7] VAP-1 acts as a traditional inflammation-inducible adhesion molecule in supporting leukocyte rolling under physiological shear.[8–10] On the other hand, it belongs to a distinct group of cell-surface enzymes [semicarbazide-sensitive amine oxidases (SSAOs), EC 1.4.3.6] that catalyze oxidative deamination of primary amines into aldehydes, hydrogen peroxide, and ammonium.[11] The physiological role of SSAOs, which is clearly distinct from intracellular monoamine oxidase-A and -B in terms of structure, subcellular localization, substrates, cofactors, and inhibitors, has remained enigmatic for decades. Only very recently, SSAO activity has been implicated in the regulation of adipocyte differentiation and glucose transport[12–15] as well as in leukocyte-endothelial cell adhesion.[9–11]

Apart from the membrane-associated form of VAP-1, a soluble form of this molecule is found in serum.[16] Analyses of soluble forms of other endothelial adhesion molecules have been driven by the expectation that they may be used as indicators of ongoing inflammatory processes in the vascular system, which is not directly amenable for sampling.[17,18] When compared to soluble forms of classical endothelial adhesion molecules such as P-selectin or ICAM-1, VAP-1 displays many properties that render it especially attractive in relation to atherosclerotic processes. First, VAP-1 expression is not limited to the endothelial cells, but is also strongly synthesized on smooth muscle cells and adipocytes,[19,20] which are crucial players in atherosclerosis. Moreover, increased levels of soluble VAP-1 have so far been detected only in certain liver disorders[21] whereas sICAM-1 and sP-selectin seem to increase nonspecifically in a multitude of various inflammatory diseases. Finally, VAP-1 could be involved in atherogenesis either by its direct adhesive function or via its intrinsic enzymatic activity,

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which can result in the formation of potentially vasculopathic reaction products. For instance, the aldehydes produced might be involved in the formation of advanced glycosylation end products or in cross-linking of proteins in the vascular wall.

Here we determined whether sVAP-1 is associated with coronary heart disease and its risk factors in a Finnish population. Interestingly, the results showed that sVAP-1 is highly elevated in patients with diabetes. Therefore, we went on to study the mechanisms by which diabetes regulates sVAP-1 levels in man. The results revealed that hypoinsulinemia rather than elevated glucose or ketone body levels control shedding of VAP-1 into serum. Together these data suggest that increased sVAP-1 concentrations in patients with diabetes may contribute to the vasculopathic complications of the disease by virtue of the enzymatic activity of this molecule.

Materials and Methods

Study Population

Two patient series were collected for this study: a cross-sectional case-control material for determining the association of sVAP-1 with atherosclerosis and its risk factors (atherosclerosis series) and a cohort of patients with newly diagnosed type 1 diabetes (diabetes series) and their nondiabetic relatives. Both study protocols were approved by institutional committees for human studies and informed consent was obtained from every participant.

The Atherosclerosis Series

The current study (the Finnish Platelet Aggregation and Inflammation Study) was added to the FINNRISK'97 survey to examine the role of inflammation in the development of cardiovascular diseases.[22,23] The series was comprised of 2000 males aged 45 to 74 years from Southern Finland (Helsinki and Vantaa) and from Eastern Finland (province of North Karelia). It was randomly drawn from the population register and stratified by 10-year age groups. In the two youngest 10-year age groups (45 to 54 years and 55 to 64 years), the cell size was 250 men in both areas and in the oldest age group (65 to 74 years) contained 500 men in both areas. Of these, 1571 (79%) participated. In this series, 136 men had prevalent coronary heart disease. The diagnosis was established by self-reporting and verified with drug reimbursement records of the Social Insurance Institute for coronary heart disease (CHD) drugs prescribed by medical doctors. This was done by linking the data sets together using each patient's unique social security code. For each case, two controls were chosen so that they were frequency matched with cases by age and study area. The controls had to be free of CHD both on the basis of a self-report and register linkage with the drug reimbursement records. Persons with a self-reported history of stroke or intermittent claudication were also excluded from the control group.

Patients with Type 1 Diabetes

Nineteen children and adolescents (10 males) with newly diagnosed type 1 diabetes were sampled at diagnosis and 6 days later, when the metabolic decompensation had been corrected. Their mean age at disease presentation was 10.6 years (range, 1.4 to 15.0 years).

