Clin. Cardiol. 30, 295–300 (2007)

Effects of Insulin Dependence on Inflammatory Process, Thrombotic Mechanisms and Endothelial Function, in Patients With Type 2 Diabetes Mellitus and Coronary Atherosclerosis

Charalambos Antoniades, m.d., Dimitris Tousoulis, m.d., ph.d., facc., Kyriakoula Marinou, m.d., Nikos Papageorgiou, m.s., Erini Bosinakou, m.d., Costas Tsioufis, m.d., Elli Stefanadi, m.d., George Latsios, m.d., Costas Tentolouris, m.d., Gerasimos Siasos, m.d., Christodoulos Stefanadis, m.d., fesc., facc.

A' Cardiology Department, Athens University Medical School, Athens-Greece

Summary

Background: Type 2 diabetes mellitus (T2DM) is characterized by endothelial dysfunction, increased thrombogeneisity and abnormal inflammatory response.

Hypothesis: We hypothesizsed that insulin dependence/exogenous insulin administration may affect thrombotic/inflammatory status and endothelial function in patients with T2DM and coronary artery disease (CAD).

Methods: Fifty-five patients with T2DM + CAD (26 insulin-treated (INS) and 29 under oral biguanide + sulphonylurea (TABL)) were recruited. Endothelial function was assessed by gauge-strain plethysmography, and serum levels of inflammatory and thrombotic markers were determined by enzyme linked immunosorbent assay.

Results: There was no significant difference in endothelium-dependent dilation (EDD) between the study groups, while EDD was correlated with fasting glucose levels in both INS ($r = -0.776$, $p = 0.0001$) and TABL ($r = -0.702$, $p = 0.0001$). Patients in INS group had higher levels of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), monocyte chemoattractant protein (MCP-1) and vascular cell adhesion molecule (sVCAM-1), compared to TABL. However, TNF-α was negatively correlated with protein C (PrtC) only in INS ($r = -0.726$, $p = 0.01$) but not in TABL group ($r = -0.066$, $p = 0.738$). Similarly, sVCAM-1 was correlated with PrtC only among INS patients ($r = -0.451$, $p = 0.046$) but not in TABL ($r = 0.069$, $p = 0.727$). In multivariate analysis, insulin dependence was a predictor of IL-6, TNF-α, MCP-1 and sVCAM-1 levels independently from the patients' demographic characteristics, the angiographic extend of CAD or the duration of diabetes.

Conclusions: Insulin treatment in patients with type 2 diabetes mellitus affects the expression of inflammatory cytokines and subsequently modifies the thrombotic mechanisms in patients with coronary atherosclerosis, independently from the duration of diabetes and the extend of coronary artery disease.

Key words: endothelium, inflammation, coronary artery disease, diabetes mellitus

Clin. Cardiol. 2007; 30: 295–300.

Introduction

Coronary artery disease (CAD) is the leading cause of mortality in diabetic patients.[1] Glycemic control seems to decrease cardiovascular risk in type 2 diabetic patients (T2DM) with CAD.[1] However, it is still unclear whether insulin treatment is superior to oral hypoglycemic agents, since several oral antidiabetic medications seem to have additional beneficial effects on cardiovascular system, further to the decrease of blood glucose levels.[2]

Evidence suggests that inflammation is a key feature in atherogenesis in T2DM.[3] Proinflammatory cytokines (such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α)), are produced by adipose tissue in large

Address for reprints:
Charalambos Antoniades, M.D.
Athens University Medical School
Hippokration Hospital
Vasilissis Sofias 114, 115 28
Athens Greece
e-mail: antoniades@panafonet.gr

Received: May 17, 2006
Accepted: December 11, 2006

Published online in Wiley InterScience
(www.interscience.wiley.com).
DOI:10.1002/clc.20101

amounts in obese individuals and especially in patients with T2DM.[3] These inflammatory molecules are elevated in patients with insulin resistance, and have been shown to predict cardiovascular risk. Diabetic patients have also increased thrombogeneicity,[4] a factor contributing to the increased risk for atherothrombosis observed in these patients. Clear links between inflammation and thrombosis in these subjects are now well established, since proinflammatory cytokines regulate the synthesis of several endothelium- or liver- derived components of the thrombosis fibrinolysis system.

