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Alteration of serum adhesion molecules and cutaneous endothelium-dependent vasodilatation in insulin resistant obese patients

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Abstract

Insulin resistance (IR) is associated with decreased endothelium-dependent vasodilatation. Adhesion molecules are regarded as endothelial dysfunction biomarkers, which are markedly up-regulated in obesity-linked diseases, including coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM). The purpose of the study was to evaluate the relationships between IR, vascular cell adhesion molecule-1 (sVCAM-1), intercellular cell adhesion molecule-1 (sICAM-1), sE-selectin, and endothelium-dependent vasodilatation in metabolic syndrome (MetS) patients who were categorized as having T2DM, both T2DM and CAD, or neither. Obese MetS patients with dyslipidemia were classified into three groups: 34 patients with T2DM (D), 20 patients with T2DM and CAD (DC), and 26 patients with MetS alone (M). Eighteen healthy subjects were selected as controls (C). The study groups were matched for age and sex. IR was assessed by HOMA-IR method, and serum sVCAM-1, sICAM-1, and sE-selectin levels were measured by xMAP technology. Laser Doppler imaging with iontophoretic application of 1% acethylcholine (LDI-Ach) solution was used for the evaluation of cutaneous endothelium-dependent vasodilatation in the hand. Serum levels of sVCAM-1, sICAM-1, and sE-selectin were significantly higher in the group of DC patients compared with the levels in other groups ($p < 0.01$), except for sICAM-1 in the D group. Also, sVCAM-1, sICAM-1, and sE-selectin concentrations were significantly correlated with HOMA-IR indexes ($p < 0.0001$). Only D and DC patient groups demonstrated a significant and similar decline in LDI-Ach marker compared to the group of healthy subjects ($p < 0.001$). LDI-Ach values were significantly correlated with HOMA-IR indexes, sVCAM-1, sICAM-1, and sE-selectin levels ($p < 0.01$). Our findings show that obese MetS patients with T2DM have more higher serum levels of adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin), simultaneously with both higher IR and lower endothelium-dependent vasodilatation than those with MetS alone, and the presence of

CAD in these patients is associated with greater changes in the endothelial dysfunction markers. IR was observed to be a closely related to endothelial dysfunction.

Key words: adhesion molecule, endothelium-dependent vasodilatation, insulin resistance, metabolic syndrome.

Introduction

Adhesion molecules are vascular inflammatory markers for endothelial dysfunction (Ponthieux et al. 2004). They mediate the binding of circulating leukocytes to endothelial cells and their subsequent migration into the blood vessel wall, which is an important step in the initiation of atherosclerotic lesions. Focal expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) has been demonstrated in human atherosclerotic plaques (Davies et al. 1993). The systemic inflammatory markers C-reactive protein (CRP), oxidized low-density lipoprotein, IL-1, and NF-κB can initiate VCAM-1 and ICAM-1 expression (van der Meer et al. 2002). The levels of soluble adhesion molecules like sICAM-1 and sVCAM-1 reflect the expression of membrane-bound adhesion molecules and vascular inflammation of the vessel wall (Gearing, Newman 1993). E-selectin binds neutrophils, monocytes, eosinophils, basophils, natural killer cells, and subsets of lymphocytes. This adhesion molecule is important in the initial steps of leukocyte extravasation into inflamed tissues (Wagers et al. 1996).

Adipose tissue is thought to be able to produce inflammatory markers such as IL-6 and TNF-α, which in turn are able to stimulate the expression of cellular adhesion molecules. Higher levels of sE-selectin in obesity have been described in both men (Hwang et al. 1997) and women (Ito et al. 2002), in relation with body mass index (BMI) (Weyer et al. 2002). Conflicting results exist for the relation of sVCAM-1 and sICAM-1 levels with obesity. Some reports have shown an association between both sVCAM-1 and sICAM-1 on the one hand, and BMI on the other, while others have not. When obesity is accompanied by type-2 diabetes mellitus (T2DM), levels of sE-selectin are associated with measures of obesity (Schram, Stehouwer 2005).