Five nondiabetic siblings (one male) of children with type 1 diabetes underwent an intravenous glucose tolerance test (IVGTT) to assess their insulin secretory capacity. Their mean age was 16.9 years (range, 13.9 to 20.0 years).

Enzyme Immunoassays (EIAs) for sVAP-1

sVAP-1 levels were analyzed using an in-house EIA exactly as described.[16] In brief, anti-VAP-1 monoclonal antibody TK8-18 was absorbed onto the bottom of wells in White Cliniplates. The serum samples were diluted 1:25 (and 1:50 with patients with high sVAP-1 concentrations) in the blocking solution and incubated in the wells in triplicates. The bound VAP-1 was detected using biotinylated monoclonal antibody TK8-14 against another epitope of VAP-1. The reaction was developed using peroxidase-conjugated streptavidin and Chemiluminescence ELISA Reagent (Boehringer-Mannheim, Mannheim, Germany). Light absorbance was read using Luminoskan 360 apparatus (Labsystems, Vantaa, Finland). sVAP-1 concentrations were assessed by subtracting the mean absorbance values from those of the same samples obtained using an irrelevant detecting monoclonal antibody. The absorbance values were converted to ng/ml using a known standard included in each assay and linear regression. In the patients with atherosclerosis the levels of serum P-selectin and ICAM-1 were also determined as controls using commercially available sandwich EIAs (R&D Systems, Minneapolis, MN).

Analyses of Glucose, Ketone Bodies, Insulin, and IVGTT

The IVGTTs were performed after a preceding fast of 10 to 16 hours by infusing glucose 0.5 g/kg body weight in a 20% solution throughout a period of 3 minutes ± 15 seconds. Blood samples were taken before infusion (0 minutes) and at 1, 3, 6, 10, 20, 30, 40, 50, and 60 minutes after it had been completed. Serum insulin concentrations were determined by radioimmunoassay from 0-, 1-, 3-, 6-, 10-, 30-, and 60-minute samples. Blood glucose concentrations were measured from all time points by the glucose oxidase method and the amount of ketone bodies was determined by testing serum samples for β-hydroxybutyrate with a MediSense Precision Xtra Plus sensor (Abbot Oy, Espoo, Finland).

Hyperinsulinemic Clamp Studies

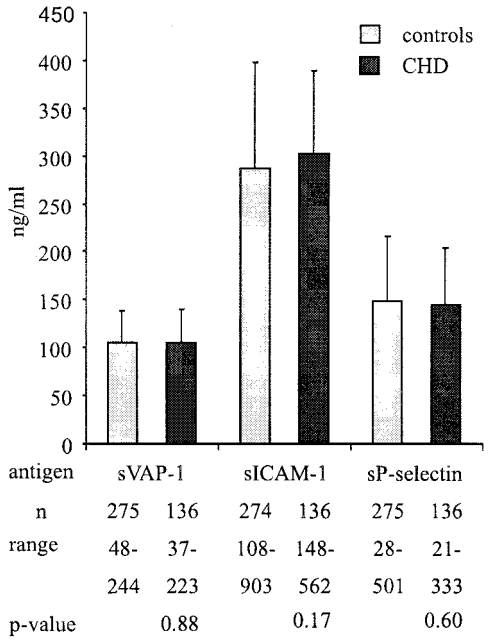
Eight adults (mean age, 30 ± 6 years; three males) with type 1 diabetes fasted overnight. They adjusted their evening blood glucose to 10 to 16 mmol/L by food or short-acting insulin, as appropriate. During the test, catheters were inserted into antecubital veins of both hands. Insulin and glucose were infused into the one vein, and blood samples were collected from the other. Insulin was infused at 4 mU/kg/min (t = 0 to 4 minutes), 2 mU/kg/min (t = 4 to 7 minutes), and 1 mU/kg/minute (from 7 minutes onward). Glucose infusion (20% solution) started according to the starting plasma glucose levels with the objective of maintaining stable glucose level of 6.5 to 7 mmol/L. Plasma sVAP-1, glucose, and insulin (EIA Assay, Crystal Chemicals) were measured before starting the clamp (0 minutes) and at 5 and 10 minutes.

SSAO Enzyme Analyses

SSAO activity was measured from serum samples (atherosclerosis and diabetes series, and healthy volunteers in the laboratory) using a modification of a radiochemical method as described.[21] In brief, the standard assay was performed at 37°C for 60 minutes in a final volume of 0.4 ml of Krebs-Ringer phosphate buffer containing 25 μl of human serum and 5 μmol/L of benzylamine with tracer [^{14}C]-benzylamine (40,000 dpm) as substrate. After stopping the catalytic reaction with citric acid, the ^{14}C-labeled benzaldehyde was extracted into toluene containing 0.35 g/L diphenyloxazole and quantified using scintillation counting. The activity of the enzyme was expressed as nanomoles of benzaldehyde formed by a milliliter of serum per hour. In serum samples a specific monoamine oxidase inhibitor clorgyline (0.3 mmol/L) had no effect on the monoamine oxidase activity, confirming that all monoamine oxidase activity in serum is derived from SSAO (data not shown). The contribution of VAP-1 to serum SSAO activity was studied by depleting all VAP-1 (or a control antigen) from the serum with immunoaffinity chromatography, and then measuring the SSAO activities as above. sVAP-1 levels were measured from the same samples with EIA.

Statistical Analyses

Standard statistical methods were used. The cross-sectional associations of soluble adhesion molecules with continuous variables were examined using Pearson product-moment correlation coefficients and analyses of covariance. Chi-square tests were computed for categorical variables. Multivariate associations of the concentrations of soluble adhesion molecules with prevalent CHD were examined using conditional logistic regression analyses. Statistical significance was set at $P < 0.05$. Correlation of SSAO activity with sVAP-1 levels were done using Spearman rank correlation and GrapPad Prism program (version 3.0). All other analyses were performed using the SAS statistical package (version 6).[24]



antigen	sVAP-1		sICAM-1		sP-selectin	
n	275	136	274	136	275	136
range	48-	37-	108-	148-	28-	21-
	244	223	903	562	501	333
p-value		0.88		0.17		0.60

Figure 1. Levels of soluble forms of endothelial adhesion molecules in CHD patients and controls. The level of sP-selectin, sICAM-1, and sVAP-1 were determined from patients with established CHD and from matched controls.

Results

Soluble VAP-1, Coronary Heart Disease, and Atherosclerosis Risk Factors

In the control group, sVAP-1 levels averaged 106 ng/ml (Figure 1), which is slightly more than in young adult volunteers (~80 ng/ml).[16] In the CHD patients, the mean concentration was 105 ng/ml for sVAP-1. When compared to controls, there was no significant association between the serum level of sVAP-1 (or sICAM-1 or sP-selectin) and established CHD. Only if the highest decile of sICAM-1 levels (37% of persons had CHD) was compared to the lowest decile (17% of persons with CHD) a slightly (P = 0.046) increased risk was found. The validity of the patient series was confirmed by finding statistically significant associations between CHD and established risk factors such as CRP, HDL-cholesterol concentrations, diastolic blood pressure, and body mass index (data not shown).

When different risk factors for CHD were correlated to sVAP-1 levels certain significant associations were found. Systolic blood pressure showed a positive correlation with sVAP-1 in the study population (Pearson correlation coefficient, r = 0.136; $P < 0.01$). In closer analyses it was seen that in the highest decile of sVAP-1 systolic blood pressure was significantly higher than in the lowest decile (Table 1). This difference was consistent also when the controls were analyzed separately (145 ± 22 mmHg in the lowest decile and 161 ± 19 mmHg in the highest, P = 0.01). Moreover, when patients were classified as hypertensives (systolic pressure >140 mmHg, diastolic pressure ≥90 mmHg, and/or using anti-hypertensive medication) and normotensives, significantly more hypertensive patients were found to have their sVAP-1 levels in the

Table 1. Correlation between sVAP-1 Cohorts and Cardiovascular Risk Factors

	Lowest*	Highest	*P* value
Systolic blood pressure (mmHg)	144 ± 21†	158 ± 20	0.0027
Diabetes no (*n*)	40	31	0.001
Diabetes yes (*n*)	0	10	
Hypertonia no (*n*)	14	5	0.015
Hypertonia yes (*n*)	26	36	