Insulin is a key feature in the regulation of all these proatherogenic mechanisms,[5] since insulin resistance is largely dependent on the inflammatory status of adipose tissue and the local production of proinflammatory cytokines such as TNF-α[6] while as a anabolic hormone, it has proatherogenic properties.[7] Although insulin induces nitric oxide (NO) synthesis in healthy endothelium,[8] it may have an opposite effect in the presence of insulin resistance, observed in T2DM.[9]

We examined whether exogenous insulin administration in patients with type 2 diabetes mellitus affects the expression of proinflammatory cytokines, the thrombogenic status and endothelial function in patients with T2DM and CAD.

Methods

Participants

Fifty-five patients with T2DM + CAD (26 under insulin treatment and 29 under oral antidiabetic treatment) were recruited in this study (Table 1). All subjects were selected from the registry of the Cardiology Department in Hippokration Hospital of Athens, and they had angiographically documented CAD, with at least one coronary stenosis >50%. Diabetes mellitus was defined in accordance with the National Data Group Criteria.[10] Patients under oral treatment were receiving sulfonylurea plus diguanides only, while patients in the (INS) insulin-treated group were exclusively under insulin treatment, for at least 1 year before recruitment. All patients had HbA1c<8.0 mg/dL at the beginning of the protocol. Patients in the two groups had similar glycemic control, and were under the same diet. The exclusion criteria were tobacco use within the past 5 years, evidence for hepatic or hematologic abnormalities and coronary event during the last 3 months before recruitment. Blood samples were obtained after overnight fasting, before the patients received their morning medication. The protocol was approved by the Institutional Ethics Committee, and an informed consent was given by each subject.

Forearm blood flow measurements

Forearm blood flow (FBF) was measured using gauge-strain plethysmography (EC-400, D.E. Hokanson Inc) and analyzed by a personal computer (Hokanson NIVP3 software), as we have previously described.[11] Forearm vasodilatory response to reactive hyperemia (endothelium-dependent dilation-(EDD)), was defined as the percentage change of FBF from baseline to the maximum flow during reactive hyperemia. Forearm vasodilatory response to nitrate (EID) was defined as the percentage change of FBF from baseline to the maximum flow after sublingual administration of 0.8 mg nitroglycerine.[11] EDD was considered as an index of endothelium-dependent dilation, while EID was considered as an index of endothelium independent dilation, as previously established.[11]

Biochemical Analyses

Plasma levels of the examined markers of thrombosis/fibrinolysis, system were determined as follows: von Willebrand factor vWF (von Willebrand Reagent, DADE BEHRING Inc Germany), forearm vasodilatory fV(Coagulation factor V activity, DADE BEHRING Inc Germany), fVII (Coagulometric method for determination of factors II, VII and X activities, by DADE BEHRING Inc Germany), protein C (PrtC) (Reagents for the determination of protein C activity, by DADE BEHRING Inc Germany) and prtS (clotting assay of protein S by STA analyzers, STACLOT PROTEIN S kit, Diagnostica Stago, France). Enzyme linked immunosorbent assays were used for the determination of serum levels of vascular cell adhesion molecule (sVCAM-1), monocyte chemoattractant protein-1 (MCP-1), TNF-α and IL-6 (ELISA kits by R&D Systems Inc. USA).

Statistical Analysis

The statistical analysis was performed using a personal computer and SPSS 9.0 statistical package. Normally distributed variables are expressed as means $\pm$ Standard Error of the Mean (SEM), while non-normally distributed variables were log-transformed for analysis and are presented in the nonlogarithmic format as median (25th −75th percentile). Unpaired t-test or Mann−Whitney U test were used as appropriate, to evaluate the differences in variables between the two groups. Univariate analysis was performed to assess correlations between variables. To examine the role of insulin dependence on the examined parameters, we performed multivariate analysis by using as dependent variables the inflammatory and thrombotic markers and as independent variables insulin treatment as well as those of the clinical risk factors (age, gender, dyslipidemia, body mass index, hypertension, duration of diabetes and HbA1c), which showed a significant association with the dependent variables in univariate analysis at 15% significance level. A backward elimination procedure was applied in all multivariate models (using $p<0.05$ as the threshold for removing a variable from the model).

