Circulating Adhesion Molecules in Humans
Role of Hyperglycemia and Hyperinsulinemia

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Background—We assessed the role of glucose and insulin in the regulation of circulating levels of soluble intercellular adhesion molecule-1 (sICAM-1) and vascular adhesion molecule-1 (sVCAM-1) in normal subjects and in patients with type 2 diabetes.

Methods and Results—Plasma glucose concentrations were acutely raised in 10 normal subjects and 10 newly diagnosed, complication-free type 2 diabetic patients and maintained at 15 mmol/L for 2 hours. In normal subjects, plasma sICAM-1, but not sVCAM-1, levels rose significantly (P<0.01) at 1 hour and returned to basal values at 2 hours. In another study, octreotide was infused during the hyperglycemic clamp to block the release of endogenous insulin; this prevented the late fall of plasma sICAM-1 levels observed in under control clamp conditions. The diabetic patients had plasma sICAM-1 levels significantly higher (P<0.01) than those of the control subjects; plasma sVCAM-1 levels were similar. Both sICAM-1 and sVCAM-1 concentrations did not change significantly during the control hyperglycemic clamp; however, octreotide infusion increased plasma sICAM-1 levels, which remained significantly (P<0.05) above baseline during the whole clamp. In an additional 10 type 2 diabetic patients, overnight euglycemia (plasma glucose 5.5 mmol/L) obtained with the aid of an artificial pancreas or supplementation with L-arginine (10 g PO for 30 days), the natural precursor of NO, normalized the increased plasma sICAM-1 levels.

Conclusions—Acute hyperglycemia increases circulating sICAM-1 levels in normal subjects, whereas the correction of hyperglycemia with insulin or L-arginine supplementation restored to normal levels the increased plasma sICAM-1 levels of type 2 diabetic patients. (Circulation. 2000;101:2247-2251.)

Key Words: cell adhesion molecules A glucose A insulin A diabetes mellitus A L-arginine

The adhesion of circulating leukocytes to the endothelial cells plays an important role in the initiation of atherosclerosis.1 Cellular adhesion molecules (CAMs) are poorly expressed by the resting endothelium, but they are upregulated during atherogenesis.2 Soluble forms of some CAMs can be found in plasma; it has been suggested that elevated plasma levels of some CAMs may be an index of endothelial activation3 or even a molecular marker of early atherosclerosis.4

Type 2 diabetes mellitus is associated with an increased risk of premature atherosclerosis,5,6 and the circulating levels of some CAMs are higher in the diabetic patient.7–13 The relation between plasma glucose or insulin and circulating CAMs is poorly understood.

The aim of the present study was to test whether circulating levels of CAMs are regulated by glucose or insulin levels. The study protocol was designed to measure serum-soluble intercellular adhesion molecule-1 (sICAM-1) and vascular adhesion molecule-1 (sVCAM-1) concentrations during acute hyperglycemia, with or without the accompanying hyperinsulinemia, in normal subjects and in newly diagnosed, complication-free type 2 diabetic patients. Because changes in soluble CAMs (sCAMs) are influenced by glycemic control,11–13 we also assessed the behavior of sCAMs after the normalization of blood glucose levels in diabetic patients. Finally, we tested the effect of raised NO availability on increased sICAM-1 levels in diabetic patients. There is evidence that NO may have an inhibitory effect on the expression of some CAMs in human vascular endothelial cells.14

