High Serum Concentrations of Soluble E-Selectin Correlate With Obesity But Not Fat Distribution in Patients With Type 2 Diabetes Mellitus

Kazunari Matsumoto, Yasunori Sera, Yasuyo Abe, Tan Tominaga, Kensaku Horikami, Koichi Hirao, Yukitaka Ueki, and Seibei Miyake

Serum concentrations of soluble adhesion molecules, eg, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin are elevated in patients with type 2 diabetes. However, little is known about the role of obesity or abnormal fat distribution in inducing upregulation of adhesion molecules. To investigate this issue, soluble ICAM-1, VCAM-1, and E-selectin levels were evaluated in 40 obese and 30 nonobese patients with type 2 diabetes. Both groups were matched for age, sex, and glycosylated hemoglobin (HbA_{1c}) levels. Computed tomography (CT) was used to measure the abdominal subcutaneous and visceral fat areas. Soluble ICAM-1 and VCAM-1 levels did not differ significantly between obese and nonobese patients. However, serum concentrations of soluble E-selectin were significantly higher in obese than in nonobese patients (90 ± 7 *v* 56 ± 4 ng/mL, *P* < .01). Soluble E-selectin levels significantly correlated with body mass index, subcutaneous fat area, and visceral fat area (Rho = 0.48, 0.37, and 0.30, respectively). Stepwise multiple regression analysis showed that body mass index (F = 16.7), but not subcutaneous and visceral fat areas (F = 0.29 and 0.01, respectively), significantly and independently correlated with soluble E-selectin levels. Our results suggest that obesity may induce endothelial activation or increased shedding of cell surface E-selectin that leads to subsequent increase in soluble E-selectin levels. The high serum concentrations of E-selectin closely correlated with increased total fat volume, but not with regional fat distribution.

LEUKOCYTE ADHESION molecules are thought to play important roles in the development of atherosclerosis through leukocyte adhesion, migration, and foam cell formation.[1] Indeed, upregulation of various adhesion molecules is observed in atherosclerotic plaques.[2,3] In patients with type 2 diabetes, high serum levels of soluble adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin have been reported in many studies.[4-7] The exact mechanism of the elevation of soluble adhesion molecules is not fully understood, but some investigators suggest the contribution of hyperglycemia, hyperinsulinemia, and insulin resistance.[8-11]

Obesity is strongly related to both insulin resistance and type 2 diabetes and is an important risk factor for atherosclerosis.[12,13] The metabolic abnormalities in obesity are known to correlate with total fat volume, as well as fat distribution.[14,15] However, whether obesity and abnormal fat distribution induce upregulation of adhesion molecules in patients with type 2 diabetes remains unknown. In this regard, computed tomography (CT) scan can precisely measure cross-sectional abdominal subcutaneous and visceral fat areas.[16] In the present study, we measured serum concentrations of soluble ICAM-1, VCAM-1, and E-selectin in nonobese and obese patients with type 2 diabetes and investigated the correlation between adhesion molecules and fat distribution assessed by the CT scan.

From the Departments of Diabetes and Metabolism, Endocrinology, Radiology, and Rheumatology, Sasebo Chuo Hospital, Nagasaki, Japan.

Submitted November 14, 2001; accepted January 8, 2002.

Address reprint requests to Kazunari Matsumoto, MD, Department of Diabetes and Metabolism, Sasebo Chuo Hospital, 15 Yamato-cho, Sasebo, Nagasaki 857-1195, Japan.

0026-0495/02/5107-0018$35.00/0
doi:10.1053/meta.2002.33354

SUBJECTS AND METHODS

A total of 70 Japanese patients with type 2 diabetes gave informed consent to participate in this case-control study. The study protocol was approved by the Ethics Committee of Sasebo Chuo Hospital. Type 2 diabetes was diagnosed based on the criteria of the World Health Organization.[17] Seventeen patients were treated with diet alone, 41 patients were treated with oral hypoglycemic agents, and 12 patients were treated with insulin. Obesity represented body mass index of more than 25 kg/m^2. Forty obese patients served as the obese group. Thirty nonobese patients who were matched for age, sex, and glycosylted hemoglobin (HbA_{1c}) served as the control. The clinical characteristics of the nonobese and obese patients are listed in Table 1.

