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Development of a disease-specific model to evaluate endothelial dysfunction in patients with diabetes mellitus

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Abstract

Diabetic patients have an increased cardiovascular risk. We propose to characterize the endothelial dysfunction in a disease-specific *in vitro* model. Human saphenous vein endothelial cells (HSVEC) were isolated from coronary artery bypass patients without and with non-insulin-dependent diabetes mellitus. Growth kinetics and proinflammatory responses (expression of adhesion molecules, cytokines) were documented under non-stimulating conditions. Diabetic HSVEC showed delayed growth kinetics with reduced cell densities of about 40%. During exponential growth of diabetic EC, the surface expression of adhesion molecules was increased 10-fold ($p \leqslant 0.05$). However, in a monolayer the expression adapted to low levels of non-diabetic EC. In addition, diabetic EC produced significantly more soluble E-selectin, VCAM-1, IL-6 and MCP-1. Our results suggest a link between the pathologically proinflammatory basic state of diabetic EC and the endothelial dysfunction in diabetic disease. Therefore, this *in vitro* model could be used for investigating early dysfunction and environmental effects on pathological endothelium.

Keywords: Endothelial dysfunction; *In vitro* model; Diabetes mellitus; Endothelial cells

Insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM) are associated with an increased risk for atherosclerotic cardiovascular disease [1]. Endothelial dysfunction, defined as an imbalance between relaxing and contracting factors, procoagulant and anticoagulant factors, and inflammatory and anti-inflammatory factors [2,3], are a condition sine qua non to vascular complications of diabetes mellitus [4]. The precise mechanisms regulating the initiation and progression of vascular lesions in diabetic patients remain unclear.

Inflammation, through overexpression of adhesion molecules and cytokines and leukocyte adhesion to endothelial cells, is thought to participate in the pathogenesis of atherosclerosis and also in the development of non-insulin-dependent diabetes mellitus [5]. It has been reported that exposure of vascular endothelium to elevated glucose concentration induces expression of adhesion molecules *in vitro* [6–8]. In addition, serum concentrations of soluble adhesion molecules (sE-selectin, sVCAM-1, sICAM-1) are elevated in patients with NIDDM [9–13].

In this study we presented for the first time a disease-related *in vitro* model to verify the endothelial dysfunction in the diabetic vascular disease.

Materials and methods

Cell culture. EC from saphenous veins (HSVEC) were prepared from diabetic (NIDDM, $n = 8$) and non-diabetic ($n = 8$) patients undergoing coronary artery bypass surgery. Subjects who self-reported having diabetes mellitus type 2 up to ten years (3/8 with oral antidiabetics, 3/8 insulin, 2/8 dietetic adjusted) or who had fasting glucose levels $\geqslant 7.0$ mM were designated as having diabetes mellitus. Informed consent was obtained from each patient, and the protocol for isolation of endothelial cells was approved by the Institutional Review Board of the University of

Abbreviations: EC, endothelial cells; NIDDM, non-insulin-dependent diabetes mellitus; IL-6, interleukin-6; IL-8, interleukin-8; MCP-1, monocyte chemoattractant protein-1; sVCAM-1, soluble vascular cellular adhesion molecule; sICAM-1, soluble intracellular adhesion molecule.

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Regensburg Medical Center. Study and preparation protocol were approved by the local human ethics committee (No. 99/133).

HSVEC were isolated and cultured by a modified method according to Jaffe et al. [14]. