Glycoconj J (2008) 25:573–579
DOI 10.1007/s10719-008-9118-8

The markers of inflammation and endothelial dysfunction in correlation with glycated haemoglobin are present in type 2 diabetes mellitus patients but not in their relatives

José Manuel Gómez · Ramon Vila · Pablo Catalina · Juan Soler · Lina Badimón · Manel Sahún

Received: 5 November 2007 / Revised: 13 February 2008 / Accepted: 14 February 2008 / Published online: 18 March 2008

Abstract The aim of this study is to test several biomarkers of inflammation, of endothelial dysfunction, glycated haemoglobin, and their reflection in arterial dilatation, in patients with type 2 diabetes mellitus and in their relatives, in order to demonstrate if relatives present markers as a form of precocious indicators of diabetes mellitus. Individuals between 30 and 55 years of age and without clinical arterial disease were divided in three groups: type 2 diabetes mellitus patients without complications (12 men and 18 women); first degree relatives of type 2 diabetes mellitus (14 men and 20 women); and control individuals (9 men and 16 women). Body composition was measured with a bioelectrical impedance analyzer and endothelial function with an eco-Doppler device. We determined glucose, insulin, C-peptide, glycated haemoglobin, fibrinogen, E-selectin, P-selectin, soluble intercellular cell adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), C-reactive protein (CRP) in plasma. We also studied endothelium independent dilatation and endothelium dependent dilatation. The results: ICAM-1 and VCAM-1 were significantly higher in the diabetic group (237.5±43.4 and 692.5±168.6 ng/l) than in controls (197.4±51.2 and 573.5±121.1 ng/l, p=0.011 and 0.013, respectively), but were not higher in the family group (224.5±45.2 and 599.8±150.4 ng/l). CRP was higher in the diabetic group (3.35±3.27 mg/l) than in the other groups (1.28±1.29 and 1.61±1.54 mg/l, p=0.002) and correlated with glycated haemoglobin. The non-endothelium mediated dilatation was lesser in the diabetic group than in the family group (17.3±6.1 vs. 24±8, p=0.029) and controls. In conclusion patients with uncomplicated type 2 diabetes, but not their relatives, have biochemical markers of sub-clinical inflammation in relationship with glycated haemoglobin and dysfunction of the endothelial cells markers. In these patients endothelium independent dilatation is more affected than endothelium dependent dilatation.

Keywords Diabetes mellitus · Glycated haemoglobin · Endothelial dysfunction · C-reactive protein · Soluble intercellular cell adhesion molecule-1 · Soluble vascular cell adhesion molecule-1

J. M. Gómez · J. Soler · M. Sahún
Department of Endocrinology and Nutrition,
University Hospital of Bellvitge,
Barcelona, Spain

R. Vila
Department of Angiology and Vascular Surgery,
University Hospital of Bellvitge,
Barcelona, Spain

J. M. Gómez · J. Soler · M. Sahún
CIBERDEM, Catalan Institute of Cardiovascular Sciences,
Barcelona, Spain

P. Catalina · L. Badimón
ICCC–CSIC, Hospital of Santa Creu i Sant Pau,
Barcelona, Spain

J. M. Gómez (✉)
c/ Sabino de Arana 40, 3°, 2a,
08028 Barcelona, Spain
e-mail: jmgs@csub.scs.es

Introduction

The theory of the implication of an inflammatory process in the pathophysiology of insulin resistance and atherosclerosis has grown in recent years [1, 2], and several studies showing the increase of inflammation markers in type 2 diabetes mellitus have reinforced this hypothesis [3–5].

This sub-clinical inflammation could cause changes in the function of the endothelium, which is a biologically active inner layer of the blood vessels, and endothelial cells secrete several substances that ensure the integrity of the blood vessel, prevent the accumulation of leukocytes and the occurrence of thrombosis on the surface of the endothelium, and promote fibrinolysis to aid in the dissolution of the micro-thrombi which may form [6]. Several of these substances, such as E-selectin, P-selectin, soluble intercellular cell adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (VCAM-1), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) have been studied in patients with type 2 diabetes mellitus and insulin-resistance, showing that these substances are probably implicated in the pathophysiology of the disease [2, 7–12]. On the other hand, high glycated haemoglobin impairs nitric oxide-mediated vascular responses by a mechanism involving superoxide anions but not cyclooxygenase derivatives [13].

The measurement of the flow-dependent dilatation of the brachial artery has proved to be a good non-invasive procedure for testing endothelial function [14, 15]. It has been demonstrated its usefulness in the early detection and prognosis of atherosclerotic disease [16]. It has been used in several studies in patients with type 2 diabetes mellitus and in their relatives, demonstrating an early affectation in these individuals [17, 18].