TABLE 1 Demographic characteristics, forearm blood flows and biochemical measurements

	Insulin-treated group	Group under oral hypoglycemic agents
Number of patients	26	29
Age (years)	66.0 ± 2.04	66.41 ± 1.89
Sex (male/female)	22/4	27/2
BMI (Kg/m^2)	27.00 ± 0.64	27.21 ± 0.47
Duration of diabetes (years)	16.69 ± 2.14	16.72 ± 2.02
Hypercholesterolemia (n)	14	11
Family history of CAD (n)	5	12
Hypertension (n)	15	14
HbA1c (%)	7.03 ± 0.21	7.05 ± 0.16
Fasting glucose (mg/mg)	170.76 ± 10.82	167.21 ± 10.81
Fasting cholesterol (mg/mL)	188.07 ± 3.94	188.14 ± 2.62
Triglycerides (mg/mL)	65.86 ± 2.49	69.66 ± 3.07
HDL (mg/mL)	31.34 ± 1.09	31.31 ± 1.249
Medication		
Aspirin (n)	26	29
Statins (n)	19	14
ACEi (n)	14	18
Biguanides (n)	—	29
Sulphonylurea (n)	—	29
Insulin (n)	26	—
FBF-1 (mL/100 mL tissue/min)	4.2 ± 0.3	4.1 ± 0.3
RHF (mL/100 mL tissue/min)	6.5 ± 0.4	6.1 ± 0.3
EDD (%)	50.4 ± 5.9	53.7 ± 5.5
FBF-2 (mL/100 mL tissue/min)	4.1 ± 0.2	3.9 ± 0.2
NTG-Flow (mL/100 mL tissue/min)	6.9 ± 0.4	6.4 ± 0.3
EID (%)	72.2 ± s5.8	65.5 ± 6.6
Von Willebrand factor (%)[a]	114(88–151)	132(99–174)
Protein C (%)[a]	96.4(62.9–130.4)	100(84.7–114.6)
Protein S (%)[a]	82.0(71.0–92.3)	98.0(70.0–103.5)
Factor V (%)[a]	140(110–158)	150(115–206)
Factor VII (%)[a]	92.3(60.1–107.3)	94.8(68.17–106.3)

BMI, Body mass index; HDL, High density lipoprotein; ACEi, Angiotensin converting enzyme inhibitor; FBF-1 and -2, Resting forearm blood flow 1- and 2-; EDD, Hyperemic %change of forearm blood flow; EID, % change of forearm blood flow after nitrate; RHF, Maximum flow during reactive hyperemia; NTG-Flow, Maximum flow after nitrate; Continuous values are expressed as means ± SEM.
[a] Values expressed as median(25th–75th percentile).
* $p<0.05$, ** $p<0.01$ vs. insulin-treated group.

Results

Forearm Blood Flow

There was no significant difference in resting or hyperemic FBF between patients treated with oral antidiabetic medication and those under exogenous insulin treatment (Table 1). Similarly, there was no significant difference in EDD or EID between the two types of diabetic patients. EDD was significantly correlated with fasting glucose levels at the time of the examination in both patients under insulin ($r = -0.776$, $p = 0.0001$) and those under oral medication ($r = -0.702$, $p = 0.0001$).

Inflammatory and Thrombotic Markers

INS patients, had higher levels of IL-6 and TNF-α ($p<0.05$ for both, Table 1, Fig. 1) as well as higher levels of MCP-1 ($p<0.05$) and sVCAM-1 ($p<0.01$) (Table 1, Fig. 2) compared to those under oral treatment. Serum TNF-α was correlated with sVCAM-1 in both patients under insulin ($r = 0.404$, $p = 0.041$) and those under oral treatment ($r = 0.566$, $p = 0.001$).