*The lowest and the highest 10% percentiles of sVAP concentrations in the whole study population.
†Mean ± SD.

highest decile (Table 1). When only the use of antihypertensive drugs was selected as the classification parameter, the correlation was lost. As previously reported, increased sVAP-1 levels also correlated with liver dysfunction (Pearson correlation coefficient with carbohydrate-deficient transferrin, $r = 0.108$; $P < 0.05$). sVAP-1 levels did not correlate with age, C-reactive protein, serum amyloid A, fibrinogen, cholesterol levels, HDL, body mass, triglyserides, or smoking (data not shown).

Patients with Diabetes Show Elevated sVAP-1

In our series there were 48 participants with a diagnosis of diabetes. In these patients, sVAP-1 levels were elevated when compared to nondiabetic patients (116.7 ± 34.3 *versus* 105.3 ± 31.9, $P = 0.02$). Also, if controls and CHD patients were analyzed separately, a significant association between sVAP-1 and diabetes was established in both groups (Table 2). Strikingly, there were no patients with diabetes in the lowest decile of VAP-1, whereas 32% of the participants belonging to the highest sVAP-1 decile suffered from this disease (Table 1). Among the participants with diabetes and insulin treatment only ($n = 7$) the sVAP-1 levels (148 ± 114 ng/ml) were significantly higher than in the patients with diabetes on other treatments ($n = 41$; sVAP, 113 ± 6; $P = 0.02$). Hence, a prominent increase in the amount of sVAP-1 was observed in the patients with diabetes.

sVAP-1 Levels Are Increased in Patients with Newly Diagnosed Type 1 Diabetes and Are Restored to Normal Levels on Correction of the Metabolic Decompensation

Because the atherosclerosis series did not allow a more detailed analyses of the correlation between sVAP-1 and diabetes, we collected serum samples from 19 children and adolescents with newly diagnosed type 1 diabetes.

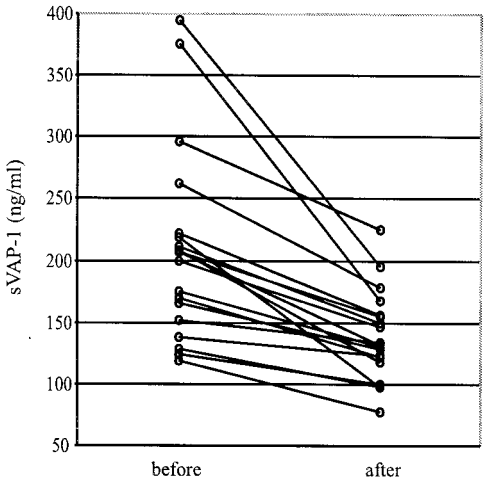


Figure 2. Increased levels of sVAP-1 in type 1 diabetes. The sVAP-1 levels in 19 patients with newly diagnosed type 1 diabetes were measured before and after metabolic compensation.

They had a mean serum glucose value of 26.5 mmol/L (range, 12.2 to >33.3 mmol/L) at diagnosis and a mean pH value of 7.34 (range, 7.20 to 7.45). Six patients (40%) had diabetic ketoacidosis (pH < 7.30 and serum Ketostix positive at a dilution of 1:4 or more) at diagnosis. sVAP-1 levels were highly elevated at presentation (209 ± 18 ng/ml) (Figure 2).

To study the mechanisms leading to increased sVAP-1 concentrations in type 1 diabetes, serum samples from the same patients were measured after correction of the metabolic imbalance. The mean serum glucose at the second sampling was 12.1 mmol/L (range, 5.1 to 15.9 mmol/L) (Table 3). sVAP-1 levels normally remain very constant in a given individual.[16] Notably however, during treatment of type 1 diabetes the sVAP-1 levels significantly decreased in all patients (from 209 ± 18 ng/ml to 139 ± 8 ng/ml) in 6 days (Figure 2 and Table 3), whereas concomitantly blood serum ketone bodies and glucose decreased in all but two patients. There was a highly significant positive correlation between the blood glucose level and sVAP-1 concentration (Table 3), and a less marked correlation with sVAP-1 and ketone bodies. A statistically significant inverse correlation was also found between the insulin level and sVAP-1 (Table 3).

