Relation Between Soluble Adhesion Molecules and Insulin Sensitivity in Type 2 Diabetic Individuals

Role of adipose tissue

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OBJECTIVE — The purpose of this study was to explore the relation between insulin resistance and plasma levels of soluble adhesion molecules and to examine the effects of acute hyperinsulinemia on these molecules in type 2 diabetic individuals.

RESEARCH DESIGN AND METHODS — Intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and E- and P-selectin plasma concentrations were measured in 36 nonobese type 2 diabetic patients without cardiovascular disease and in 7 healthy subjects. Insulin sensitivity was assessed by a 4-h euglycemic (~5 mmol/l)-hyperinsulinemic (~300 pmol/l) clamp performed in combination with [^{3}H]3-D-glucose infusion.

RESULTS — Diabetic subjects were insulin resistant but did not show plasma concentrations of adhesion molecules that were significantly higher than control subjects. In diabetic subjects, plasma ICAM-1 and E-selectin were negatively correlated with total glucose disposal during the insulin clamp ($r = -0.432$, $P < 0.01$; and $r = -0.375$, $P < 0.05$, respectively), whereas plasma VCAM-1 and P-selectin were not. Plasma ICAM-1 as well as E- and P-selectin were positively correlated with BMI, total body fat (TBF), and waist girth ($P < 0.05$–0.001). In multiple regression analyses, the relation of plasma ICAM-1 and E-selectin with insulin sensitivity was lost after adjustment for potential confounders, including HbA_{1c}, blood pressure, and/or LDL cholesterol. In these analyses, BMI was the only independent predictor of plasma ICAM-1 ($R^2 = 0.244$, $P < 0.002$), whereas TBF was the only independent predictor of plasma E-selectin ($R^2 = 0.202$, $P = 0.01$). The 4-h insulin infusion during the glucose clamp did not significantly change plasma levels of adhesion molecules.

CONCLUSIONS — Overall adiposity, rather than insulin resistance, may be a determinant of plasma levels of ICAM-1 and E-selectin in type 2 diabetic individuals. In these patients, acute hyperinsulinemia does not exert any significant effect on plasma adhesion molecules. These findings support the possibility that adipose tissue releases one or more factors that may adversely affect endothelial function on one hand and insulin sensitivity on the other.

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Cellular adhesion molecules mediate attachment and transmigration of leukocytes across the endothelial surface and are thought to play a crucial role in the early steps of atherogenesis (1–3). Accordingly, increased plasma concentrations of intercellular adhesion molecule (ICAM)-1 and P-selectin independently predicted myocardial infarction in healthy individuals (4,5).

Several studies (2,3,6–8) demonstrated that plasma levels of adhesion molecules are increased in patients with obesity, dyslipidemia, hypertension, and type 2 diabetes, which are well-established risk factors for cardiovascular diseases (CVDs). Resistance to insulin-mediated glucose disposal and hyperinsulinemia are common features of these clinical conditions. This notion raises the possibility that the increased plasma levels of adhesion molecules found in obesity, dyslipidemia, hypertension, and type 2 diabetes are somehow related to insulin resistance and/or compensatory hyperinsulinemia. However, acute insulin infusion does not change plasma levels of adhesion molecules in both healthy subjects (9,10) and type 2 diabetic patients (11). This finding supports the conclusion that insulin resistance, rather than hyperinsulinemia, may be a factor contributing to the increased plasma levels of adhesion molecules in the clinical conditions mentioned above (12). Consistent with such a conclusion is the significant relation between the degree of insulin resistance and plasma levels of adhesion molecules recently found in healthy individuals (13).

The purpose of the present study was to explore the relation between plasma levels of adhesion molecules and insulin sensitivity and to examine the acute effect of hyperinsulinemia on plasma levels of these molecules in type 2 diabetic individuals without any clinical evidence of CVD.