A blood sample was drawn in the morning after 12-hour fast. Plasma glucose was measured with the glucose oxidase method (Kyoto-Daiichi Kagaku, Kyoto, Japan). HbA_{1c} was measured with a high-performance liquid chromatography (HPLC) method (Tosoh, Tokyo, Japan). Total cholesterol and triglycerides were measured with the enzymatic method (Kokusai Shiyaku, Kobe, Japan). High-density lipoprotein (HDL) cholesterol was determined after isolation by a precipitation method (Kyowa, Tokyo, Japan). Soluble ICAM-1, VCAM-1, and E-selectin were measured by commercially available enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN).

All patients underwent CT (HiSpeed Advantage; General Electric Medical Systems, Milwaukee, MI) to measure cross-sectional abdominal subcutaneous and visceral fat areas. Patients were examined in the supine position, and CT scans were performed at the umbilical levels. Adipose tissue areas were determined using commercially available software (Fat Scan; N2 System, Osaka, Japan).[16]

Data are presented as mean ± SEM. Comparisons between nonobese and obese patients were conducted by Mann-Whitney *U* test or contingency table analysis. Correlation coefficients were calculated by Spearman's method. Stepwise multiple regression analysis was used to examine the relationships between soluble adhesion molecules and adiposity. Differences were considered statistically significant at *P* less than .05. In multivariate analysis, F values ≥ 4 were considered as significant. Statistical analysis was performed using Statview 5.0 (SAS, Cary, NC).

RESULTS

By definition, obese patients had significantly greater body mass index (Table 1), together with larger subcutaneous and

Table 1. Clinical Characteristics, Adiposity, Biochemical Data, and Soluble Adhesion Molecules in Nonobese and Obese Patients With Type 2 Diabetes

	Nonobese Patients	Obese Patients
No. (men/women)	30 (18/12)	40 (24/16)
Age (yr)	60.6 ± 1.9	59.5 ± 2.0
Duration of diabetes (yr)	6.9 ± 1.0	5.9 ± 0.7
Body mass index (kg/m^2)	22.9 ± 0.3	29.4 ± 0.7*
Subcutaneous fat area (cm^2)	131.1 ± 9.0	222.7 ± 15.8*
Visceral fat area (cm^2)	104.6 ± 8.0	165.9 ± 7.8*
HbA_{1c} (%)	7.7 ± 0.3	7.8 ± 0.3
Fasting glucose (mmol/L)	7.6 ± 0.3	7.2 ± 0.3
Total cholesterol (mmol/L)	5.1 ± 0.2	5.1 ± 0.2
Triglyceride (mmol/L)	1.3 ± 0.1	1.8 ± 0.2*
HDL cholesterol (mmol/L)	1.4 ± 0.1	1.3 ± 0.1
ICAM-1 (ng/mL)	206 ± 14	241 ± 15
VCAM-1 (ng/mL)	866 ± 41	830 ± 24
E-selectin (ng/mL)	56 ± 4	90 ± 7*

NOTE. Data are mean ± SEM of number of patients.
*$P < .01$ *v* nonobese patients.

visceral fat areas, compared with nonobese patients. Fasting glucose and total and HDL cholesterol levels were comparable between the groups. Triglyceride levels were significantly higher in obese patients than in nonobese patients. Serum levels of soluble ICAM-1 tended to be higher in obese patients than nonobese patients, but the difference did not reach statistical significance ($P = .07$). Soluble VCAM-1 levels were comparable between the 2 groups ($P = .286$). Soluble E-selectin levels were significantly higher in obese patients than nonobese patients ($P < .001$).