Hypoinsulinemia Results in Increased sVAP-1 Levels

The findings of highly elevated sVAP-1 levels in uncontrolled type 1 diabetes suggest that blood glucose or insulin levels could regulate the production of sVAP-1. To

Table 2. Two-Way Analysis of Variance of sVAP-1 Levels (Mean + SD, ng/ml) by Diabetes and CHD Status

CHD+		CHD−	
Diabetes + (*n* = 21)	Diabetes − (*n* = 113)	Diabetes + (*n* = 27)	Diabetes − (*n* = 244)
111.1 ± 36.6	104.7 ± 34.5	123.2 ± 40.1	104.4 ± 30.2

P values: diabetes, 0.01; CHD, 0.25; diabetes × CHD interaction, 0.23.

Table 3. Diabetic Parameter Levels During Metabolic Correction and Their Correlation with Each Other

Treatment	Mean	SEM		sVAP	GLU	Ketone bodies	Insulin
sVAP							
Before	209.2	17.8		1			
After	138.5	8.3					
Glucose							
Before	26.5	1.7	r	0.57	1		
After	12.1	1.2	p	0.0002			
Ketone bodies							
Before	3.4	0.6	r	0.34	0.55	1	
After	0.0	0.0	p	0.04	0.0003		
Insulin							
Before	0.4	0.1	r	−0.45	−0.44	−0.47	1
After	1.7	0.3	p	0.004	0.005	0.003	

test these hypotheses directly, sVAP-1 levels were measured from five unaffected siblings of children with type 1 diabetes who were undergoing IVGTT (Figure 3). The results unambiguously showed that an acute increase in blood glucose actually resulted in a 15% decrease in sVAP-1 levels (Figure 3A). The change in sVAP-1 concentration was seen in each individual, and the levels returned to baseline after 1 hour. The infused glucose solution cannot cause a dilution effect on sVAP-1, because the volume infused is only ~3 to 4% of the total blood volume. Thus, elevated blood glucose levels per se do not explain the increased sVAP-1 values in patients with type 1 diabetes.

To establish the cause of increased sVAP-1 in diabetes, we analyzed the amount of ketone bodies and insulin during the IVGTT. A negative correlation was found between insulin levels and sVAP-1 concentration ($r = -0.48$, $P = 0.003$) (Figure 3E). Moreover, sVAP-1 levels also correlated inversely with C-peptide levels ($r = -0.45$, $P = 0.006$) (Figure 3D). In contrast, the amount of ketone bodies did not correlate with sVAP-1 levels (Figure 3C). Thus hypoinsulinemia rather than ketone bodies or hyperglycemia may be the principal trigger of increased shedding of sVAP-1 into the peripheral circulation in patients with type 1 diabetes.

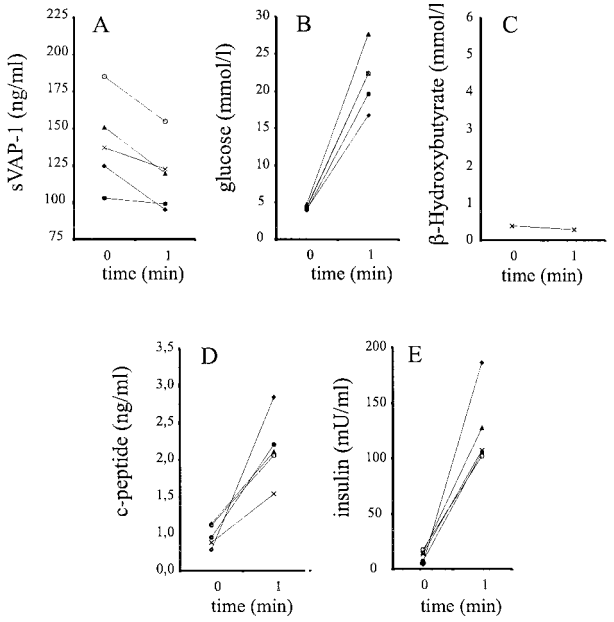


Figure 3. IVGTTs show that insulin is a regulator of sVAP-1 production. The concentrations of sVAP-1 (**A**), blood glucose (**B**), blood β-hydroxybutyrate (**C**), serum C-peptide (**D**), and serum insulin (**E**) were measured during the IVGGT. Data from the time points $t = 0$ and $t = 1$ minute are shown. All of the parameters were measured from all five patients (although the lines may overlap in the figure; the β-hydroxybutyrate values for the other four patients were zero at both time points).