Methods

Study Population
The normal control subjects were recruited from the medical and paramedical staff of the Department of Geriatrics and Metabolic Diseases at the Second University of Naples. The diabetic patients had newly diagnosed (within 6 months) type 2 diabetes mellitus and were managed with diet alone. The clinical and metabolic characteristics of all subjects are shown in the Table. The control subjects had no evidence of present or past hypertension, hyperlipidemia, diabetes, or any systemic conditions. The diabetic patients had normal blood pressure and lipid levels and a urinary albumin excretion of <30 mg/24 h. They were screened on the basis of
clinical history, physical examination, ECG, chest radiography, ophthalmological and neurological examinations, and routine chemical analyses and had no evidence of any cardiovascular complications. All subjects were on weight-maintaining diets with 250 g of carbohydrates/d, had no recent change in body weight or intercurrent illness, and were taking no medications. Particular care was taken to exclude subjects with infectious or inflammatory diseases, as confirmed with measurement of a C-reactive protein level of <5 mg/L. None of the subjects were engaged in physical activity for >3 h/wk or smoked. The protocol of the study was approved by the ethics committee of our institution. All subjects gave informed consent before being tested.

Study Protocol
After a 12-hour overnight fast, subjects were placed in a supine comfortable position with a room temperature between 20° and 24°C. Intravenous lines were inserted into a large antecubital vein of 1 arm for infusions and into a dorsal vein of the contralateral arm for blood sampling. Patency was preserved with a slow saline infusion (0.9% NaCl). The study began after the subjects had rested for 30 minutes.

Study 1
Ten control subjects and 10 diabetic patients underwent the following tests in random order and separated by at least a 3-day interval:

1. In the hyperglycemic glucose clamp test, plasma glucose concentrations were acutely raised with a bolus injection of 0.33 g/kg glucose followed by a varying 30% glucose infusion to achieve steady state plasma glucose concentrations of ~15 mmol/L for 120 minutes. To prevent hypokalemia, 0.26 mmol/L KCl was added to the glucose. The test was performed with the aid of an artificial pancreas (Biorstatator; Life Science).

2. The other test consisted of the hyperglycemic clamp as described earlier plus octreotide infusion (25 μg IV bolus followed by a 0.5 μg/min infusion, Longastatina; Italfarmaco) to block the release of endogenous insulin. The octreotide infusion was started 5 minutes before the priming glucose pulse and was interrupted at the end of the clamp, 125 minutes later.

Five control subjects and 5 diabetic patients received an additional infusion of octreotide alone at the dose administered earlier, in the absence of the hyperglycemic clamp.

Study 2
The diabetic patients were hospitalized on the day before the study. Two hours after dinner (8 PM), they were connected to an artificial pancreas to obtain normalization of blood glucose levels (~5.5 mmol/L) during the night. Regular insulin was infused into each diabetic patient at varying rates through a plastic catheter inserted into a large hand or antecubital vein. Euglycemia was attained within the first 2 hours of insulin infusion and maintained for the next 10 hours. The study ended on the next morning (8 AM), when the patients were disconnected from the artificial pancreas and venous blood samples were taken for the measurement of plasma sCAMs.

On another occasion, the same diabetic patients were administered L-arginine at a daily dose of 10 g PO for 30 days.

Analyses
Samples for analysis of plasma glucose were collected in tubes containing a trace of sodium fluoride, and samples for analysis of insulin were collected in tubes containing a mixture (0.1 mL/mL blood) of EDTA-aprotinin (Trasylol) solution (500 U/mL Trasylol [Bayer], 1.2 g/L disodium EDTA). Plasma glucose was determined according to the glucose oxidase method with an autoanalyzer (Beckman Instruments). Labile and stable forms of glycated hemoglobin A₁ (HbA1) were determined in duplicate according to the method of Compagnucci et al.,13 as previously described.14 Plasma insulin levels were determined with radioimmunoassay.16 Serum samples for sCAM levels were stored at −20°C until assay. Serum concentrations of sICAM-1 and sVCAM-1 were determined in duplicate with commercially available immunosorbent kits (R&D Systems). Dilution curves of serum samples were parallel those of standard. Intra-assay and interassay coefficients of variation were 3.5% and 5.7%, respectively, for sVCAM-1 and 4.1% and 5.9%, respectively, for sICAM-1. No cross-reactivity of human IgG or recombinant soluble E-selectin was observed, according to the manufacturer’s protocol.