The results of Spearman's correlation coefficient between soluble adhesion molecules and total adiposity (body mass index) and fat distribution are listed in Table 2. Adiposity did not correlate with soluble ICAM-1 and VCAM-1 levels. However, total adiposity and regional adiposity significantly correlated with soluble E-selectin levels (Table 2 and Fig 1). Stepwise multiple regression analysis showed that the soluble E-selectin level was predicted by body mass index (F = 16.7) and HbA_{1c} (F = 8.8). However, subcutaneous (F = 0.3) and visceral (F = 0.1) fat areas did not predict soluble E-selectin level. Thus, soluble E-selectin level was significantly related to total adiposity (body mass index), but not to regional fat distribution.

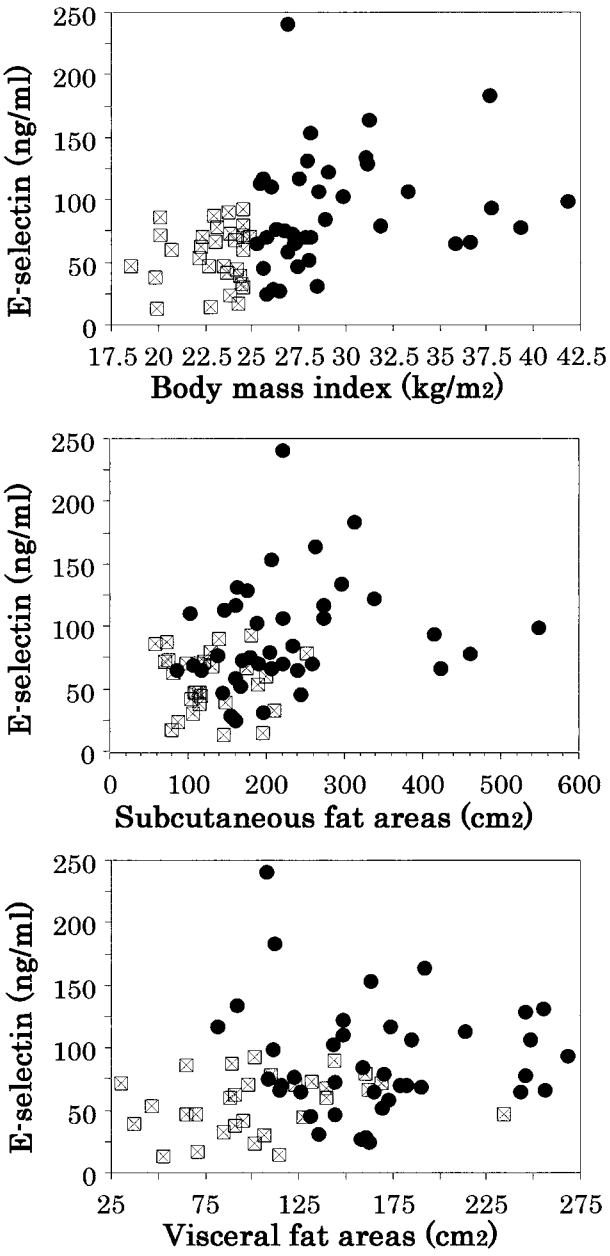

Fig 1. The relationships between soluble E-selectin levels and body mass index, subcutaneous fat areas, and visceral fat areas in patients studied. (☒, nonobese patients with type 2 diabetes mellitus; ●, obese patients with type 2 diabetes mellitus. Spearman's correlation coefficients are listed in Table 2.