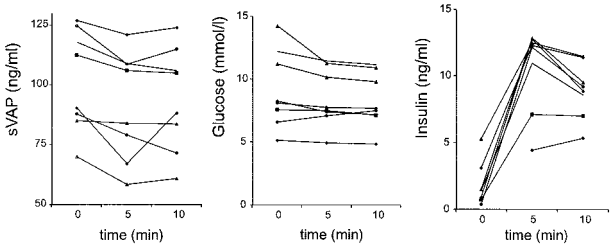


Figure 4. Hyperinsulinemic clamp tests show that insulin is a regulator of sVAP-1 levels. The concentrations of sVAP-1 (**A**), plasma glucose (**B**), and plasma insulin (**C**) were measured during the tests. Data from time points $t = 0$, $t = 5$, and $t = 10$ minutes are shown for eight diabetic patients.

To verify that sVAP-1 levels are regulated by insulin and not glucose, we analyzed these parameters in diabetic patients undergoing a hyperinsulinemic clamp. In this test fasted diabetic patients are infused with both insulin and glucose. The rate of infusion creates a rapid change from hypoinsulinemic to a hyperinsulinemic state ($t = 0$ to 5 minutes) while at the same time a relatively stable blood glucose level is maintained (Figure 4). A negative correlation was found between the change in insulin and sVAP-1 levels at $t = 0$ to 5 minutes ($r = -0.828$, $P = 0.02$) and $t = 5$ to 10 minutes ($r = -0.745$, $P = 0.03$). In contrast, no correlation was found between sVAP-1 and glucose. Hence, insulin seems to be a main regulator of sVAP-1 level in the acute setting.

SSAO Enzyme Activity in Diabetes Is Derived from VAP-1

Finally we wanted to elucidate whether increased sVAP-1 level in patients with diabetes is accompanied by a corresponding increase in SSAO activity. The SSAO activity in the serum of 47 patients with diabetes from the atherosclerosis series (from the remaining ones enough serum was not available for enzymatic analyses) was 3.5 ± 0.3 nmol/ml/hour, whereas that from nine randomly drawn controls from the same study population was 2.8 ± 0.5 nmol/ml/hour and that from six healthy laboratory workers 2.9 ± 0.5 nmol/ml/hour. These results show that there is a tendency for increased SSAO activity in diabetics.

Among the children and adolescents with the newly diagnosed type 1 diabetes the SSAO activity was 6.2 ± 0.4 nmol/ml/hour ($n = 10$) before the treatment. After metabolic decompensation was corrected the SSAO activity decreased to 5.1 ± 0.4 nmol/ml/hour ($P = 0.03$). Thus, simultaneously with the decrease in sVAP-1 levels, a significant decrease in SSAO enzyme activity took place on restoration of metabolic balance in patients with type 1 diabetes.