Studies on the association of endothelial cell adhesion molecules with insulin resistance (IR) in non-diabetic individuals showed that the concentrations of sVCAM-1, sICAM1 and sE-selectin were significantly elevated in IR individuals (Chen et al. 1999; Hak et al. 2001), although not all studies demonstrated this for sVCAM-1 (Weyer et al. 2002). Some studies showed no positive relationship between high levels of adhesion molecules and coronary artery disease (CAD) risk, but most have demonstrated this (Blake, Ridker 2002). Concentrations of soluble adhesion molecules have been consistently shown to be increased in diabetic patients and in subjects with IR. They correlate with various cardiovascular risk factors such as smoking, hypertension, low high-density lipoprotein (HDL)-cholesterol and hypercholesterolemia (Blankenberg et al. 2001).

Metabolic syndrome (MetS) is considered to be a state of chronic inflammation closely associated with endothelial dysfunction causing an increased incidence of ischemic cardiovascular events and high mortality. Low-grade inflammation is observed in patients with increased plasma levels of sVCAM-1, sICAM-1 and sE-selectin (Gonzalez, Selwyn 2003), which are correlated with inflammatory markers e.g., CRP, TNF-α, IL-6, and IL-1 (Ruotsalainen et al. 2008).

The purpose of the study was to evaluate the relationships between IR, sVCAM-1, sICAM-1, sE-selectin, and cutaneous endothelium-dependent vasodilatation in MetS patients who were categorized as having T2DM, both T2DM and CAD, or neither.

Materials and methods

Subjects

Obese MetS patients with dyslipidemia, who were recruited in the study, were classified into three groups: 34 patients with T2DM (D); 20 patients with T2DM and CAD (DC), and 26 patients with MetS alone (M). Eighteen healthy subjects were selected as controls (C). The study groups were matched for age and sex.

MetS was diagnosed according to the International Diabetes Foundation criteria with specific reference to the European population (Alberti et al. 2005). Patients were not included if their systolic blood pressure was ≥ 160 mm Hg or diastolic ≥ 95 mm Hg and if they were treated with antihypertensive drugs other than angiotensin-converting enzyme inhibitors. Diabetes was defined as a reported history of diabetes and treatment with antidiabetic drugs. Duration of T2DM was 8 ± 5 years and glycated hemoglobin HbA1c was less than 7.5 %. The diabetics did not undergo insulin therapy and lacked pronounced diabetic complications.

The diagnosis of CAD was substantiated by coronary angiography. Digital coronary angiography was performed by means of a GE Medical System X-ray digital angiography system. Results of coronary angiography were accepted as positive if stenosis ≥ 50 % of at least one of the three main epicardial branches of coronary arteries was detected. Patients with acute coronary syndrome and those who had evidence of peripheral vascular disease or cerebral ischemia were not included. Other exclusion factors were acute inflammatory condition or chronic inflammatory states such as rheumatoid arthritis, systemic lupus erythematosus, vasculitis, inflammatory bowel disease, surgery and trauma within the preceding 30 days, and other diseases known to be associated with significant changes of cytokines. Malignancy, alcoholism and smoking were also exclusion criteria. We did not include patients who were using COX-2 inhibitors, nonsteroidal antiinflammatory agents or corticosteroids, or had used them within the preceding 30 days.

All subjects gave their informed consent to the protocol, which was approved by the local Medical Ethics Committee of the University of Latvia for Biomedical Research.