DISCUSSION

Upper body fat distribution or visceral fat accumulation was reported to be associated with various metabolic abnormalities in nondiabetic and diabetic patients.[18-20] In contrast, Abate et al[21] reported that subcutaneous rather than intraperitoneal or retroperitoneal fat volume was associated with insulin resistance in patients with type 2 diabetes. With regard to adhesion molecules, to our knowledge, no studies have demonstrated a correlation between fat distribution and serum levels of soluble adhesion molecules in patients with type 2 diabetes. In the present study, we have shown that obesity may induce endothelial activation and subsequently increase serum levels of soluble E-selectin. The

Table 2. Spearman's Correlation Coefficient Between Soluble Adhesion Molecules and Total, Subcutaneous, and Visceral Adiposity

	ICAM-1		VCAM-1		E-Selectin	
	Rho	*P* Value	Rho	*P* Value	Rho	*P* Value
Body mass index	0.19	.108	0.03	.829	0.48	<.001
Subcutaneous fat area	0.18	.143	0.04	.759	0.37	.002
Visceral fat area	0.12	.310	0.09	.476	0.30	.012

elevated levels of E-selectin closely correlated with total fat volume rather than regional fat distribution.

The reason for high serum concentrations of E-selectin in obese subjects may be partly explained by adiponectin. Adiponectin is an adipocyte-specific secretory protein, which is reduced in obesity.[22] Ouchi et al[23] reported that adiponectin inhibits the expression of adhesion molecules on human aortic endothelial cells. Thus, an increase of soluble E-selectin in the serum may be related to a decrease in adiponectin in obesity. Another possibility is that the same amount of E-selectin surface expression may be occurring throughout the vasculature, but the shedding of E-selectin, although the mechanisms are poorly understood, may be sensitive to fat-derived mediator. Further examination is needed to clarify these hypotheses.

The reason for the lack of effect of obesity on serum levels of ICAM-1 and VCAM-1 is presently unknown. In our recent study, ICAM-1 and VCAM-1 did not correlate with body mass index, but only E-selectin correlated significantly in 150 Japanese patients with type 2 diabetes.[24] Hwang et al[25] also reported that soluble E-selectin, but not ICAM-1 and VCAM-1, correlate with body mass index in subjects in The Atherosclerosis Risk In Communities Study. Therefore, the impact of obesity may be stronger on E-selectin than ICAM-1 and VCAM-1.

We recently reported that serum levels of E-selectin decreased following diet therapy, as well as oral hypoglycemic agents and insulin, in patients with type 2 diabetes.[26] Patients treated by diet alone showed significant reductions of body weight and E-selectin levels.[26] These results suggest that reduction of body weight in obese patients with type 2 diabetes may also result in reduction of serum concentrations of soluble E-selectin.

In conclusion, although our study was performed in a limited number of patients, our results indicated that obesity can induce endothelial activation and increase serum levels of soluble E-selectin in patients with type 2 diabetes. In these patients, elevation of E-selectin seems to be related to total fat volume rather than fat distribution.

REFERENCES

1. Carter AM, Grant PJ: Vascular homeostasis, adhesion molecules, and macrovascular disease in non-insulin-dependent diabetes mellitus. Diabet Med 14:423-432, 1997

2. Richardson M, Hadcock SJ, DeReske M, et al: Increased expression in vivo of VCAM-1 and E-selectin by the aortic endothelium of normolipemic and hyperlipemic diabetic rabbits. Arterioscler Thromb 14:760-769, 1994

3. Iiyama K, Hajra L, Iiyama M, et al: Patterns of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in rabbit and mouse atherosclerotic lesions and at sites predisposed to lesion formation. Circ Res 85:199-207, 1999

4. Cominacini L, Pasini AF, Grabin U, et al: Elevated levels of soluble E-selectin in patients with IDDM and NIDDM: Relation to metabolic control. Diabetologia 38:1122-1124, 1995

5. Ceriello A, Falleti E, Bortolotti N, et al: Increased circulating intercellular adhesion molecule-1 levels in type II diabetic patients: The possible role of metabolic control and oxidative stress. Metabolism 45:498-501, 1996

6. Otsuki M, Hashimoto K, Morimoto Y, et al: Circulating vascular cell adhesion molecule-1 (VCAM-1) in atherosclerotic NIDDM patients. Diabetes 46:2096-2101, 1997