RESEARCH DESIGN AND METHODS

— We examined 36 nonobese patients with type 2 diabetes who regularly attended our Diabetes Clinic. They were recruited over a period of 6 months to participate in a clinical trial on the effects of antihypertensive treatment on insulin sensitivity (14). The inclusion criteria were as follows: age 30–70 years,

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Abbreviations: AER, albumin excretion rate; CVD, cardiovascular disease; GIR, glucose infusion rate; ICAM, intercellular adhesion molecule; IL-6, interleukin-6; TBF, total body fat; TGD, total glucose disposal; TNF, tumor necrosis factor; VCAM, vascular cell adhesion molecule; WHR, waist-to-hip ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

BMI <30 kg/m^2, no treatment with insulin, no recent acute illness or clinical evidence suggestive of kidney or liver diseases, and no severe chronic diabetic complications (proliferative retinopathy, macroalbuminuria, symptomatic neuropathy, or clinically manifest CVD). Altogether, 5 patients were treated with diet only, and the remaining 31 were treated with diet and oral hypoglycemic agents (sulfonylureas alone, $n = 12$; sulfonylureas plus metformin, $n = 19$). Hypoglycemic medications were withheld on the morning of the study. A total of 16 diabetic patients had normal blood pressure and 20 had arterial hypertension, according to conventional criteria (15). Seven hypertensive subjects were treated with antihypertensive drugs (four with ACEIs and three with calcium channel blockers). None of the subjects were taking β-blockers, diuretics, or other medications known to interfere with glucose metabolism or endothelial function. In subjects treated for hypertension, the medications were discontinued at least 4 weeks before the study was performed. A group of seven healthy nondiabetic, nonobese subjects served as control subjects. Both diabetic and control subjects gave written informed consent. The protocol was reviewed and approved by the Ethical Committee of the University of Verona Medical School.

Blood pressure, anthropometric, and behavioral parameters

Blood pressure was measured with a standard mercury manometer. The mean of three measurements, taken at 5-min intervals, were averaged and used for the analysis. BMI was calculated by dividing weight (kilograms) into height (meters) squared (2). Waist circumference (widest between the lower rib margin and the iliac crest) and hip circumference (widest over the great trochanters) were measured in duplicate and used to calculate the waist-to-hip ratio (WHR), which was used as an index of regional fat distribution. A tetrapolar bioimpedance analyzer (BIA-103; Akern, Florence, Italy) was used to measure body electrical resistance and to derive an estimate of total body water, fat-free mass, and total body fat (TBF). The measures achieved with this technique strongly correlate with those generated by more sophisticated methods, including isotope water dilution (16). Information on smoking status was obtained through a standardized questionnaire. Subjects were categorized as those who had never smoked or quit smoking and those who were current smokers.

Glucose clamp studies

The study consisted of a 4-h euglycemic-hyperinsulinemic clamp associated with [^{3}H]3-D-glucose infusion, as previously reported (17). Briefly, teflon cannulas were inserted into an antecubital vein for infusion of insulin, glucose (20% dextrose), and [^{3}H]3-D-glucose and into a contralateral heated (~60°C) hand vein for arterialized blood sampling. After blood sampling for plasma glucose, insulin, lipids, and adhesion molecules in the basal state, a prime-constant (20 mU · min · m^2 body surface area) insulin infusion was started and continued for 240 min. The prime dose consisted of two subsequent 5-min periods of insulin infusion at the rate of 80 and 40 mU · min · m^2, respectively. In diabetic patients, plasma glucose was left to drop until euglycemia (~5 mmol/l) was reached (within 60–120 min in all subjects), maintained at that level by a variable glucose infusion, and adjusted every 5–10 min according to the change in plasma glucose. A prime-constant infusion of [^{3}H]3-D-glucose was initiated 2 h after the beginning of the glucose clamp at the rate of 0.45 μCi/min and continued until the end of the study. The prime dose of labeled glucose was calculated by dividing the glucose pool (plasma glucose concentration multiplied by glucose distribution volume, assumed to be 25% of body weight) by the product of 1.1 by the glucose infusion rate (GIR) in the 100–120 min period of the clamp and then multiplying the result by the tracer infusion rate. The GIR was multiplied by 1.1 to take into account the expected 10% average increase in GIR from 100–120 min to 180–240 min of the glucose clamp. As previously reported (17), with this methodological approach, a good steady state of the tritiated glucose specific activity is obtained during the last 60 min of the clamp (coefficient of variation <10%). During this period, blood was withdrawn every 10 min to measure plasma concentrations of glucose, insulin, and tritiated glucose specific activity. At 240 min of the glucose clamp, plasma levels of adhesion molecules were also measured. The insulin-mediated total glucose disposal (TGD) rate was calculated by dividing the [^{3}H]3-D-GIR by the steady-state [^{3}H]3-D-glucose specific activity (17).