Biochemical measurements

Blood samples (5 mL) for the determination of cytokines were collected after a 12-h fast and allowed to coagulate for 20 to 30 min at room temperature. Sera were separated by centrifugation at 4 °C for 20 min at 1600 × g. All specimens were immediately aliquoted, frozen, and stored at –80 °C. sVCAM-1, sICAM-1, and sE-selectin concentrations were measured by xMAP multiplex immunobead assay technology (Luminex200 analyzer, Luminex Corp., Austin, TX) (Kofoed et al. 2006). We used homeostasis model assessment (HOMA-IR) to quantify IR (fasting glucose × fasting insulin / 22.5) (Matthews et al. 1995). HOMA-IR values have been shown to correlate well with values obtained using the "gold standard" clamp technique (Bonora et al. 2000). Fasting concentrations of lipids, insulin, and glucose were analyzed by standard methods.

Blood flow measurements

Measurement of cutaneous endothelium-dependent vasodilatation was performed by Laser Doppler imaging (LDI; moorLDI2, Moor Instruments Ltd., UK) in conjunction with iontophoretic application of 1 % acethylcholine (LDI-Ach) solution on the dorsum of the hand (Turner et al. 2008).

Statistical analysis

After testing the normality of data distribution, statistical differences between the four groups were assessed by one-way ANOVA using Fisher's multiple comparison test. Data were recorded as the means ± SD and two-tailed values of $p < 0.05$ were considered to be significant. Correlation analyses were performed using one-factor linear regression analysis. All analyses were performed using STATISTICA 6.0 software (StatSoft Inc, USA).

Results

All patient groups demonstrated significantly higher HOMA-IR values than the group of healthy controls. The value of HOMA-IR in the diabetic group was higher than that in the group of patients with MetS alone (D 5.77 ± 3.06 vs M 3.87 ± 1.86, $p < 0.05$), but did not differ from the group of patients with both T2DM and CAD (Fig. 1).

Serum levels of sVCAM-1, sICAM-1, and sE-selectin in patients with T2DM and CAD ($p < 0.01$) were significantly higher than those in other groups ($p < 0.01$), except for sICAM-1 in the group of diabetics (Fig. 2 and 3). Also, sVCAM-1, sICAM-1, and sE-selectin concentrations were significantly correlated with HOMA-IR indexes ($p < 0.0001$)

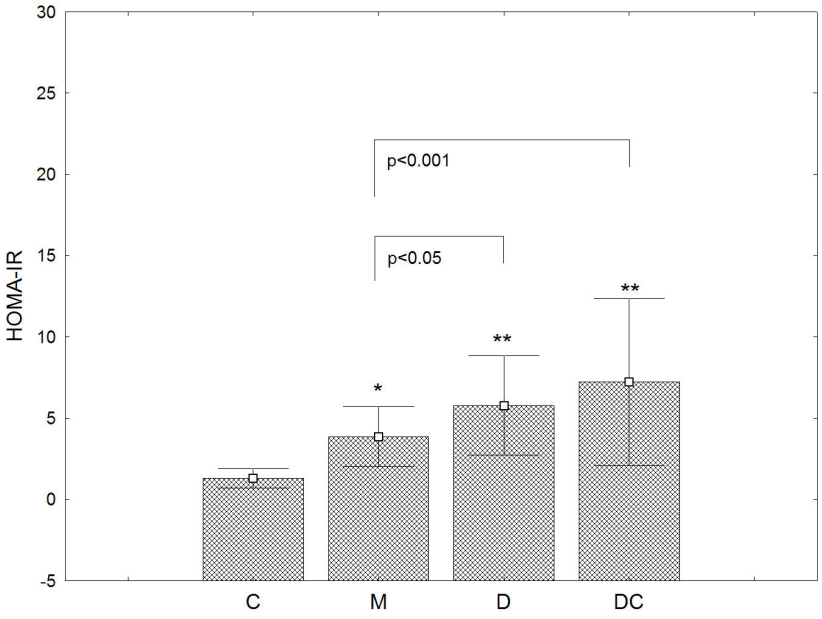

Fig. 1. Insulin resistance (HOMA-IR) in healthy subjects (C), obese metabolic syndrome patients (M), MetS patients with type 2 diabetes mellitus (D), and MetS patients with diabetes and coronary artery disease (DC). Data are expressed as mean ±SD. *, $p < 0.01$ vs. healthy subjects.