There was no significant difference in plasma PrtC, PrtS, FV, FVII and vWF between patients receiving insulin and those under oral treatment ($p =$ ns for all, Table 1). However, TNF-α was negatively correlated with PrtC only in patients under insulin treatment ($r = -0.726$, $p = 0.01$, Fig. 3(a)) but not in patients receiving oral treatment ($r = -0.066$, $p = 0.738$, Fig. (b)). Similarly, sVCAM-1 was correlated with PrtC levels only among patients under insulin ($r = -0.451$, $p = 0.046$) but not among those under oral treatment ($r = 0.069$, $p = 0.727$).

Multivariate Analysis

To examine whether insulin dependence is associated with the examined inflammatory markers independently

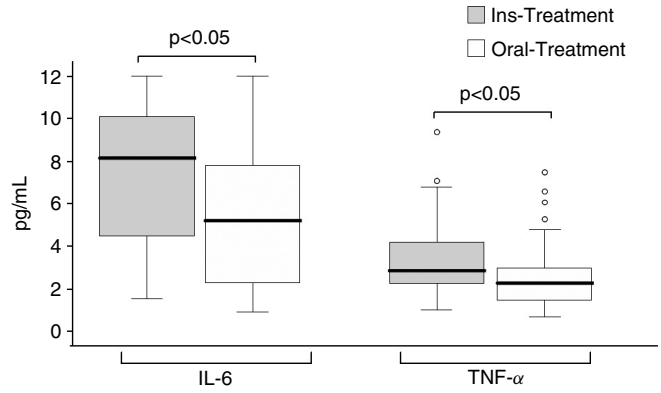

FIG. 1 Patients with coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) treated with exogenous insulin (Ins-treatment) had significantly higher levels of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) compared to those under oral treatment (oral treatment), despite the similar glycemic control and duration of diabetes. Values expressed as median (horizontal line), 25th and 75th percentiles (box), and the minimum and maximum observed values that are not statistically outlying (bars). Outliers/extreme values are presented as open circles.

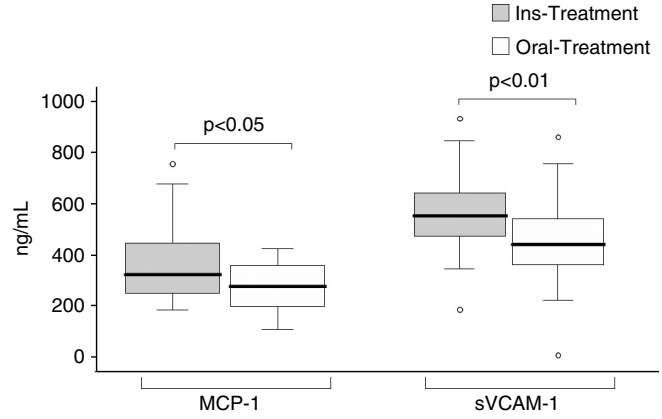

FIG. 2 Patients with coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) treated with exogenous insulin (Ins-treatment) had significantly higher levels of monocyte chemoattractant protein-1 (MCP-1) and soluble vascular cell adhesion molecule (sVCAM-1) compared to those under oral medication (oral-treatment), despite the similar glycemic control and duration of diabetes. Values expressed as median (horizontal line), 25th and 75th percentiles (box), and the minimum and maximum observed values that are not statistically outlying (bars). Outliers/extreme values are presented as open circles.

from the patients demographic characteristics, body mass index (BMI), the duration of diabetes, the glycemic control or the angiographic extend of coronary artery disease, we performed multivariate analysis. Indeed, the only independent predictors for IL-6 levels were insulin dependence ($\beta = 2.07$ (SE:0.76), $p = 0.022$) and smoking ($\beta = 2.31$ (SE:1.02), $p = 0.023$) (R^2 for the model = 0.157), while for TNF-α the independent predictors were insulin dependent ($\beta = 1.23$ (SE:0.50) $p = 0.018$), smoking ($\beta = 1.67$ (SE:0.60), $p = 0.008$), the duration of diabetes ($\beta = 0.74$ (SE:0.024), $p = 0.003$) and arterial hypertension ($\beta = 1.12$ (SE:0.52), $p = 0.036$) (R^2 for the model = 0.323). Similarly the only independent predictors of MCP-1 were insulin dependent ($\beta = 78.8$ (SE:29.9), $p = 0.011$) and the angiographic extend of CAD ($\beta = 98.4$ (SE:20.9, $p = 0.0001$) (R^2 for the model = 0.407), while for sVCAM-1 levels the independent predictors were again insulin dependent ($\beta = 131.2$ (SE:39.3), $p = 0.003$) the duration of diabetes ($\beta = 5.76$ (SE:1.84), $p = 0.003$) and the presence of arterial hypertension ($\beta = 130.8$ (SE:40.7), $p = 0.002$) (R^2 for the model = 0.387).