The aim of this study was to test the several biomarkers of inflammation (C-reactive protein [CRP], IL-6), of endothelial dysfunction (E-selectin, P-selectin, ICAM-1, VCAM-1 and MCP-1), glycated haemoglobin (HbA_{1c}), and disordered coagulation, such as fibrinogen, and their reflection in arterial dilatation, in patients with type 2 diabetes mellitus and in their relatives, subjects at high risk of diabetes mellitus but who are otherwise healthy; thus, it was performed in order to demonstrate if they present these markers as a form of precocious parameters of diabetes mellitus in this population.

Patients and methods

Assessment of patients and control characteristics

This cross-sectional design study was carried out in our ambulatory type 2 diabetes mellitus patients, in their family members and voluntary control individuals. Three groups of individuals were studied and in all groups the age was between 30 and 55 years; their members were life-long non-smokers (more than 10 years), and the body mass index (BMI) was between 22 and 32 kg/m^2, without clinical arterial disease. For the diabetic group, individuals without severe complications (proliferative or pre-proliferative retinopathy, microalbuminuria and polyneuropathy) were selected. In the family group, first-degree relatives of type 2 diabetic patients were selected. For the control group, individuals without diabetes mellitus or a family history of diabetes mellitus were also selected. Individuals in the family and control group had no known arterial hypertension, dyslipidemia or nephropathy. The study was completed in 30 patients (12 men and 18 women) in the diabetic group. The time of diagnosis of diabetes mellitus was 8.7± 6.1 years (range 1–25 years). Diabetes mellitus was treated only by diet in 8 cases and by insulin in 14 cases or oral hypoglycemic agents in 8 cases. In the family group 34 individuals (14 men and 20 women) were studied, and 25 (9 men and 16 women) in the control group. The following variables were recorded: age, body weight, BMI, waist-to-hip ratio, blood pressure and body composition parameters. In all cases after an overnight fast, blood was obtained for measurement of serum concentrations of glucose, basal insulin, C-peptide, HbA_{1c}, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, fibrinogen, E-selectin, P-selectin, soluble ICAM-1, soluble VCAM-1, IL-6, MCP-1 and CRP. Blood samples were obtained in the morning (0800–0900), and serum was frozen at −80°C until analysis.

BMI was calculated as body weight divided by height squared (kg/m^2) and we determined waist-to-hip ratio; blood pressure was measured in the supine position after 5 min of rest. A bioelectrical impedance analyzer using a formula provided by the manufacturers (Holtain BC Analyzer, UK) determined body composition. The results obtained were: total water in l, free fat mass in kg, fat mass in kg, and body fat in percentage.

All patients gave their informed consent for the study, which was approved by the ethics committee at the hospital.

Haemodynamic measurements

Throughout the period of measurements, the subject rested supine in a climatized room (22°C), and the system comprises a Doppler continuous wave with an 8-MHz linear phased-array transducer to determine systolic blood pressure in brachial, humeral and posterior tibial arteries. For intima-media thickness assessment, the examination was performed in common carotid and femoral arteries with the 8-MHz linear phased-array transducer; the images were measured in an automated analyzing system and the intima-media thickness was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall. The mean of two separately analyzed images from each vessel area was used in the statistical analysis.

Endothelial function *in vivo* was measured with a Doppler device (Ultramark 9 HDI, Advances Technology

Table 1 Anthropometrical, blood pressure, and body composition characteristics of patients, family individuals and controls

	Diabetic (n=30)	Family (n=34)	Control (n=25)	Difference (p)
Age (years)	48.5±5.8 (36–55)	43.6±7.6 (29–54)	44.9±9.1 (30–55)	0.001[a,b]
BMI (kg/m^2)	28.6±2.5	28.2±3.2	26.0±3.9	0.013[b]
Waist/hip ratio	0.92±0.08	0.87±0.08	0.86±0.08	0.013[a,b]
Systolic blood pressure (mm Hg)	135.0±14.6	115±16.7	111.6±12.9	0.0001[a,b,c]
Diastolic blood pressure (mm Hg)	88.4±9.2	73.4±12.1	70.5±9.0	0.0001[a,b,c]
Total body water (l)	38.2±6.5	34.7±7.1	38.0±7.5	0.126
Fat free mass (kg)	53.4±9.0	46.8±9.7	52.0±10.0	0.058
Fat mass (kg)	23.7±5.4	24.4±6.7	15.5±8.3	0.0001[a,b,c]
Body fat (%)	31.3±7.2	33.9±8.6	22.7±11.1	0.0001[a,b,c]