Analytical determinations

Plasma glucose was measured by the glucose oxidase method on a Beckman Glucose Analyzer (Fullerton, CA). HbA_{1c} was measured by high performance liquid chromatography (Bio-Rad Diamat, Milan, Italy); normal range values in our laboratory were 3.0–5.5%. Plasma lipids were determined by an automatic colorimetric method (DAX 96; Bayer Diagnostics, Milan, Italy). LDL cholesterol was calculated by the Friedewald's equation, except when triglycerides exceeded 5 mmol/l. Urinary albumin excretion rate (AER) was measured on a timed (24 h) urine collection by a immunonephelometric method (Beckman Analyzer). Patients were classified as normoalbuminuric (AER <20 μg/min) or microalbuminuric (AER 20–200 μg/min). No patients had macroalbuminuria. Insulin was measured by a double antibody radioimmunoassay, with an antibody not crossreacting with proinsulin (Linco Research, St. Louis, MO). Plasma [^{3}H]3-D-glucose specific activity was determined as described in detail elsewhere (16). The assessment of plasma ICAM-1, vascular cell adhesion molecule (VCAM)-1, and E- and P-selectin concentrations was performed in duplicate by commercially available enzyme-linked immunosorbent assay kits (Bender MedSystems, Vienna). Intra- and interassay coefficients of variation were 5.1 and 6.9% for ICAM-1, 5.3 and 7.7% for VCAM-1, 4.8 and 7.4% for E-selectin, and 5.5 and 6.9% for P-selectin, respectively.

Statistical analysis

All data are presented as the means ± SD. The following statistical tests were used: paired Student's t test, χ^2 test, Pearson's simple correlation, and stepwise multiple regression analysis. Because of skewness and kurtosis of frequency distribution, adhesion molecules, triglycerides, insulin, and insulin-mediated TGD were logarithmically transformed for statistical analyses and then back-transformed to their natural units for presentation in the text and tables. Nonparametric statistical tests were also used, but because the results were very similar to those obtained by parametric procedures, only the latter were presented. $P < 0.05$ was considered statistically significant.

Table 1—*Main clinical and biochemical characteristics of type 2 diabetic patients and control subjects*

	Diabetic patients	Control subjects	*P*
Sex (M/F)	30/6	5/2	NS
Age (years)	55 ± 6.0 (44–67)	52 ± 4.0 (47–60)	NS
Weight (kg)	75 ± 10 (52–96)	67 ± 8.0 (59–80)	<0.05
BMI (kg/m^2)	26.5 ± 2.5 (20–29.8)	24.5 ± 2.1 (22–28)	<0.05
TBF (kg)	22 ± 6.0 (9–35)	19 ± 4.0 (6–25)	<0.05
FFM (kg)	53 ± 7.0 (34–67)	48 ± 9.0 (35–58)	<0.05
Waist girth (cm)	96 ± 7.0 (81–111)	84 ± 9.0 (74–91)	<0.05
WHR	0.97 ± 0.05 (0.83–1.05)	0.81 ± 0.11 (0.68–0.89)	<0.01
Systolic BP (mmHg)	148 ± 19 (105–187)	121 ± 13 (105–135)	<0.01
Diastolic BP (mmHg)	91 ± 12 (68–114)	79 ± 6.0 (65–88)	<0.01
Fasting insulin (pmol/l)	70 ± 7.0 (35–110)	40 ± 5.0 (10–49)	<0.01
Fasting glucose (mmol/l)	10.3 ± 2 (7–16.5)	5.1 ± 1.0 (4.8–6.0)	<0.01
HbA_{1c} (%)	6.7 ± 1.2 (4.2–9.0)	ND	ND
LDL cholesterol (mmol/l)	3.7 ± 1.1 (1.6–6.9)	3.2 ± 0.5 (1.4–5.7)	NS
HDL cholesterol (mmol/l)	1.07 ± 0.41 (0.4–1.8)	1.23 ± 0.15 (0.7–2.1)	NS
Triglycerides (mmol/l)	2.2 ± 1.12 (0.6–5.3)	1.6 ± 0.51 (0.5–2.9)	NS
ICAM-1 (ng/ml)	278 ± 81 (159–473)	266 ± 74 (155–396)	NS
VCAM-1 (ng/ml)	630 ± 175 (375–1,103)	551 ± 109 (452–782)	NS
E-selectin (ng/ml)	42 ± 20 (12–114)	48 ± 31 (18–109)	NS
P-selectin (ng/ml)	89 ± 61 (28–326)	99 ± 30 (73–160)	NS
TGD during clamp (μmol · min^{-1} · kg FFM^{-1})	22 ± 11 (8.0–61)	53 ± 13 (35–76)	<0.01