Discussion

In the present study, we showed that diabetic patients with CAD receiving insulin, had higher levels of IL-6, TNF-α and of sVCAM-1 compared to those receiving oral treatment, independently from patients' demographic characteristics, glycemic control or the angiographic extend of CAD. Furthermore, TNF-α was negatively correlated with protein C only in insulin-treated patients but not in those under oral hypoglycemic treatment, suggesting that insulin administration affects the

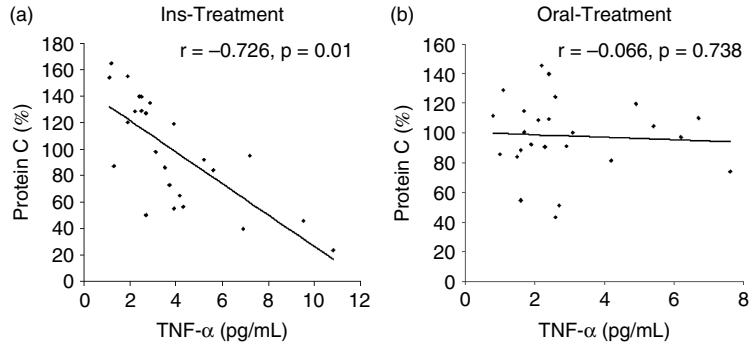

FIG. 3 Among patients with coronary artery disease (CAD) and type 2 diabetes mellitus (T2DM) treated with exogenous insulin (Ins-treatment), serum tumor necrosis factor alpha (TNF-α) was negatively correlated with protein C plasma levels (a), while no such correlation was observed among patients treated with oral medication (b).

expression of inflammatory cytokines and subsequently modifies the thrombotic mechanisms.

Insulin is an independent predictor for cardiovascular risk in T2DM.[12] Subjects with insulin glucose tolerance and normal glucose levels, have increased cardiovascular risk, as a result of increased insulin levels.[13] As an anabolic molecule, insulin seems to have proatherogenic properties,[5] although it can also be hypothesized that insulin levels could be just a marker of insulin resistance, and is not directly involved in atherogenesis at a clinical level.

In contrast, insulin may have beneficial effects on cardiovascular system, since it stimulates NO release, via the L-arginine-nitric oxide pathway, a mechanism with a central role in the vasodilator action of insulin,[8] which is, however, impaired in obesity.[9] Furthermore, it may have antiinflammatory effects,[14] since it decreases the expression of proinflammatory cytokines from vascular wall, by modulating the regulatory role of nuclear factor kappa B pathway.[15] Indeed, *in vitro* data obtained from human aortic endothelial cells showed that insulin suppressed intracellular adhesion molecule 1 expression and secretion through its effects on NO synthesis,[16] while at a clinical level, insulin may have antiinflammatory effects in obese humans.[17] However, recent evidence suggested that insulin induces TNF-α secretion in human subcutaneous adipose cells,[18] implying that insulin may affect cytokine synthesis, in a tissue-specific way.

We hypothesized that in human T2DM, where the main source of proinflammatory cytokines is adipose tissue and where insulin resistance is a key feature in atherogenesis, exogenous insulin administration may induce proinflammatory cytokines synthesis.

We found that patients treated with insulin, had significantly higher levels of all the examined inflammatory markers, an effect that was independent from glycemic control, the angiographic extend of CAD or the duration of diabetes. However, it is also likely that TNF-α and IL-6 may induce insulin resistance,[19] while previous studies have shown that a low dose of insulin infusion benefit the clinical outcomes of both diabetic and nondiabetic patients with acute myocardial infarction.[20,21] In either case, our findings imply that in T2DM, the improvement of insulin resistance (e.g. weight loss or by using insulin sensitizers) could be a more useful strategy to decrease proinflammatory cytokines synthesis, than controlling glucose levels by exogenous insulin administration.