Data are expressed as the mean±standard deviation, and the range in parenthesis. Difference is among the three groups
BMI: body mass index
[a] Difference between diabetic and family groups
[b] Difference between diabetic and control group
[c] Difference between family and control group

Laboratories, Bothel, USA) with a linear phased-array transducer giving high axial resolution. The brachial artery was identified using the ultrasound transducer and after a 10-min rest a two-dimensional longitudinal M-mode image of the brachial artery was obtained simultaneously to R wave of the ECG. Blood artery flow was calculated by multiplying the mean blood velocity corrected for Doppler angle by the internal brachial artery diameter measured by wall tracking. The measurement was made at baseline and afterwards a sphygmomanometer was inflated at the wrist over systolic pressure (250 mm Hg for 5 min). Blood flow was recorded from 30 s after cuff release, and internal artery brachial diameter was measured at 90 s. All measurements were repeated 10 min later and 3 min after the administration of sublingual trinitrate (400 μg), an endothelium-independent vasodilator; the difference between the two determinations was the non-endothelial dependent vasodilatation [14].

Assays

Plasma glucose was measured using a glucose analyzer (Hitachi autoanalyzer); serum insulin and C-peptide with enzymochemiluminescence assay in the solid phase (Medgenix Diagnostics and Daichii Laboratories, respectively). The inter-assay and intra-assay coefficient of variation was 7.3% and 6% for insulin, and 12.8% and 4.2% for C-peptide, respectively. HbA_{1c} by high resolution liquid chromatography (Bio-Rad Diamat, München, Germany). Insulin resistance was calculated through the homeostasis model assessment function (HOMA) from fasting plasma glucose and insulin concentrations [19]. Total serum cholesterol was measured through the reaction of cholesterol esterase/cholesterol oxidase/peroxidase. HDL-cholesterol was quantified after precipitation with polyethylene glycol at room temperature. Total serum triglycerides were measured through the reaction of glycerol-phosphate-oxidase and peroxidase. LDL-cholesterol concentrations were calculated from total serum cholesterol and HDL-cholesterol. Fibrinogen was measured by a functional method (IL Test fibrinogen C, Instrumentation Laboratory, Milan, Italy). MCP-1 and IL-6 concentrations were measured by enzyme-linked immunoabsorbent assay (ELISA, R&D Systems). Plasma PCR protein was measured by a highly sensitive immunonephelometry kit (Dade Behring, Marburg, Germany). E-selectin, P-selectin, ICAM-1 and VCAM-1 were determined by enzyme-linked immunoabsorbent assays (Bender Medsystems, Kits BMS205, BMS192, BMS201 and BMS232, respectively). To define the specificity of these determinations, several polypeptides were tested and there was no cross-reactivity determined for any the tested proteins and no interference with other members of the immunoglobulin family.

Statistical analysis and power calculations

The usual statistics (mean and standard deviation) have been used to describe the data, and the Kolmogorov–Smirnov test was applied to check the normality of the variables. The ANOVA test was used to compare quantitative data among groups for independent samples and after analysis using parametric methods, the Bonferroni, Scheffe and Student's t-test were used to compare paired normally distributed variables. In no normally distributed variables, the Kruskal–Wallis test was used. The relationships among variables were sought by Pearson's and Spearman's correlation coefficients. The study was designed with 80% power to detect differences among diabetic patients, family and control individuals higher of 5%, in biochemical and markers determinations (α value of 0.05), based in standard deviation provided for our previous studies [15, 20, 21]; we calculated that a total sample size of 20 subjects in each group would be required. The estimated minimum number

Table 2 Biochemical characteristics, homeostasis model assessment (HOMA), and coagulation parameters of patients, family individuals and controls

	Diabetic (n=30)	Family (n=34)	Control (n=25)	Difference (p)
Glucose (mmol/l)	9.8±3.4	5.2±0.5	5.1±0.6	0.0001[a,b]
Basal insulin (pmol/l)	113.7±72.8	72.7±30.9	65.1±23.3	0.001[a,b]
Basal C-peptide (nmol/l)	0.81±0.45	0.86±0.27	0.68±0.16	0.109
HbA_{1c} (%)	7.2±1.7	4.9±0.3	4.8±0.4	0.0001[a,b]
HOMA	7.20±6.04	2.38±1.16	2.09±0.80	0.0001[a,b]
Total cholesterol (mmol/l)	5.23±0.77	5.28±0.99	5.0±0.9	0.485
HDL-cholesterol (mmol/l)	1.53±0.35	1.52±0.36	1.51±0.28	0.967
LDL-cholesterol (mmol/l)	3.06±0.64	3.38±0.95	3.08±0.83	0.307
Triglycerides (mmol/l)	1.45±1.05	1.11±0.57	0.83±0.29	0.015[b]
Fibrinogen (g/l)	2.88±0.73	2.99±0.62	3.01±0.65	0.717