Data are means ± SD (range). BP, blood pressure; FFM, fat-free mass; ND, not determined; NS, not significant.

RESULTS — The main clinical and biochemical characteristics of type 2 diabetic patients and control subjects are summarized in Table 1. On average, diabetic patients were mildly overweight, hypertensive, and dyslipidemic. Their average glycometabolic control was good. Compared with healthy control subjects, diabetic patients were markedly insulin resistant and had only slightly but not significantly higher plasma levels of ICAM-1 and VCAM-1. E- and P-selectin were similar in the two groups.

Simple correlations of adhesion molecules with clinical and biochemical features in diabetic patients are reported in Table 2. ICAM-1 significantly correlated with BMI, waist girth, diastolic blood pressure, and HbA_{1c}. E-selectin significantly correlated with BMI, TBF, waist girth, WHR, LDL cholesterol, and HbA_{1c}. P-selectin significantly correlated with TBF, waist girth, and WHR. VCAM-1 did not significantly correlate with any of the study variables.

Figure 1 shows the scattergrams of simple correlations between adhesion molecules and TGD during the euglycemic clamp. ICAM-1 and E-selectin were inversely and significantly correlated with insulin sensitivity, whereas VCAM-1 and P-selectin were not.

When diabetic subjects were divided into subgroups according to the median value of insulin sensitivity, those below the median (i.e., the most insulin-resistant subgroup) had significantly higher plasma levels of ICAM-1 and E-selectin but not VCAM-1 and P-selectin compared with those above the median (data not shown).

In stepwise multiple regression analyses, the relation of ICAM-1 and E-selectin with insulin-mediated TGD was lost after adjustment for the other variables that significantly correlated with ad-

Table 2—*Simple correlations between plasma adhesion molecules and main clinical and biochemical features in type 2 diabetic patients*

	ICAM-1	E-selectin	P-selectin	VCAM-1
Sex	0.064	−0.004	0.006	−0.149
Age	0.017	−0.060	−0.161	0.047
BMI	0.522*	0.516*	0.283	0.094
TBF	0.287	0.565†	0.336‡	0.173
Fat-free mass	0.285	0.184	0.056	0.036
Waist girth	0.427*	0.517*	0.393‡	0.188
WHR	0.266	0.336‡	0.370‡	−0.024
Systolic BP	0.140	0.070	0.169	0.177
Diastolic BP	0.330‡	0.009	0.047	0.225
LDL cholesterol	−0.031	0.338‡	0.189	−0.285
HDL cholesterol	−0.253	−0.049	0.099	−0.318
Total/HDL cholesterol	0.152	0.298	0.129	0.114
Triglycerides	0.135	0.251	0.240	0.233
Fasting insulin	0.180	0.155	−0.003	0.234
Fasting glucose	0.065	0.257	0.137	0.196
HbA_{1c}	0.379‡	0.424*	0.111	0.201
Smoking	0.088	0.301	0.147	−0.197

BP, blood pressure. *$P < 0.01$; †$P < 0.001$; ‡$P < 0.05$.