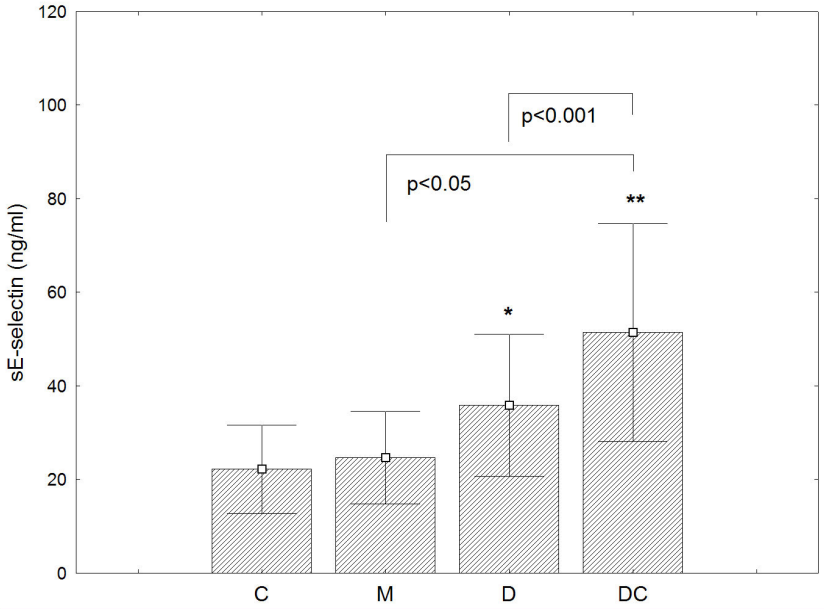

Fig. 2. Soluble E-selectin (sE-selectin) in healthy subjects (C), obese metabolic syndrome patients (M), MetS patients with type 2 diabetes mellitus (D), and MetS patients with diabetes and coronary artery disease (DC). Data are expressed as mean ±SD. *, $p < 0.01$ vs. healthy subjects.

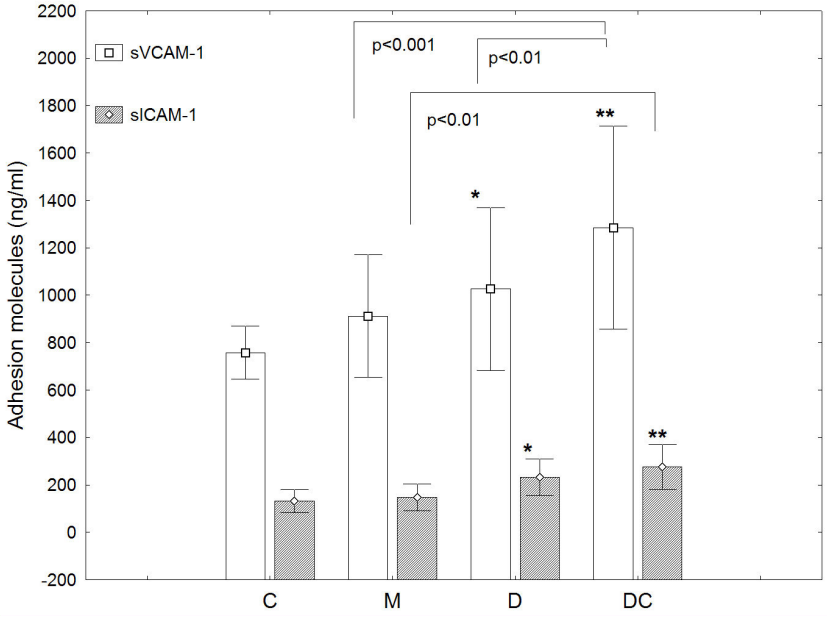

Fig. 3. Soluble vascular cell adhesion molecule-1 (sVCAM-1) and intercellular cell adhesion molecule-1 (sICAM-1) in healthy subjects (C), obese metabolic syndrome patients (M), MetS patients with type 2 diabetes mellitus (D), and MetS patients with diabetes and coronary artery disease (DC). Data are expressed as mean ±SD. *, $p < 0.01$ vs. healthy subjects.