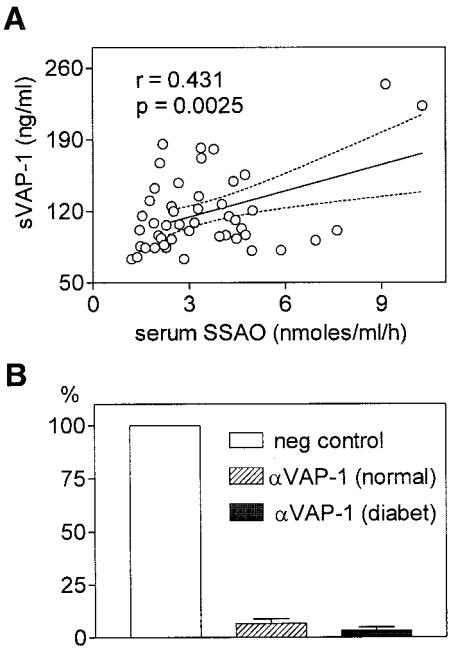


Figure 5. sVAP-1 accounts for elevated SSAO enzyme activity in patients with diabetes. **A:** Correlation of sVAP-1 and SSAO enzyme activity in the 47 diabetic patients from the atherosclerosis series. **B:** Depletion of sVAP-1, but not a control antigen, from serum by immunoaffinity chromatography results in concomitant disappearance of SSAO activity (mean ± SEM, n = 3). The SSAO activity in the sample after depleting a control antigen is defined as 100%. After depleting VAP-1 from normal samples [αVAP-1 (normal)] or from the diabetic samples [αVAP-1 (diabet)] practically all SSAO activity disappears.

A close correlation between the sVAP-1 levels and SSAO activity was evident in the patients with diabetes (Figure 5A). Moreover, if sVAP-1 was depleted from the diabetic or control sera by immunoaffinity chromatography using anti-VAP-1antibodies, almost all SSAO activity disappeared simultaneously (Figure 5B). Thus, more than 95% of the increased SSAO activity in diabetes is derived from the sVAP-1 molecule.

Discussion

Here we found that sVAP-1 does not correlate with coronary heart disease at the population level, but is instead significantly elevated in patients with diabetes. In patients with type 1 diabetes sVAP-1 concentrations correlated positively with blood glucose levels and rapidly normalized after correction of the metabolic decompensation. Molecularly, sVAP-1 accounted for the increased oxidative SSAO activity in patients with diabetes. IVGTTs and hyperinsulinemic clamp tests showed that it was hypoinsulinemia rather than blood glucose or ketone bodies that regulated the level of sVAP-1. Thus, lack of insulin (type 1 diabetes) or its effects (type 2 diabetes) could lead to increased sVAP-1 levels in diabetics.

In nonobese diabetic mice, we have previously shown induction of VAP-1 in pancreatic vessels simultaneously with lymphocytic infiltration.[25] Hence VAP-1 can be envisioned to play multiple pathogenetic roles in diabetes because of its dualistic nature: endothelial VAP-1 may support increased recruitment of leukocytes to the endocrine pancreas and to the vascular wall in atherogenesis[20,26,27] and the intrinsic enzymatic activity of VAP-1 may result in reaction products that are cytotoxic at high concentrations.[6] The aldehydes are involved in nonenzymatic crosslinking of sugar residues to proteins (advanced glycosylation end products) as well as in crosslinking of proteins to each other. This can lead to the stiffening of arterial media in hypertension and diabetic vasculopathy. The hydrogen peroxide and ammonium, in addition to functioning as signaling molecules, can also be cytotoxic at high concentrations.[28] Interestingly, in animal models, SSAO inhibitors have been successfully used to block the development of vascular complications in chemically induced diabetes,[29] suggesting that excessive SSAO activity does indeed play a pathogenetic role in this disease. In line with this, transgenic VAP-1 mice, in which VAP-1 transcription is directed to vascular endothelium, produce increased sVAP-1 levels on induction of chemical diabetes (C Stolen, manuscript in preparation) showing a direct relationship between the destruction of the endocrine pancreas and increased sVAP-1 production.

VAP-1 may not be solely detrimental in diabetes. Recent reports suggest that the SSAO activity conferred by VAP-1 results in differentiation of adipocytes, which also express this molecule, and alteration of their metabolism. Thus when exogenous amine substrates were provided for SSAO/VAP-1, glucose uptake was improved via GLUT 4 transporter in an H_2O_2-dependent manner.[14,15] Interestingly, the SSAO-driven activity also had anti-lipolytic effects in adipocytes.[15] The magnitude of both effects were approximately one third of those seen with insulin, but the effects were additive suggesting partially different signaling mechanisms. Based on these experiments, addition of SSAO substrates (and low levels of vanadate) has been shown to improve acute and chronic glycemic control in diabetic rats.[30] Because we have shown that soluble VAP-1 is mainly produced by shedding VAP-1 in sinusoidal endothelium of the liver,[21] these results may suggest that this organ attempts to improve its capacity to use glucose and concomitantly more VAP-1 is shed into the circulation. While in blood, the soluble VAP-1 may display deleterious vasculopathic effects by virtue of producing cytotoxic compounds from circulating amines. The nature of the physiological soluble substrate of VAP-1 still remains open, but at least methylamine and aminoacetone, both formed during intermediary metabolism in man, are potent substrates for this enzyme.[7] Thus, the role of VAP-1 in diabetic pathophysiology depends critically on the cell type that expresses VAP-1.