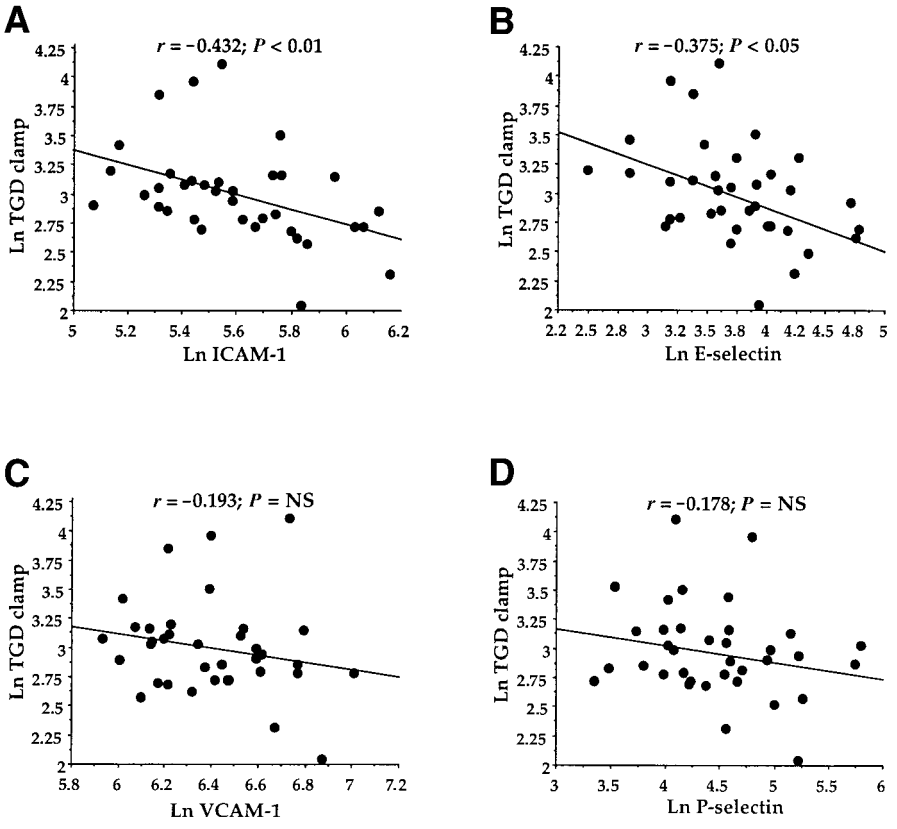

Figure 1—*Relation between insulin-mediated total glucose disposal and plasma adhesion molecules (ICAM-1 [A], E-selectin [B], VCAM-1 [C], and P-selectin [D]) in type 2 diabetic patients.*

hesion molecules in univariate analyses (BMI or TBF, waist girth, diastolic blood pressure, LDL cholesterol, and/or HbA_{1c}). These analyses confirmed that BMI was the only independent predictor of ICAM-1 ($R^2 = 0.244$, $P < 0.002$), whereas TBF was the only independent predictor of E-selectin ($R^2 = 0.202$, $P = 0.01$).

The 4-h insulin infusion during euglycemic clamp, which increased plasma insulin levels approximately fourfold above basal (from 70 ± 7 to 265 ± 10 pmol/l), did not significantly change the plasma levels of any adhesion molecule (ICAM-1 272 ± 89 vs. 278 ± 81 ng/ml; VCAM-1 614 ± 146 vs. 630 ± 175 ng/ml; E-selectin 41 ± 21 vs. 42 ± 20 ng/ml; P-selectin 87 ± 53 vs. 89 ± 61 ng/ml at the end of insulin infusion and at baseline, respectively).

CONCLUSIONS — The main findings of this study, which was performed in type 2 diabetic subjects without any clinical evidence of CVD, are as follows: *1*) insulin sensitivity is inversely correlated with plasma ICAM-1 and E-selectin, *2*) this relation is lost after adjustment for BMI or TBF, *3*) BMI and TBF are independently correlated with ICAM-1 and E-selectin, and *4*) acute insulin infusion does not significantly change circulating adhesion molecules in type 2 diabetes.