Data are expressed as the mean±standard deviation. Difference is among the three groups
[a] Difference between diabetic and family group
[b] Difference between diabetic and control group

of individuals required to show a difference of 10% in flow-mediated dilatation with a statistical power of 90% (α value of 0.05), was also 20 subjects in each group. If the possibility of chance occurrence was $p<0.05$, it was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS/Windows version 10.0, SPSS inc., Chicago IL, USA).

Results

The clinical and anthropometric characteristics of all the groups are shown in Table 1. The age was higher in diabetic patients than in family and control individuals. The BMI was significantly greater in diabetics than in controls. The waist-to-hip ratio and the systolic and diastolic blood pressure were greater in the diabetic group than in the other two groups; the control group had lesser systolic and diastolic blood pressure, fat mass and body fat than the family group, and these were the only differences between family and control group. In Table 2 the main biochemical parameters are set out. As expected, basal glycemia and insulinemia, HOMA and HbA_{1c} were higher in the diabetic group than in family members and controls. Triglycerides were also higher in diabetics than in controls. No differences in fibrinogen were observed among the three groups.

In the endothelial parameters (Table 3), no difference was observed in E-selectin. P-selectin, IL-6 and MCP-1. ICAM-1 and VCAM-1 were significantly higher in the diabetic group than in the family and control group, but not higher in the family group than in controls. CRP was higher in the diabetic group than in the other groups. The data

Table 3 Endothelial and inflammatory parameters and data obtained following high-resolution studies of the brachial arteries in patients, family individuals and controls

	Diabetic (n=30)	Family (n=34)	Control (n=25)	Difference (p)
E-selectin (μg/l)	14.6±12.1	14.6±8.8	10.6±6.2	0.223
P-selectin (μg/l)	54.8±16.2	61±24.3	67.3±42.6	0.318
ICAM-1(ng/l)	237.5±43.4	224.5±45.2	197.4±51.2	0.011[a,b]
VCAM-1 (ng/l)	692.5±168.6	599.8±150.4	573.5±121.1	0.013[a,b]
IL-6 (ng/l)	4.6±1.6	3.3±1.3	3.8±1.4	0.21
MCP-1 (ng/l)	140.9±39	136.5±21.7	151.8±40.8	0.236
C-reactive protein (mg/l)	3.35±3.27	1.28±1.29	1.61±1.54	0.002[a,b]
Brachial dilatation mediated by endothelium (%)	8.6±5.92	10.5±7	10.7±5.1	0.5597
Brachial dilatation non-mediated by endothelium (%)	17.3±6.1	24±8.2	22.5±6.7	0.029[a,b]
Right vessel caliber (mm)	0.78±0.38	0.56±0.31	0.5±0.34	0.314
Left vessel caliber (mm)	0.7±0.36	0.54±0.26	0.52±0.39	0.324

Data are expressed as the mean±standard deviation. Difference is among the three groups
ICAM-1: intracellular adhesion molecule-1, *VCAM-1*: vascular adhesion molecule 1, *IL-6*: interleukine-6, *MCP-1*: monocyte chemoattractant protein 1
[a] Difference between diabetic and family group
[b] Difference between diabetic and control group

of the brachial arteries (Table 3) showed a lesser non-endothelium mediated dilatation in the diabetic group than in the family and control group, with no differences in endothelium mediated dilatation or in vessel calibre in both the left and the right arteries.

In all groups, E-selectin correlated positively with systolic blood pressure (r=0.291, p=0.009), basal C-peptide (r=0.32, p=0.006), HOMA (r=0.261, p=0.026), triglycerides (r=0.439, p=0.001), fibrinogen (r=0.262, p=0.023) and negatively with HDL-cholesterol (r=−0.244, p=0.038), endothelium mediated dilatation (r=−0.390, p=0.006) and non-endothelium mediated brachial dilatation (r=−0.310, p=0.032). P-selectin correlated negatively with total cholesterol (r=−0.269, p=0.016), fat mass (r=−0.281, p=0.016) and body fat (r=−0.251, p=0.032). ICAM-1 correlated positively with basal glycemia (r=0.272, p=0.016) and systolic blood pressure (r=0.235, p=0.037). IL-6 correlated positively with basal glycemia (r=0.400, p=0.001), basal insulinemia (r=0.425, p=0.001), HbA_{1c} (r=0.428, p=0.001) and HOMA (r=0.691, p=0.001). MCP-1 correlated positively with HOMA (r=0.291, p=0.012). CRP correlated positively with BMI (r=0.327, p=0.003), diastolic blood pressure (r=0.265, p=0.018), systolic blood pressure (r=0.302, p=0.007), basal glycemia (r=0.449, p=0.001), HbA_{1c} (r=0.467, p=0.001), HOMA (r=0.346, p=0.003), triglycerides (r=0.277, p=0.017), fibrinogen (r=0.344, p=0.003) and body fat (r=0.303, p=0.009).

