Improvement of Glycemic Control by 1 Year of Insulin Therapy Leads to a Sustained Decrease in sE-Selectin Concentrations in Type 2 Diabetes

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OBJECTIVE — To examine whether and how improvement of glycemic control by long-term insulin therapy decreases endothelial activation as measured by serum levels of the soluble adhesion molecules sE-selectin and vascular cell adhesion molecule (VCAM-1) and whether the drug used to lower blood glucose in addition to insulin influences such a response.

RESEARCH DESIGN AND METHODS — Circulating adhesion molecules were measured before and after 3 and 12 months of therapy in 81 patients with type 2 diabetes and 41 subjects without diabetes. The patients were treated with bedtime administration of NPH insulin combined with either glibenclamide (n = 19), metformin (n = 17), glibenclamide and metformin (n = 17), or morning administration of NPH insulin (n = 23).

RESULTS — Before insulin therapy, serum sE-selectin level was 71% higher in the patients with type 2 diabetes (77 ± 4 ng/ml) than in the normal subjects (45 ± 3 ng/ml, P < 0.001), whereas levels of sVCAM-1 were comparable (420 ± 25 vs. 400 ± 11 ng/ml, respectively). Glycemic control in all patients improved as judged from a decrease in HbA1c from 9.7 ± 0.7% to 7.6 ± 0.6% (P < 0.001). sE-selectin decreased to 67 ± 4 ng/ml by 3 months (P < 0.001 vs. 0 months) and then remained unchanged until 12 months (70 ± 4 ng/ml, P < 0.001 vs. 0 months). sVCAM-1 levels at 12 months was similar to those at 0 months (416 ± 25 ng/ml). The change in glycemic control, measured by HbA1c, but not in other parameters, was correlated with the change of sE-selectin (r = 0.41, P < 0.001) within the patients with type 2 diabetes. The decreases in sE-selectin were not different between the various treatment groups.

CONCLUSIONS — We conclude that improvement in glycemic control by administration of insulin alone or insulin combined with either glibenclamide, metformin, or both agents induces a sustained decrease in sE-selectin, the magnitude of which seems to be dependent on the degree of improvement in glycemia. These data suggest that sE-selectin might provide a marker of effects of treatment of chronic hyperglycemia on endothelial activation.

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Hypoglycemia promotes leukocyte adhesion to endothelial cells through upregulation of cell-surface expression of E-selectin, intercellular adhesion molecule (ICAM-1), and vascular cell adhesion molecule (VCAM-1) (1). Stimulation of endothelial cells with glycated albumin (advanced glycation end product/bovine serum albumin) also increases expression of these adhesion molecules (1,2), which can be detected in soluble (3) forms (sE-selectin, sICAM-1, and sVCAM-1) in the circulation (4,5). In patients with type 2 diabetes, concentrations of serum sE-selectin, which is expressed exclusively on endothelial cells, have been increased in most (3,6–11) but not all (12,13) comparisons and have correlated with glycemia in five studies (6–10). Serum sICAM-1 concentrations have been increased in most comparisons between groups of patients with type 2 diabetes and normal subjects (8,9,11–14). A positive correlation with glycemic control was reported in two studies (12,14). Serum sVCAM-1 concentrations have been increased in seven comparisons (6,9,10,12,14–16) and unchanged in seven comparisons (3,7,8,12,13,17). A correlation with glycemic control was found in one study (14) but not in other studies reporting data on the existence of such an association (6–10,12,17,18). These cross-sectional data suggest that sE-selectin may be a more sensitive indicator of hyperglycemia-induced endothelial activation in vivo than sVCAM-1 or sICAM-1, although the expression of all three is regulated by glucose in vitro.

Data are limited with respect to the effects of various treatments on circulating adhesion molecules in type 2 diabetes (7,10). Effects of insulin treatment on circulating adhesion molecules have only been investigated in one study, in which 16 patients with type 2 diabetes received 2 weeks of insulin treatment with continuous subcutaneous insulin infusion (10). In this study, both sE-selectin and sVCAM-1 concentrations decreased significantly (10). There are, however, no data on effects of long-term insulin therapy on levels of these adhesion molecules in larger groups of patients. It is also unknown whether administration of different oral agents in addition to administration of insulin modifies the response of circulating markers of endothelial activation to improved glycemia. In the present study, serum sE-selectin and sVCAM-1 concent-
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Patients and study design
Patients were recruited from regional health centers to four trial centers by using the following inclusion criteria: age 40–70 years, BMI <35 kg/m², fasting blood glucose level >8 mmol/l (>144 mg/dl), duration of diabetes >3 years, previous oral therapy with either glibizide (>15 mg/day) or glyburide (>10 mg/day), and fasting serum C-peptide level >0.33 nmol/l (reference range 0.33–0.69 nmol/l or 1.0–2.0 ng/ml). Exclusion criteria included the following: congestive heart failure, myocardial infarction, or stroke in the past 6 months; epilepsy or other severe disease; liver disease, serum creatinine concentration >120 μmol/l (1.36 mg/dl), or macroalbuminuria; proliferative retinopathy or severe maculopathy; previous insulin therapy for >2 weeks; excessive alcohol consumption (>20 g/day); and night work as previously described (19).