Hyperglycemia in diabetes mellitus induces endothelial dysfunction through several mechanisms.[22] Further to hyperglycemia, insulin resistance and obesity are additional components of endothelial dysfunction in T2DM.[22] Although insulin stimulates NO release by acting directly on endothelial cells,[8] in the presence of insulin resistance (a common finding in T2DM), the net effect of exogenous insulin administration could be proatherogenic.[9] Indeed, hyperinsulinaemia under conditions where pathways leading to production of NO are impaired but pathways related e.g. to endothelin production are intact, has an adverse effect on vascular endothelium.[9] In addition, other pathological effects of hyperinsulinaemia in the vasculature such as proliferation of vascular smooth muscle cells and accelerated atherosclerosis[23] are also hyperactive in the presence of insulin resistance.

In the present study, we have shown that exogenous administration of insulin does not improve endothelial function in patients with T2DM, compared to patients treated with oral hypoglycemic agents, even in the presence of similar glycemic control. Similarly, plasma vWF levels (an index of endothelial cells integrity in diabetes),[24] were also similar between patients treated with insulin or oral medication.

T2DM is associated with increased thrombogeneicity and elevated risk for thrombotic events.[25] Diabetic patients have elevated fV and fVII,[25] an effect which is closely related with insulin resistance, although the underlying mechanisms connecting diabetes/insulin resistance with these prothrombotic molecules, are unknown.

Proteins C and S are vitamin K-dependent proteins produced by hepatocytes and they inactivate factor V_a, having in this way anticoagulant effects.[25] In diabetes mellitus, factor V is increased while proteins C and S plasma levels are decreased.[25] Evidence suggests that TNF-α depresses the gene expression of PrtC mRNA in the liver,[26] while the exact mechanisms of this association are unclear. Furthermore, insulin itself may depress protein C-antigen synthesis, since protein C is decreased in patients with type 1 diabetes[27] although it is unclear whether there is any difference between patients with diabetes type 1 and 2.

We have shown that although none of the examined markers of thrombosis was different between the two study groups, plasma protein C was correlated with TNF-α and sVCAM-1 only in those patients who were under insulin treatment, but not in those who were under oral antidiabetic medication, suggesting that proinflammatory stimuli and especially TNF-α, may be connective links between exogenous insulin administration and protein C levels in patients with T2DM and CAD.

The observational design is a limitation of the study. As in all observational studies, it is difficult to know whether the choice of treatment is really causative in affecting the levels of these inflammatory markers, or whether patients who are able to be controlled with oral agents have lower circulating levels of these inflammatory markers for other reasons.

In conclusion, we have shown for the first time that in patients with CAD, insulin-dependent T2DM is associated with higher levels of IL-6, TNF-α and sVCAM-1, compared to T2DM treated with oral antidiabetic medication, independently from glycemic control, the angiographic extend of CAD or the duration of diabetes. Furthermore, the negative correlation observed between inflammatory markers and protein C in patients receiving exogenous insulin treatment, suggests that proinflammatory cytokines may be a link between insulin administration and increased thrombogeneicity in patients with T2DM. These findings imply that insulin sensitization should be the first priority in patients with CAD and T2DM, while exogenous insulin should be administered with caution.