Statistical Analysis
Results are given as mean±SD. One-way ANOVA was used to compare baseline data, followed by Scheffe’s test for pairwise comparisons. Multiple comparison tests were made with ANOVA, followed by post hoc analysis (Student-Newman-Keuls test) to locate the significant difference indicated with ANOVA. A value of P<0.05 was considered statistically significant.

Results
Hyperglycemic Clamps in Control Subjects
Fasting plasma glucose and insulin levels were 5.0±0.7 mmol/L and 71±18 pmol/L, respectively. During the clamp, plasma glucose stabilized at 15 mmol/L. Oscillations of plasma glucose during the clamp did not exceed 5% of the prefixed value. Labile HbA1 levels increased from the fasting value of 0.15±0.02% to 0.95±0.10% (P<0.05) at 120 minutes. Stable HbA1 levels did not show any significant change during the hyperglycemic clamp. A biphasic pattern of insulin release was observed, with an early rise at 10 minutes (371±110 pmol/L), followed by a gradual and sustained increase thereafter (120-minute value 415±120 pmol/L). Plasma sICAM-1 levels rose from a basal value of 170±29 to 253±39 ng/mL at 60 minutes (P<0.01) and returned to basal levels at 120 minutes (160±39 ng/mL). Octreotide infusion blocked insulin secretion almost completely (10 minutes 98±31 pmol/L, 120 minutes 69±18 pmol/L, P<0.01 versus control subjects) and prevented the late fall of sICAM-1 at 120 minutes, which remained significantly above the prestimulatory level (baseline 185±26 ng/mL, 120 minutes 240±40 ng/mL, P<0.05) (Figure 1). Plasma sVCAM-1 levels did not show any significant change during the clamp (Figure 1).
The infusion of octreotide alone in 5 control subjects produced a reduction in basal plasma insulin levels (baseline 64±21 pmol/L, 120 minutes 34±16 pmol/L, P<0.01) and an increase in fasting glucose from 5.5±0.7 to 6.2±0.8 mmol/L (P<0.01). Plasma levels of sICAM-1 and sVCAM-1 showed no significant change during octreotide infusion (data not shown).

Hyperglycemic Clamps in Diabetic Subjects
Plasma glucose stabilized around 15 mmol/L during the clamping with no significant difference from values obtained in nondiabetic subjects. Oscillations in plasma glucose from the prefixed target did not exceed 5%. The absolute increase in labile HbA1 levels during the clamping (+0.89±0.21%) in diabetic patients was not different from that observed in control subjects (+0.78±0.2%). The diabetic subjects had a markedly reduced first-phase insulin secretion in response to glucose (10 minutes 103±35 pmol/L, P<0.01 versus control subjects) with a preserved second-phase secretion (120 minutes 395±103 pmol/L). Plasma sICAM-1 levels were higher than those in control subjects (Table) and did not show any significant variation during the clamping (Figure 1). The octreotide infusion completely blocked glucose-induced insulin secretion (10 minutes 52±21, 120 minutes 89±41 pmol/L) and caused significant increases in sICAM-1 concentrations; in fact, sICAM-1 levels rose from a basal value of 242±29 to 298±37 (P<0.05) and 281±27 (P<0.05) ng/mL after 60 and 120 minutes of clamping, respectively. Plasma sVCAM-1 levels in diabetic patients were not different from those obtained in nondiabetic subjects (Table), nor was there any change during the hyperglycemic clamps (with or without the infusion of octreotide) (Figure 1). The infusion of octreotide alone did not cause any significant variation in the plasma concentration of either sICAM-1 or sVCAM-1 (data not shown).