Eligible patients were randomized for treatment with four different bedtime insulin regimens as described below. All patients gave written informed consent to participate in the study, which was approved by the respective ethical committees for human investigation.

Randomization
Patients were randomly assigned to four groups in the four centers by using minimization of differences between the treatment groups as previously described (20). The original study included 96 patients, 88 of which completed the 1-year study (19). Of these, serum was available for measurement of sE-selectin and sVCAM concentrations at 0, 3, and 12 months in 81 patients. A group of 41 normal subjects were studied as a control group.

Physical and biochemical characteristics of the study subjects are given in Table 1. Insulin therapy consisted of an injection of NPH insulin (Orion, Espoo, Finland) given at bedtime. In addition, the patients treated with bedtime insulin plus sulfonylurea (BI+SU) used glyburide (Euglucon; Orion), 10.5 mg, given as one 3.5-mg tablet before dinner; the patients treated with bedtime insulin plus sulfonylurea plus metformin (BI+SU+MET) used both 2 g metformin and 10.5 mg glyburide as described for the BI+SU and BI+MET groups; and the patients treated with bedtime insulin and morning insulin (BI+MI) received a second injection of NPH insulin before breakfast. The trial was blinded with respect to the oral agents (i.e., all groups using oral agents took seven tablets per day that were identical in appearance). Insulin was injected subcutaneously in the abdomen. Because data on effects of the various insulin regimens on metabolic parameters have been reported previously (19) and because changes in adhesion molecule concentrations were not dependent on the bedtime insulin regimen chosen (see RESULTS), data will mostly be presented as a comparison between all patients with type 2 diabetes and normal subjects. The patients were taught to self-adjust the insulin dose based on home glucose-monitoring results. The recommendation was to increase the bedtime insulin dose by 2 U if three consecutive fasting blood glucose concentrations exceeded 6 mmol/l.

Follow-up visits took place at 3 and 6 weeks and every 3 months for 1 year. During these visits, body weight, blood pressure, insulin dose, glycosylated hemoglobin, and side effects were recorded. Before and 3 and 12 months after initiation of insulin therapy, blood samples were also taken after an overnight fast for measurement of serum free insulin, C-peptide, triglyceride, cholesterol, HDL cholesterol, sE-selectin, and sVCAM-1 concentrations. In addition, body weight and composition, waist-to-hip circumference, and blood pressure were recorded (19).

Analytical procedures
Levels of serum free insulin, glycosylated hemoglobin (reference range 4.0–6.0%), serum C-peptide, HDL cholesterol, total cholesterol, and triglycerides were measured as previously described (19). Serum sE-selectin (catalog no. BBE 2B; R&D Systems, Minneapolis, MN) and sVCAM-1 (catalog no. BBE 3; R&D Systems) were measured by solid-phase enzyme-linked immunosassays. Fat-free mass was measured in vivo.

Table 1—Characteristics of the study groups

<table>
<thead>
<tr>
<th>Patients with type 2 diabetes (n = 81)</th>
<th>Normal subjects (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 months</td>
<td>3 months</td>
</tr>
<tr>
<td>Age (years)</td>
<td>58 ± 1</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>52/29</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.4 ± 1.7</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>58.5 ± 1.3</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>HbA1c(%)</td>
<td>9.7 ± 0.2</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>11.9 ± 0.3</td>
</tr>
<tr>
<td>Serum triglyceride level (mmol/l)</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Serum HDL cholesterol level (mmol/l)</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Serum LDL cholesterol level (mmol/l)</td>
<td>3.5 ± 0.1</td>
</tr>
</tbody>
</table>

Data are n or means ± SEM. *Reference range 4.0–6.0%. †P < 0.05, ‡P < 0.01, §P < 0.001 for 3 or 12 vs. 0 months; ||P < 0.05, ¶P < 0.01, #P < 0.001 for 12 vs. 3 months; **P < 0.05, †P < 0.01 for patients with type 2 diabetes versus normal subjects.
Table 2—Metabolic parameters in the patients with type 2 diabetes grouped according to the treatment regimen