Discussion

In the early 1990s, several authors proposed the theory that inflammation could participate in the pathophysiology of obesity and insulin resistance [22]. Since then, several studies have been made to demonstrate the increase of several inflammation markers, such as CRP, IL-6, or fibrinogen, in individuals with diabetes mellitus or with high risk of diabetes mellitus or insulin resistance. In our study, CRP was significantly higher in diabetic patients than in diabetic relatives and controls, and correlated with basal glycemia, HbA1c and HOMA. IL-6 was not significantly elevated, but correlated with basal insulinemia, glycemia, HbA_{1c} and HOMA. Other studies have demonstrated an increase of CRP in type 2 diabetes mellitus and even in patients with impaired glucose tolerance [4], and its correlation with insulin resistance [3, 5, 23]. However, some studies contend that CRP is more closely related to obesity than to insulin resistance *per se* [24]. IL-6 also has been seen to be elevated in some studies in type 2 diabetes mellitus [25], but it also seems more closely related to fat mass [26]. In this situation, sub-clinical inflammation can change the milieu of the arterial wall and prompt the production of adhesion molecules and chemokines; furthermore, it has been recently shown that highly HbA_{1c} induces endothelial dysfunction in rat vessels by generating superoxide anions that interfere with nitric oxide mediated response [13]; so, other mechanisms for its interaction with endothelial dysfunction may be in correlation with markers of inflammation as HbA_{1c}.

The selectins expressed on the endothelial cells would slow down mononuclear cells that could be further recruited and activated by MCP-1. The action of ICAM-1 and VCAM-1 leads to the firm adhesion of mononuclear cells [1]. Elevated concentrations of these factors have been described in several cardiovascular diseases [27, 28]. Several studies of the biomarkers of endothelial dysfunction have been done in patients with diabetes mellitus or insulin resistance. Although most of them have demonstrated an increased concentration of E-selectin, P-selectin, ICAM-1 and VCAM-1, there is a broad heterogeneity in the results; mainly in regard to which factor is more highly altered [2, 7, 9–12]. Furthermore, the results in patients with impaired glucose tolerance are discordant [8, 12] and some studies have described, in the general population, an increased risk of diabetes mellitus in people with higher biomarkers of endothelial dysfunction [2, 13, 29, 30]. In our study, only VCAM-1 and ICAM-1 were significantly elevated in the diabetic group than those observed in controls, but not in the family group. Interestingly, in our series the only differences between the family and control group were that controls presented lesser systolic, diastolic blood pressure, fat mass and body fat.

There are several factors that can influence their concentrations and age does not seem to be a strong determinant, because high concentrations of selectins and soluble adhesion molecules have been described in children and adolescents with a high risk of atherosclerosis [31].

There are contradictory data about the effect of metabolic control in previous studies [32, 33]; in type-2 diabetics there is an increase in E-selectin and ICAM-1 in those with peripheral arterial insufficiency, with respect to those without such disease [10]. Moreover, a close relationship between increased urinary albumin excretion, endothelial dysfunction, and inflammation and death risk in diabetic patients has been described [34]. On the other hand, diabetic patients who were smokers had higher concentrations of E-selectin, ICAM-1 and VCAM-1 than non-smokers as has been shown in one report [7].

Some drugs used frequently in diabetic patients, such as estatins, fibrates, angiotensin receptor blockers, rosiglitazone, repaglinide and metformin could influence the concentrations of some of these factors [35–41]; this fact is a limitation of our study because eight patients were under treatment with hypoglycemic agents.

The endothelium dependent blood-flow responses have been impaired in diabetic patients in most, although not in

all, studies [17, 29, 39–41]. Reponses to sodium nitroprusside have been subnormal in many, but not in all, reports [17, 41, 42]. Many factors could influence the results; hyperglycemia does not seem to be an important determinant [41], and the presence of microalbuminuria has been demonstrated to lower the endothelium response [43]. This fact, and the presence of other diabetic complications, could have influenced the results of previous studies. In our study, the patients were non-smokers and had no complications of diabetes mellitus and those with known arterial disease or microalbuminuria were excluded and in these patients, non-endothelium mediated reactivity seems more affected. Although statistically not significant, it is important to note that low power probability in our study could explain that endothelium mediated reactivity is not impaired in diabetic patients.