7. Albertini JP, Valensi P, Lormeau B, et al: Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule-1 in NIDDM. Diabetes Care 21:1008-1013, 1998

8. Cominacini L, Pasini AF, Garbin U, et al: E-selectin plasma concentration is influenced by glycemic control in NIDDM: Possible role of oxidative stress. Diabetologia 40:584-589, 1997

9. Morigi M, Angioletti S, Imberti B, et al: Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-κB dependent fashion. J Clin Invest 101:1905-1915, 1998

10. Matsumoto K, Miyake S, Yano M, et al: High serum concentrations of soluble E-selectin in patients with impaired glucose tolerance with hyperinsulinemia. Athrosclerosis 152:415-420, 2000

11. Matsumoto K, Sera Y, Abe Y, et al: Serum concentrations of soluble vascular cell adhesion molecule-1 and E-selectin are elevated in insulin-resistant patients with type 2 diabetes (Letter). Diabetes Care 24:1697-1698, 2001

12. Kissebah AH, Krakower GR: Regional adiposity and morbidity. Physiol Rev 74:761-811, 1994

13. Steinberg HO, Chaker H, Leaming R, et al: Obesity/insulin resistance is associated with endothelial dysfunction. J Clin Invest 97:2601-2610, 1996

14. Fujioka S, Matsuzawa Y, Tokunaga K, et al: Contribution of intra-abdominal fat accumulation to the impairment of glucose and lipid metabolism in human obesity. Metabolism 36:54-59, 1987

15. Despres JP, Lamarche B: Effects of diet and physical activity of adiposity and body fat distribution: Implications for the prevention of cardiovascular disease. Nutr Rev 6:137-159, 1993

16. Yoshizumi T, Nakamura T, Yamane M, et al: Abdominal fat: Standardized technique for measurement at CT. Radiology 211:283-286, 1999

17. Alberti KGMM, Zimmet PZ for the WHO Consultation, Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Daibet Med 15:539-553, 1998

18. Kissebah AH, Vydelingum N, Murray R, et al: Relation of body fat distribution to metabolic complication of obesity. J Clin Endocrinol Metab 54:254-260, 1982

19. Kanai H, Matsuzawa Y, Kotani K, et al: Close correlation of intra-abdominal fat accumulation to hypertension in obese women. Hypertension 16:484-490, 1990

20. Banerji MA, Chaiken RL, Gordon D, et al: Does intra-abdominal adipose tissue in black men determine whether NIDDM is insulin-resistant or insulin-sensitive? Diabetes 44:141-146, 1995

21. Abate N, Garg A, Peshock RM, et al: Relationship of generalized and regional adiposity to insulin sensitivity in men with NIDDM. Diabetes 45:1684-1693, 1996

22. Arita Y, Kihara S, Ouchi N, et al: Paradoxical decrease of an adipocyte-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79-83, 1999

23. Ouchi N, Kihara S, Arita Y, et al: Novel modulator for endothelial adhesion molecules. Adipocyte-derived plasma protein adiponectin. Circulation 100:2473-2476, 1999

24. Matsumoto K, Sera Y, Nakamura H, et al: Serum concentrations of soluble adhesion molecules are related to degree of hyperglycemia and insulin resistance in patients with type 2 diabetes mellitus. Diab Res Clin Pract 55:131-138, 2002

25. Hwang SJ, Ballantyne CM, Sharrett AR, et al: Circulating adhesion molecules VCAM-1, ICAM-1, and E-selectin in carotid atherosclerosis and incident coronary heart disease cases. The Atherosclerosis Risk In Communities (ARIC) Study. Circulation 96:4219-4225, 1997

26. Matsumoto K, Nakamura H, Ueki Y, et al: Correction of hyperglycemia reduces insulin resistance and serum soluble E-selectin levels in patients with type 2 diabetes mellitus. Diabet Med 18:224-228, 2001