Table 1. Correlations of adhesion molecules (sVCAM-1, sICAM-1 and E-selectin) with markers of insulin resistance (HOMA-IR) and acethylcholine induced endothelium-dependent vasodilatation (LDI-Ach) in the total study clinical material. * $p < 0.01$; ** $p < 0.0001$

Adhesion molecules	HOMA-IR	LDI-Ach
sVCAM-1	r = 0.48**	r = –0.36*
sICAM-1	r = 0.59**	r = –0.51**
E-selectin	r = 0.60**	r = –0.55**

(Table 1).

A significant and similar decline in endothelium dependent vasodilatation (LDI-Ach) was observed only for patients with T2DM and those who were diagnosed with both T2DM and CAD ($p < 0.001$; Fig. 4). LDI-Ach values were significantly correlated with HOMA-IR indexes, sVCAM-1, sICAM-1, and sE-selectin concentrations ($p < 0.01$; Fig. 5 and Table 1).

Discussion

The results of this study indicate that obese MetS patients with T2DM, independently of CAD, have higher serum levels of sICAM-1, sVCAM-1, and sE-selectin than those with MetS alone, whilethese patients had higher insulin resistance (HOMA-IR) and lower microvascular (cutaneous) endothelium-dependent vasodilatation (LDI-Ach). The

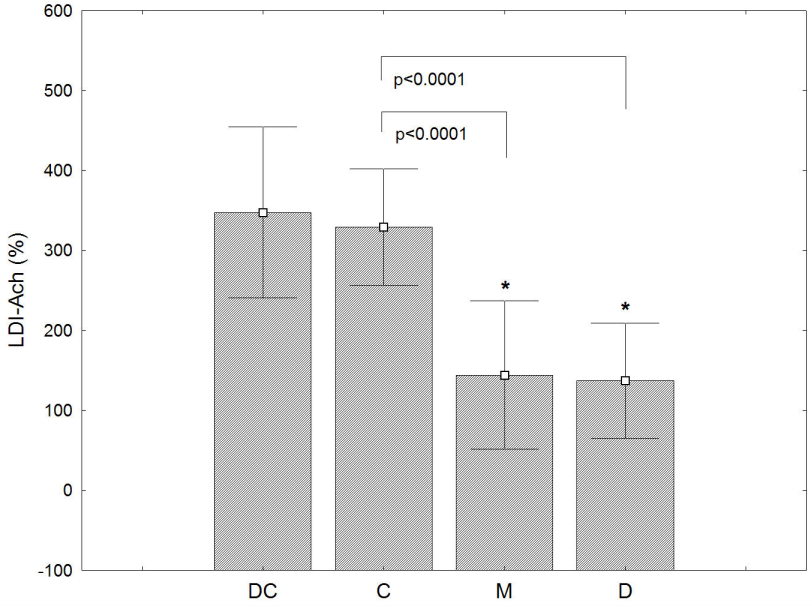

Fig. 4. Acethylcholine-induced endothelium-dependent vasodilatation (LDI-Ach) in healthy subjects (C), obese metabolic syndrome patients (M), MetS patients with type 2 diabetes mellitus (D), and MetS patients with diabetes and coronary artery disease (DC). Data are expressed as mean ± SD. *, $p < 0.01$ vs. healthy subjects.