So, this study proposes which comes first in type 2 diabetes mellitus, the metabolic disturbances or inflammation or endothelial markers or both will appear simultaneously; but with our data we are unable to define the prognostic value and clinical utility of these markers in relatives of diabetic patients.

In conclusion, non-smoking patients with uncomplicated type 2 diabetes mellitus have elevated concentrations of CRP that indicate sub-clinical inflammation in relationship with HbA1c. They also have increased concentrations of ICAM-1 and VCAM-1, reflecting dysfunction of the endothelial cells. In these patients, endothelium independent dilatation is more affected than is endothelium dependent dilatation. These abnormalities are not detected in diabetic relatives, indicating that endothelial alterations are not precocious in individuals with high risk of diabetes mellitus.

References

1. Yeh, E.T.H.: CRP as mediator of disease. Circulation **109**(suppl II), II-11–II-14 (2004)
2. Meigs, J.B., Hu, F.B., Rifai, N., Mansons, J.A.E.: Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. JAMA **291**, 1978–1986 (2004)
3. Temelkova-Kurktschiev, T., Siegert, G., Bergmann, S., Henkel, E., Koehler, C., Jarob, W., Hanefeld, M.: Subclinical inflammation is strongly related to insulin resistance but not to impaired insulin secretion in a high risk population for diabetes. Metabolism **51**, 743–749 (2002)
4. Henareh, L., Jogestrand, T., Agewall, S.: Glucose intolerance is associated with C-reactive protein and intima-media anatomy of the common carotid artery in patients with coronary heart disease. Diabet. Med. **22**, 1212–1217 (2005)
5. Nakanishi, N., Shiraishi, T., Wada, M.: Association between C-reactive protein and insulin resistance in a Japanese population: the Minoh Study. Intern. Med. **44**, 542–547 (2005)
6. Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P.: Endothelial dysfunction, inflammation and diabetes. Rev. Endocr. Metab. Disord. **5**, 189–196 (2004)
7. Takeuchi, N., Kawamura, T., Kanai, A., Nakamura, N., Uno, T., Hara, T., Sano, T., Sakamoto, N., Hamada, Y., Nakamura, J., Hotta, N.: The effect of cigarette smoking on soluble adhesion molecules in middle-aged patients with type 2 diabetes mellitus. Diabet. Med. **19**, 57–64 (2002)
8. Piemonti, L., Calori, G., Mercalli, A., Lattuada, G., Monti, P., Garancini, M.P., Constantino, F., Ruotolo, G., Luzi, L., Perseghin, G.: Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. Diabetes Care **26**, 2883–2889 (2003)
9. Boulbou, M.S., Koukoulis, G.N., Vasiou, K.G., Petinaki, E.A., Gourgoulianis, K.I., Fezoulidis, I.B.: Increased soluble E-selectin levels in type 2 diabetic patients with peripheral arterial disease. Int. Angiol. **23**, 18–24 (2004)
10. Elhadd, T.A., Kennedy, G., Robb, R., McLaren, M., Jung, R.T., Belch, J.J.F.: Elevated soluble cell adhesion molecules E-selectin and intercellular cell adhesion molecule-1 in type-2 diabetic patients with and without asymptomatic peripheral arterial disease. Int. Angiol. **23**, 128–133 (2004)
11. Gokulakrishnan, K., Deepa, R., Mohan, D.R., Gross, M.D.: Soluble P-selectin and CD40L levels in subjects with prediabetes, diabetes mellitus, and metabolic syndrome—the Chennai Urban Rural Epidemiology Study. Metabolism **55**, 237–242 (2006)
12. Boulbou, M.S., Koukoulis, G.N., Makri, E.D., Petinaki, E.A., Gourgoulianis, K.I., Germenis, A.E.: Circulating adhesion molecules levels in type 2 diabetes mellitus and hypertension. Int. J. Cardiol. **98**, 39–44 (2005)
13. Vallejo, S., Angulo, J., Peiró, C., Nevado, J., Sánchez-Ferrer, A., Petidier, R., Sánchez-Ferrer, C.F., Rodríguez-Mañas, L.: Highly glycated oxyhaemoglobin impairs oxide relaxation in human mesenteric microvessels. Diabetologia **43**, 83–90 (2000)
14. Celermajer, D.S., Sorensen, K.E., Gooch, V.M., Spiegelhalter, D.J., Miller, O.I., Sullivan, I.D., Lloyd, J.K., Deanfield, J.E.: Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet **340**, 111–1115 (1992)
15. Gómez, J.M., Sahún, M., Vila, R., Domenèch, P., Catalina, P., Soler, J., Badimón, L.: Elevation of E-selectin concentrations may correlate with potential endothelial dysfunction in individuals with hypopituitarism during therapy with growth hormone. Curr. Neurovasc. Res. **4**, 55–62 (2007)
16. Landmesser, U., Hornig, B., Drexler, H.: Endothelial function: a critical determinant in atherosclerosis? Circulation **109**(21 suppl II), II-27–II-33 (2004)
17. Enderle, M.D., Benda, N., Schmuelling, R.M., Haering, H.U., Pfohl, M.: Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. Diabetes Care **21**, 271–277 (1998)
18. Balletshofer, B.M., Rittig, K., Enderle, M.D., Volk, A., Maerker, E., Jacob, S., Matthaei, S., Rett, K., Haring, H.U.: Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. Circulation **101**, 1780–1784 (2000)
19. Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., Turner, R.C.: Homeostasis model assessment: insulin resistance and b-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia **28**, 412–419 (1985)
20. Fernández Castañer, M., Biarnés, J., Camps, I., Ripollés, J., Gómez, N., Soler, J.: Beta-cell dysfunction in first-degree relatives of patients with non-insulin-dependent diabetes mellitus. Diabet. Med. **13**, 953–959 (1999)
21. Fernández Castañer, M., Camps, I., Fernández-Real, J.M., Domenèch, P., Martínez-Brotons, F.: Increased prothrombin