The finding that insulin sensitivity, as assessed by euglycemic clamp, is inversely correlated with ICAM-1 and E-selectin extends recent data by Chen et al. (13), who reported a positive relation of insulin resistance, as estimated by the insulin suppression test, with plasma ICAM-1 and E-selectin concentrations in 28 healthy, nondiabetic individuals. Moreover, our data are consistent with those reported by Lim et al. (18), who found a positive relation between ICAM-1 and insulin resistance in 59 obese type 2 diabetic subjects. However, in the latter study (18), insulin resistance was estimated by the homeostasis model assessment, which is a good but only surrogate index of insulin sensitivity in vivo (19).

Overall, our data support the concept that insulin resistance might be related to endothelial dysfunction. Such a hypothesis is plausible because insulin enhances nitric oxide production within the endothelial cells (20,21), and nitric oxide modulates a number of endothelial functions, including the expression of adhesion molecules (20,22). However, the negative relation of plasma ICAM-1 and E-selectin concentrations with insulin sensitivity was lost after adjusting for BMI or TBF. This finding suggests that the relation between adhesion molecules and insulin sensitivity might be an epiphenomenon of the correlations observed between adhesion molecules and adiposity indexes on one side and adiposity indexes and insulin sensitivity on the other. In other words, adipose tissue might be the link (a common denominator) between insulin sensitivity and adhesion molecules. Such a conclusion is consistent with the notion that a significant univariate correlation between two variables is not immediate proof of a cause-and-effect relation.

The significant and independent correlations between adiposity indexes and plasma levels of E-selectin and ICAM-1 that we observed in the present study are consistent with data reported in both a large cross-sectional survey of healthy men (23) and a clinical study of obese individuals (8). In both of these studies, E-selectin and ICAM-1 were significantly correlated with BMI and were significantly higher in obese individuals than in nonobese individuals. Furthermore, it was reported that weight loss resulted in a significant reduction of plasma adhesion molecules in obese subjects (8).

The reason why plasma adhesion molecules are correlated with BMI and TBF and why they decrease with weight loss is poorly understood. It is known that an excess of adipose tissue can determine a low grade of inflammation through the release of adipocyte-secreted proteins, such as tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) (24–26). Such an adiposity-induced mild chronic inflammation might induce insulin resistance on one hand and endothelial dysfunction on the other, thus linking adiposity indexes with insulin resistance and increased plasma levels of adhesion molecules (24–26). A further possibility is that adiponectin, a novel adipocyte-derived secretory protein that is decreased in obesity, might modulate endothelial function by inhibiting TNF-α–induced expression of E-selectin and ICAM-1 (27).

In this study, a 4-h physiologic hyperinsulinemia did not significantly change plasma levels of adhesion molecules. This finding is consistent with data reported by other investigators in both nondiabetic (9) and type 2 diabetic (11) individuals. One might hypothesize that an effect of insulin might be detected with either a higher level of hyperinsulinemia or a longer duration of insulin infusion than those applied in the present study. This would be particularly true if insulin increased the expression (production), rather than the release, of adhesion molecules. However, Jilma et al. (10) showed that a 6.5-h euglycemic-hyperinsulinemic clamp, which resulted in pharmacological plasma insulin levels (~1,500 pmol/l), did not significantly change plasma levels of adhesion molecules in nondiabetic individuals. Moreover, 4 h of hyperinsulinemia is generally a time interval sufficient in length to elicit even the less prompt biological effects of the hormone (28).

In the present study, we excluded patients with clinically manifest CVD or other severe chronic diabetic complications. Because plasma adhesion molecules are increased in subjects with CVD (1–3,11), the result may have been the exclusion of patients with the highest plasma levels of ICAM-1, VCAM-1, and E- and/or P-selectin and the creation of an obstacle that prevented better correlations between the variables under study. However, it is unlikely that the results of both the univariate and multivariate analyses would have been substantially different, as the ranges of plasma adhesion molecules in patients we examined were wide.

In conclusion, the results of the present study suggest that overall adiposity, rather than insulin resistance, may be a determinant of plasma ICAM-1 and E-selectin levels in type 2 diabetic individuals without clinical evidence of CVD. These findings support the possibility that adipose tissue releases one or more factors (i.e., TNF-α and IL-6) that may adversely affect endothelial function on one hand and insulin sensitivity on the other.

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