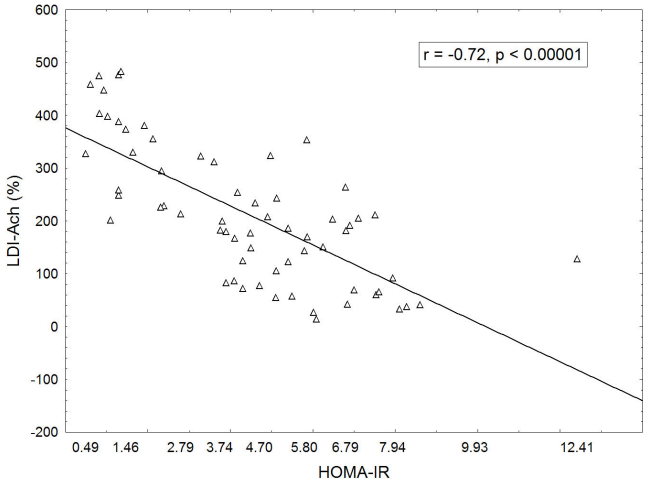

Fig. 5. Correlation between insulin resistance (HOMA-IR) and acethylcholine-induced endothelium-dependent vasodilatation (LDI-Ach) in the total study clinical material.

results also show that the presence of CAD in MetS patients with T2DM is associated with significant elevation of sICAM-1 concentrations and HOMA-IR values and with impairment of endothelium-dependent vasodilatation. Our findings suggest that in obese patients MetS can be characterized by elevated HOMA-IR. The study have confirmed correlations between HOMA-IR and serum levels of sICAM-1, and sVCAM-1, and additionaly demonstrated correlation between HOMA-IR, sE-selectin, and LDI-Ach and also between sVCAM-1, sE-selectin, and LDI-Ach (Fig. 5 and Table 1).

In recent years, much attention has been paid to the potential value of soluble adhesion molecules as biomarkers for CAD risk (Blankenberg et al. 2001). Cellular adhesion molecules may be important in atherosclerosis, inasmuch as they facilitate the immigration of leukocytes into the vessel wall (van der Meer et al. 2002). However, reports on adhesion molecules and atherosclerosis are not consistent. Several studies have reported association of sICAM-1 (Hwang et al. 1997), sVCAM-1 (Peter et al. 1997) or both sCAMs (Blankenberg et al. 2001) with measures of atherosclerosis. Our findings support the notion (Kressel et al. 2009) that adhesion molecules are excellent markers of CAD risk, especially in MetS and IR patients. Nevertheless clinical data on these soluble CAM forms are still limited and more research is necessary to elucidate the role of adhesion molecules as important risk markers in atherosclerosis and CAD.

The association of adhesion molecules with MetS can be explained by close interaction with proinflammatory cytokines which are known to be increased in MetS patients (such as IL-1ß, TNF-α, and IL-6; Rutter et al. 2005). An association between obesity and the expression of cellular adhesion molecules has also been demonstrated. This suggests that obesity is involved in the development of endothelial dysfunction. Studies on the effects of weight loss suggest that endothelial dysfunction caused by obesity is reversible. Mechanisms that may explain these relationships include increased stress to the cardiovascular system in overweight patients, increased production of inflammatory markers by adipocytes, or metabolic stimulus such as the effect of insulin on the endothelium (Schram, Stehouwer

2005).

Cellular adhesion molecules may play an important role in the development of the MetS and T2DM, as well as in their cardiovascular complications. All three cellular adhesion molecules, sVCAM-1, sICAM-1 and sE-selectin, have been investigated with the aim to improve our knowledge on endothelial function. The endothelium is thought to be the major source of the soluble forms of these molecules. Strong evidence exists that increased levels of the adhesion molecules reflect an alteration of endothelial function, which may have pathophysiological consequences. There is a growing body of evidence showing an association between cellular adhesion molecules and development of T2DM and its cardiovascular complications (Schram, Stehouwer 2005).

Our findings show that obese MetS patients with T2DM have higher serum levels of adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin), simultaneously with both higher insulin resistance and lower endothelium-dependent vasodilatation than those who have neither T2DM nor CAD. The presence of CAD in these patients is associated with greater change in the endothelial dysfunction markers. Also, insulin resistance has a close relationship to endothelial dysfunction.

Acknowledgements

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