fragment 1+2 and D-dimer in first degree relatives of type 2 diabetic patients. Acta Diabetol. **33**, 118–121 (1996)
22. Hotamisligil, G.S., Shargill, N.S., Spiegelman, B.M.: Adipose expression of tumor necrosis factor-a: direct role in obesity-linked insulin resistance. Science **259**, 87–91 (1993)
23. Freeman, D.J., Norrie, J., Caslake, M.J., Gaw, A., Ford, I., Lowe, G.D., O'Reilly, D.S., Packard, C.J., Sattar, N.: C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. Diabetes **51**, 1596–1600 (2002)
24. Greenfield, J.R., Samaras, K., Jenkins, A.B., Kelly, P.J., Spector, T.D., Gallimore, J.R., Pepys, M.B., Campbell, L.V.: Obesity is an important determinant of baseline serum C-reactive protein concentration in monozygotic twins, independent of genetic influences. Circulation **109**, 3022–3028 (2004)
25. Antoniades, C., Tousoulis, D., Tountas, C., Tentolouris, C., Toutouza, M., Vasiliadou, C., Tsioufis, C., Toutouzas, P., Stefanadis, C.: Vascular endothelium and inflammatory process: in patients with combined type 2 diabetes mellitus and coronary atherosclerosis, the effects of vitamin C. Diabet. Med. **21**, 552–558 (2004)
26. Carey, A.L., Bruce, C.R., Sacchetti, M., Anderson, M.J., Olsen, D.B., Saltin, B., Hawley, J.A., Febbraio, M.A.: Interleukin-6 and tumor necrosis factor-alpha are not increased in patients with type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. Diabetologia **47**, 1029–1037 (2004)
27. Blann, A.D., Lip, G.Y.H.: Editorial: cell adhesion molecules in cardiovascular disease and its risk factors—what can soluble levels tell us? J. Clin. Endocrinol. Metab. **85**, 1745–1747 (2000)
28. Sartipy, P., Loskutoff, D.J.: Monocyte chemoattractant protein 1 in obesity and insulin resistance. Proc. Natl. Acad. Sci. U. S. A. **100**, 7265–7270 (2003)
29. McEleavy, O.D., McCallum, R.W., Petrie, J.R., Small, M., Connell, J.M., Sattar, N., Cleland, S.J.: Higher carotid-radial pulse wave velocity in healthy offspring of patients with type 2 diabetes. Diabet. Med. **21**, 262–266 (2004)
30. Ostergard, T., Nyholm, B., Hansen, T.K., Rasmussen, L.M., Ingerslev, J., Sorensen, K.E., Botker, H.E., Saltin, B., Schmitz, O.: Endothelial function and biochemical vascular markers in first-degree relatives of type 2 diabetic patients, the effect of exercise training. Metabolism **55**, 1508–1515 (2006)
31. Glowinska, B., Urban, M., Peczynska, J., Florys, B.: Soluble adhesion molecules (sICAM-1, sVCAM-1) and selectins (sE selectin, sP selectin, sL selectin) levels in children and adolescents with obesity, hypertension, and diabetes. Metabolism **54**, 1020–1026 (2005)
32. Cominacini, L., Fratta Pasini, A., Garbin, U., Davoli, A., De Santis, A., Campagnola, M., Rigoni, A., Zenti, M.G., Moghetti, P., Lo Cascio, V.: Elevated levels of soluble E-selectin in patients with IDDM and NIDDM: relation to metabolic control. Diabetologia **38**, 1122–1124 (1995)
33. Yudkin, J.S., Panahloo, A., Stehouwer, C., Emeis, J.J., Bulmer, K., Mohamed-Ali, V., Denver, A.E.: The influence of improved glycaemic control with insulin and sulphonylureas on acute phase and endothelial markers in type II diabetic subjects. Diabetologia **43**, 1099–1106 (2000)