sVAP-1 did not simply reflect the overall inflammatory status of the persons studied, because it did not correlate with established inflammatory parameters such as CRP, fibrinogen, and amyloid A. In general, sICAM-1 and sP-selectin appear to be increased in a multitude of various disorders ranging from autoimmune diseases to various types of acute and chronic infections.[18] In contrast, so far elevated VAP-1 levels have only been detected in a subgroup of hepatic diseases. Thus, sVAP-1 is elevated in alcoholic liver cirrhosis, infectious hepatitis, primary bili-

ary cirrhosis, and liver adenocarcinoma, but not in primary sclerosing cholangitis, paracetamol poisoning, liver metastasis of colorectal cancer, or in various arthritic diseases, appendicitis, or inflammatory bowel diseases.[16,21] Hence, sVAP-1 appears to be a more specific marker of certain types of inflammatory lesions than other endothelial adhesion molecules. We are aware that an obvious limitation in the study of coronary heart disease patients and controls was that we investigated men only. However, at least among the normal population, we found no evidence of sex difference in sVAP-1 levels.[16]

The mechanism by which soluble VAP-1 is produced has remained unclear. Our unpublished observations show that soluble VAP-1 contains an N-terminal end, which corresponds exactly to the membrane distal sequence of the transmembrane form of the molecule. Thus, it is most likely produced by shedding of the transmembrane form. Until now, there has been no evidence about the mediators that regulate the production of soluble VAP-1 or SSAOs in general. Theoretically, the increased levels of sVAP-1 could be established in diabetes either by accelerated cleavage rate or by increased expression accompanied by a steady-state cleavage rate. Glucose has been shown to affect the function of another adhesion molecule (CD44) and to increase sICAM-1 levels.[31–33] Because sVAP-1 levels and blood glucose concentrations showed a positive correlation in patients with type 1 diabetes, it was the prime suspect as a regulator for sVAP-1 as well. Nevertheless, IVGTTs and hyperinsulinemic clamp tests ruled out hyperglycemia (or ketone bodies) as the regulatory mechanism. Instead, insulin correlated inversely with the production of sVAP-1 suggesting that insulin is a physiological regulator of the shedding of VAP-1. Based on the current data it is equally possible that diabetes or hypoinsulinemia would control sVAP-1 levels by regulating its clearance or sequestration.

Elevated SSAO activity has been reported previously in patients with both type 1 and 2 diabetes.[34,35] In these reports, however, only the total SSAO enzyme activity in serum has been analyzed by radioisotopic assays. Because there are at least three different SSAO genes reported so far in man,[11,36–38] our molecular definition of VAP-1 allows for the first time the measurement of a specific SSAO enzyme. Moreover, the use of EIA, which is also commercially available, instead of a radiochemical enzyme assay or high pressure liquid chromatography analyses with fluorescent detection makes it possible to determine the sVAP-1 levels in extensive clinical series in routine laboratories. Moreover, our analyses indicate that there was only a tendency toward increased SSAO activity in the patients with diabetes included in the atherosclerosis series, when a statistically significant difference with sVAP-1 EIA was already evident. We also showed that all soluble SSAO in patients with type 1 diabetes is derived from the sVAP-1 molecule. Hence, our current findings revealed that elevated sVAP-1 is the molecule responsible for the long known increase in SSAO enzyme activity in patients with diabetes.

In conclusion, sVAP-1/soluble SSAO levels are increased in diabetes. This molecule may exert harmful effects in this disease by its adhesive function and by its enzymatic properties. The level of sVAP-1 in serum is rapidly controlled by an insulin-dependent mechanism. In the future it will be interesting to study whether determination of sVAP-1 levels in patients with diabetes will give predictive information about the development of diabetic vasculopathy.

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