References

1. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M: Mortality from coronary heart disease in subjects with Type 2 diabetes and in nondiabetic subjects with and without Prior Myocardial Infarction. *N Engl J Med* 1998;339:229–234
2. Pfutzner A, Marx N, Lubben G, Langenfeld M, Walcher D, et al.: Improvement of cardiovascular risk markers by pioglitazone is independent from glycemic control: results from the pioneer study. *J Am Coll Cardiol* 2005;45:1925–1931
3. Wellen KE, Hotamisligil GS: Inflammation, stress, and diabetes. *J Clin Invest* 2005;115:1111–1119
4. Morishita E, Asakura H, Jokaji H, Saito M, Uotani C, et al.: Hypercoagulability and high lipoprotein(a) levels in patients with type II diabetes mellitus. *Atherosclerosis* 1996;120:7–14
5. Sjoholm A, Nystrom T: Endothelial inflammation in insulin resistance. *Lancet* 2005;365:610–612
6. Hotamisligil GS: Inflammatory pathways and insulin action. *Int J Obes Relat Metab Disord* 2003;27(suppl 3):S53–S55
7. Muis MJ, Bots ML, Grobbee DE, Stolk RP: Insulin treatment and cardiovascular disease; friend or foe? A point of view. *Diabet Med* 2005;22:118–126
8. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 1994;94:2511–2515
9. Laakso M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. A novel mechanism for insulin resistance. *J Clin Invest* 1990; 85:1844–1852
10. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes* 1979;28:1039–1057
11. Tousoulis D, Antoniades C, Stefanadis C: Evaluating endothelial function in humans: a guide to invasive and non-invasive techniques. *Heart* 2005;91:553–558
12. Pyorala M, Miettinen H, Laakso M, Pyorala K: Plasma insulin and all-cause, cardiovascular, and noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study. *Diabetes Care* 2000;23:1097–1102
13. Unwin N, Shaw J, Zimmet P, Alberti KG: Impaired glucose tolerance and impaired fasting glycaemia: the current status on definition and intervention. *Diabet Med* 2002;19:708–723
14. Dandona P, Aljada A, Mohanty P: The anti-inflammatory and potential anti-atherogenic effect of insulin: a new paradigm. *Diabetologia* 2002;45:924–930
15. Aljada A, Ghanim H, Saadeh R, Dandona P: Insulin inhibits NFkappaB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab* 2001;86:450–453
16. Aljada A, Saadeh R, Assian E, Ghanim H, Dandona P: Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. *J Clin Endocrinol Metab* 2000;85:2572–2575
17. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, et al.: Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 2001; 86:3257–3265
18. Harte A, McTernan P, Chetty R, Coppack S, Katz J, et al.: Insulin-mediated Upregulation of the renin angiotensin system in Human Subcutaneous Adipocytes is reduced by Rosiglitazone. *Circulation* 2005;111:1954–1961
19. Golovchenko I, Goalstone ML, Watson P, Brownlee M, Draznin B: Hyperinsulinemia enhances transcriptional activity of nuclear factor-kappaB induced by angiotensin II, hyperglycemia, and advanced glycosylation end products in vascular smooth muscle cells. *Circ Res* 2000;87:746–752
20. Malmberg K, Ryden L, Efendic S, Herlitz J, Nicol P, et al.: Randomized trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction (DIGAMI study): effects on mortality at 1 year. *J Am Coll Cardiol* 1995;26:57–65
21. Diaz R, Paolasso EA, Piegas LS, Tajer CD, Moreno MG, et al.: Metabolic modulation of acute myocardial infarction. The ECLA (Estudios Cardiologicos Latinoamerica) Collaborative Group. *Circulation* 1998;98:2227–2234
22. Calles-Escandon J, Cipolla M: Diabetes and endothelial dysfunction: a clinical perspective. *Endocr Rev* 2001;22:36–52
23. Goalstone ML, Natarajan R, Standley PR, Walsh MF, Leitner JW, et al.: Insulin potentiates platelet-derived growth factor action in vascular smooth muscle cells. *Endocrinology* 1998; 139:4067–4072
24. Vischer UM, Emeis JJ, Bilo HJ, Stehouwer CD, Thomsen C, et al.: von Willebrand factor (vWf) as a plasma marker of endothelial activation in diabetes: improved reliability with parallel determination of the vWf propeptide (vWf:AgII). *Thromb Haemost* 1998;80:1002–1007
25. Carr ME: Diabetes mellitus: a hypercoagulable state. *J Diabetes Complicat* 2001;15:44–54
26. Yamamoto K, Shimokawa T, Kojima T, Loskutoff DJ, Saito H: Regulation of murine protein C gene expression in vivo: effects of tumor necrosis factor-alpha, interleukin-1, and transforming growth factor-beta. *Thromb Haemost* 1999;82:1297–1301
27. Vukovich TC, Schernthaner G: Decreased protein C levels in patients with insulin-dependent type I diabetes mellitus. *Diabetes* 1986;35:617–619