34. Stehouwer, C.D.A., Gall, M.A., Twisk, J.W.R., Knudsen, E., Emeis, J.J., Parving, H.H.: Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes. Progressive, interrelated, and independently associated with risk of death. Diabetes **51**, 1157–1165 (2002)
35. Empen, K., Frost, R.J.A., Geiss, H.C., Otto, C., Parhofer, K.G.: Differential effects of fenofibrate versus atorvastatin on the concentrations of E-selectin and vascular cellular adhesion molecule-1 in patients with type 2 diabetes mellitus and mixed hyperlipoproteinemia: a randomized cross-over trial. Cardiovasc. Diabetol. **2**, 17 (2003)
36. Hetzel, J., Balletshofer, B., Rittig, K., Walcher, D., Kratzer, W., Hombach, V., Häring, H.U., Koenig, W., Marx, N.: Rapid effects of rosiglitazone treatment on endothelial function and inflammatory biomarkers. Arterioscler. Thromb. Vasc. Biol. **25**, 1804–1809 (2005)
37. De Jager, J., Kooy, A., Lehert, P.H., Bets, D., Wulffelé, M.G., Teerlink, T., Scheffer, P.G., Schalkwijk, C.G., Donker, A.J.M., Stehouwer, C.D.A.: Effects of short-term treatment with metformin on markers of endothelial function and inflammatory activity in type 2 diabetes mellitus: a randomized, placebo-controlled trial. J. Int. Med. **257**, 100–109 (2005)
38. Nomura, S., Shouzu, A., Omoto, S., Nishikawa, M., Fukuhara, S., Iwasaka, T.: Effect of valsartan on monocyte/endothelial cell activation markers and adiponectin in hypertensive patients with type 2 diabetes mellitus. Thromb. Res. **117**, 385–392 (2006)
39. Goodfellow, J., Ramsey, M.W., Luddington, L.A., Jones, C.J., Coates, P.A., Dunstan, F., Lewis, M.J., Owens, D.R., Henderson, A.H.: Endothelium and inelastic arteries: an early marker of vascular dysfunction in non-insulin dependent diabetes. BMJ **312**, 744–745 (1996)
40. Avogadro, A., Piarulli, F., Valerio, A.: Forearm nitric oxide balance vascular relaxation, and glucose metabolism in NIDDM patients. Diabetes **46**, 1040–1046 (1997)
41. Mäkimattila, S., Liu, M.L., Vakkilainen, J., Schlenzka, A., Lahdenpera, S., Syvanne, M., Mantysaari, M., Summanen, P., Bergholm, R., Taskinen, M.R., Yki-Jarvinen, H.: Impaired endothelium-dependent vasodilatation in type 2 diabetes: relation to LDL size, oxidized LDL, and antioxidants. Diabetes Care **22**, 973–981 (1999)
42. Natali, A., Toschi, E., Baldeweg, S., Casolaro, A., Baldi, S., Sironi, A.M., Yudkin, J.S., Ferranini, E.: Haematocrit, type 2 diabetes, and endothelium-dependent vasodilatation of resistance vessels. Eur. Heart J. **26**, 464–471 (2005)
43. Papaioannou, G.I., Seip, R.L., Grey, N.J., Katten, D., Taylor, A., Inzucchi, S.E., Young, L.H., Chyun, D.A., Davey, J.A., Wackers, F.J.T.H., Iskandrian, A.E., Ratner, R.E., Robinson, E.C., Carolan, S., Engel, S., Heller, G.V.: Brachial artery reactivity in asymptomatic patients with type 2 diabetes mellitus and microalbuminuria (from the detection of ischemia in asymptomatic diabetics-brachial artery reactivity study). Am. J. Cardiol. **94**, 294–299 (2004)