<table>
<thead>
<tr>
<th>Sex (F/M)</th>
<th>BI+SU</th>
<th>BI+MET</th>
<th>BI+SU+MET</th>
<th>BI+MI</th>
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<td>7/13</td>
<td>8/9</td>
<td>8/13</td>
<td>6/17</td>
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</table>

Serum sE-selectin and sVCAM-1 at baseline

At baseline, serum sE-selectin concentrations were 71% higher in the patients with type 2 diabetes (77 ± 4 ng/ml) than in the normal subjects (45 ± 3 ng/ml, P < 0.001, F-ratio 26.3). This difference remained essentially unchanged (P < 0.001, F-ratio 18.3) after adjusting for serum triglyceride and HDL cholesterol levels, blood pressure, BMI, waist-to-hip ratio, and age. There was no gender difference in sE-selectin concentrations between women with type 2 diabetes (82 ± 8 ng/ml) as compared with men (74 ± 4 ng/ml), whereas the serum sE-selectin level was 64% higher in normal men (52 ± 4 ng/ml) than in women (32 ± 3 ng/ml, P < 0.001). Serum sVCAM-1 concentrations were comparable between patients with type 2 diabetes (420 ± 25 ng/ml) and normal subjects (400 ± 11 ng/ml, NS). Baseline concentrations or changes in sE-selectin and sVCAM-1 did not differ significantly between the treatment groups (Fig. 2, Table 2).

Effect of insulin therapy in serum sE-selectin and sVCAM-1 concentrations

After 3 months of insulin therapy, the serum sE-selectin level had decreased to 67 ± 4 ng/ml (P < 0.001 versus baseline) and to 70 ± 4 ng/ml after 12 months (P < 0.001 versus baseline, NS for 12 vs. 3 months). The concentration at 12 months was still 55% higher than in the normal subjects (Fig. 1). Serum sVCAM-1 decreased transiently during the first 3 months from 240 ± 25 to 379 ± 19 ng/ml (P < 0.001) and then increased back to baseline concentrations by 12 months (416 ± 25 ng/ml, P < 0.001 for 12 vs. 3 months).

Interrelationships between clinical and biochemical characteristics and serum concentrations of sE-selectin and VCAM-1

At baseline, HbA1c correlated with serum sE-selectin concentrations in men with diabetes (r = 0.29, P < 0.05) but not in women with diabetes (r = −0.07, NS). None of the other clinical or biochemical parameters correlated with serum sE-selectin level at baseline before initiation of insulin therapy (data not shown). Within the normal subjects, the percentage of body fat correlated with sE-selectin both in normal men (r = 0.48, P < 0.01) and normal women (r = 0.63, P < 0.05). Serum triglycerides were also positively correlated with serum sE-selectin concent-
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Figure 2—Relationship between change in glycemic control versus serum sE-selectin concentrations within the patients with type 2 diabetes. ● BI + SU; ▲ BI + MET; ▼ BI + SU + MET; ▲ BI + MI.

Figure 1—Glycosylated hemoglobin (A) and serum sE-selectin (B) concentrations before and after 3 and 12 months of treatment of 81 patients with type 2 diabetes with various bedtime insulin regimens and in 41 normal subjects. +++P < 0.001 vs. patients with type 2 diabetes at 0, 3, and 12 months.

CONCLUSIONS — In the present study, sustained improvement in glycemic control was associated with a significant decrease in serum sE-selectin concentrations. The decrease was highly significantly correlated with improvement in glycemic control and independent of the pharmacological agent (glibenclamide, metformin, NPH insulin) used to improve glycemia. In contrast to sE-selectin, sVCAM-1 exhibited a transient decrease at 3 months and was not significantly different from concentrations in normal subjects at any time point.

In previous cross-sectional studies, sE-selectin levels have been, on average, higher in patients with type 2 diabetes than in normal subjects. In the present study, the increase before insulin therapy averaged 71%. We also found, in keeping with data by Bannan et al. (17), that normal women had lower levels than normal men and that this difference was abolished by type 2 diabetes. The gender difference in normal subjects may be explained by estrogen, because estradiol decreases sE-selectin levels (21). In contrast to sE-selectin, sVCAM-1 concentrations did not differ between patients with type 2 diabetes and normal subjects in the present study, in keeping with lack of a difference in half of previous studies (6,9,10,12,14–16) and a mean percent difference in sVCAM-1 concentrations of only 12% (25 and 75% CIs 8 and 23% [6–10,12–17]) between patients with type 2 diabetes and normal subjects in previous studies. E-selectin is specifically localized to endothelial cells, whereas VCAM-1 is widely expressed in multiple tissues in addition to endothelium (18,22). Because early vascular dysfunction seems to be localized to the endothelium rather than smooth muscle cells in type 2 diabetes (23), it is possible that sE-selectin is a more sensitive measure of early vascular dysfunction than sVCAM-1 in these patients. Along these lines, sVCAM-1 has been reported to be increased in patients with type 2 diabetes and overt macrovascular disease compared with patients who are clinically free of cardiovascular disease (12,18) and has been reported to correlate with intima-media thickness (18).

Although hyperglycemia in vitro stimulates the production of both E-selectin, VCAM-1, and ICAM-1 (1), the present study demonstrating a sustained decrease in sE-selectin but not in sVCAM-1 supports the idea that in vivo sE-selectin is a sensitive marker of hyperglycemia-induced endothelial activation. The present study also shows that sE-selectin decreases irrespective of the agent (glibenclamide, metformin, NPH insulin) used to lower blood glucose concentrations. The decrease in sE-selectin was greater in the patients using metformin than in the other groups (Table 2). This difference disappeared, however, after adjusting for baseline sE-selectin. Regarding sVCAM-1, our data suggest that this marker decreases only transiently during insulin therapy, because a decrease was observed at 3 months but not at 12 months. Thus, the decrease in the serum sVCAM-1 level observed by Albertini et al. (10) after 2 weeks of insulin therapy might have disappeared during long-term insulin therapy. In the present study, the percentage of fat was positively correlated with sVCAM-1 levels. The patients gained weight, especially after the first 3-month treatment period (Table 1), raising the possibility that weight gain or other changes not observed during a 2-week insulin treatment period counteracted a decrease in sVCAM-1. On the other hand, Cominacini et al. (7) also found a significant decrease in sE-selectin but not in sVCAM-1 or sICAM-1 during improvement of glycemic control in patients with type 2 diabetes by treatment with mostly diet and oral agents for 3 months. These data support the potential usefulness of sE-selectin as compared with VCAM-1 to monitor reversal of early vascular dysfunction by antihyperglycemic therapies.

The soluble forms of adhesion molecules measured in the present study lack the membrane-spanning and cytoplasmic...
domains of the membrane-bound forms (4,5). The circulating forms may, however, reflect expression in blood vessels. Endothelial adhesion molecule expression is enhanced in the aorta and internal mammary artery of patients with diabetes (24), and soluble forms of E-selectin (4) and ICAM-1 and VCAM-1 (5) are present in supernatants of cytokine-activated cultured endothelial cells. Furthermore, treatment of metabolic abnormalities such as hyperglycemia (7,10; present data) and hypertriglyceridemia (25) decrease both levels of soluble forms of the adhesion molecules and their expression in endothelial cells in vitro (26).

Regarding the mechanisms linking hyperglycemia and sE-selectin concentrations, it seems possible that glucose-induced increased oxidative stress is involved (1,27). Glycosylated hemoglobin concentrations are associated with reduced total plasma antioxidant trapping capacity in patients with type 2 diabetes (23), a finding consistent with the concept that clinically relevant hyperglycemia increases oxidative stress. However, antioxidant treatment (N-acetyl-L-cysteine) has decreased sVCAM-1 levels in one study (28), although it has not been possible to decrease sE-selectin or sVCAM-1 levels by tomato juice (29), α-tocopherol (29), or glaziclide (30). Because treatment of hyperglycemia has reduced sE-selectin levels in three studies (7,10; present study), it could be considered a better tool than antioxidants to lower sE-selectin concentrations in type 2 diabetes. Such lowering might be important because sE-selectin concentrations have been shown to predict restenosis in patients with intermittent claudication undergoing percutaneous transluminal angioplasty (31), and levels of soluble forms correlate with endothelial expression (5). On the other hand, no data presently exist regarding the predictive value of sE-selectin concentrations for future vascular events in patients with type 2 diabetes, and no data exist to demonstrate that lowering of sE-selectin reflects a beneficial change in vascular function. It should also be emphasized that sE-selectin is a nonspecific marker of endothelial damage or activation and is elevated in several other conditions, such as discoid lupus erythematosus (32), localized scleroderma (33), palmar and plantar pustulosis (34), and malignancies such as those in metastatic breast carcinoma (35).

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References