Overnight Euglycemia in Diabetic Patients
The clinical and metabolic characteristics of the diabetic patients evaluated in the present study were not significantly different from those of the diabetic patients who participated in the previous study (Table). The fasting concentrations of plasma glucose and insulin after overnight euglycemia in diabetic patients were 5.3±0.8 mmol/L and 169±58 pmol/L, respectively. These values were significantly (P<0.05 to 0.01) different from those obtained during hyperglycemia (Table). Plasma sICAM-1 concentrations decreased from a pretreatment level of 268±38 ng/mL to 198±31 ng/mL after insulin treatment (P<0.01), which was not different from the value observed in the nondiabetics. Plasma sVCAM-1 concentrations showed a nonsignificant trend to decrease after insulin treatment (baseline 635±85 ng/mL, posttreatment 602±79 ng/mL).

L-Arginine Supplementation in Diabetic Patients
The fasting concentrations of plasma glucose and insulin of the diabetic patients were 9.0±1.4 mmol/L and 79±23 pmol/L, respectively. These values were not significantly affected by treatment (8.8±1.3 mmol/L and 86±30 pmol/L, respectively), nor was there any difference in labile HbA1 levels before (0.43±0.05%) and after (0.39±0.045%) treatment. Plasma sICAM-1 levels decreased from 254±37 ng/mL (pretreatment) to 207±35 ng/mL (posttreatment, P<0.01) (Figure 2).

Discussion
The main findings of the present study were that (1) acute hyperglycemia in normal subjects induces an increase in plasma sICAM-1 concentrations, (2) this effect of hyperglycemia is modulated by the ambient plasma insulin concent-

![Figure 2. Circulating levels of sICAM-1 in 10 type 2 diabetic patients before and after l-arginine supplementation.](http://circ.ahajournals.org/)

### Clinical and Metabolic Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Subjects (n=10)</th>
<th>Diabetic Patients (Study 1; n=10)</th>
<th>Diabetic Patients (Study 2; n=10)</th>
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</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>48±5</td>
<td>50±4</td>
<td>51±4</td>
</tr>
<tr>
<td>Sex, M/F, n</td>
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<td>5/5</td>
<td>5/5</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>26.4±1.1</td>
<td>27.1±1.2</td>
<td>27.3±1.2</td>
</tr>
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<td>Plasma glucose, mmol/L</td>
<td>5.1±0.7</td>
<td>8.7±1.2*</td>
<td>8.9±1.3*</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>4.4±0.5</td>
<td>8.1±0.9*</td>
<td>8.0±0.8*</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>177±28</td>
<td>250±30*</td>
<td>268±38*</td>
</tr>
<tr>
<td>sVCAM-1, ng/mL</td>
<td>608±71</td>
<td>620±80</td>
<td>635±85</td>
</tr>
</tbody>
</table>

*P<0.05 vs normal subjects.
tration, and (3) overnight euglycemia and L-arginine supplementation in hyperglycemic diabetic patients normalize the increased sICAM-1 plasma levels. The results indicate that hyperglycemia is able to change the adhesive properties of endothelial cells but only when insulin is absent or present in insufficient amounts. Moreover, this effect of hyperglycemia is dependent, at least in part, on reduced NO availability.

Unlike sVCAM-1, sICAM-1 levels are elevated in newly diagnosed, complication-free type 2 diabetic patients compared with matched nondiabetic control subjects; moreover, hyperglycemia acutely raises plasma sICAM-1 levels in normal subjects to values similar to those found in diabetic patients but has no effect on plasma sVCAM-1 levels. This suggests that the soluble forms of these adhesion molecules are regulated in different ways by hyperglycemia. Contrary to normal control subjects, however, diabetic patients failed to show any significant increase in sICAM-1 levels during clamping, although this was possible with octreotide. One possible explanation for this differing behavior between control subjects and diabetic patients is the different ambient plasma sICAM-1 levels. Another possibility is that the previous exposition to hyperglycemia in the diabetic patient might have made the endothelium less susceptible to the effects of acute hyperglycemia; with this perspective, acute hyperglycemia has an effect of increasing plasma sICAM-1 levels only when endogenous insulin is fully suppressed, such as with octreotide.

Previous studies have demonstrated increased plasma levels of some CAMs in the diabetic patient, although a definite pattern has not emerged.8–13 In a very large series of type 2 diabetic patients, Otsuki et al10 found elevated plasma sVCAM-1 levels in 56 patients with an atherosclerotic change of the carotid arteries; this is in contrast to the normal values of the 45 patients without any detectable atherosclerosis of the carotid arteries. The presence of vascular complications, the type of antidiabetic treatment, the presence of concomitant diseases, and related treatments may help explain the divergent findings in the literature. The diabetic patients studied in the present report were newly diagnosed, had no evidence of vascular complications, were treated with diet, were not taking medications, and were nonsmokers, which allowed us to exclude their potential confounding effects on plasma sCAM levels.

Surprisingly, we could not find any study that assessed the role of insulin in the modulation of circulating sCAM levels in humans. Our results indicate that insulin may have this role, at least under hyperglycemic conditions, because the suppression of endogenous insulin secretion with octreotide prevented the late fall of sICAM-1 levels in normal subjects and raised sICAM-1 levels in diabetic patients. This seems to suggest that endogenous hyperinsulinemia may counterbalance, at least in part, the effects of hyperglycemia on circulating sICAM-1 levels. Although the normalization of elevated sICAM-1 levels after overnight euglycemia in diabetic patients is strongly suggestive for a major role of hyperglycemia in modulation of the plasma levels of this adhesion molecule, a contributory role of overnight (exogenous) hyperinsulinemia cannot be easily dismissed. Insulin is known to activate endothelial NO synthesis,18 which in turn may have an inhibitory effect on the expression of adhesion molecules.14 A role for NO in modulation of the levels of sICAM-1 is also suggested by the results with L-arginine supplementation, which normalized the increased sICAM-1 levels in diabetic patients without affecting the glycemic control. In acute experiments, L-arginine is able to reverse the decreased NO availability induced by acute hyperglycemia in normal subjects.19

Improved glycemic control obtained with either a 14-day continuous subcutaneous insulin infusion17 or intensification of dietary or pharmacological treatment11 reduced the level of the adhesion molecules whose concentration was increased when the glycemic control was poorer. In both studies, however, the posttreatment glycemic level was still elevated (7.5 to 7.8 mmol/L), and the diabetic patients evaluated in those studies had a long-lasting disease (≈10 years). In the present study, glucose control was normalized with an overnight insulin infusion, as also suggested by the normalization of labile HbA1 levels, which may fluctuate rapidly in response to rapid changes in plasma glucose concentrations.16

The source of sICAM-1 found in plasma is uncertain, although circulating forms of adhesion molecules may be derived from the corresponding component expressed on the surface of activated cells, including endothelial and smooth muscle cells.20,21 The endothelial cells seem a likely candidate, because they first receive the impact of acute hyperglycemia, which may reduce NO availability.19 Further support for this hypothesis comes from the results of a recent study that shows that ADP-activated platelets induce surface expression of ICAM-1 in cultured endothelial cells22: an additional effect of acute hyperglycemia in humans is to increase platelet aggregation in response to stimulants such as ADP, which, according to Grawaz et al,23 may contribute to the elevation of plasma sICAM-1 levels. Last, the increased plasma sICAM-1 levels seen after an oral glucose challenge in humans is completely prevented by the administration of the antioxidant glutathione,13 pointing to an important role for oxidative stress in mediation of the effects of hyperglycemia on sICAM-1 levels.

The present study introduces an additional aspect of how hyperglycemia may contribute to early stages of atherogenesis: high blood glucose levels might alter the adhesive properties of the endothelium by reducing NO availability. The relevance of this acute change to the chronic vascular complications of diabetes is, at present, speculative; moreover, the exact mechanism that links acute or chronic hyperglycemia with variations of sICAM-1 levels is not resolved in the present study. On the other hand, an association between the risk of future myocardial infarction and raised plasma concentrations of sICAM-1 was found in the US Physicians’ Health Study.5

References

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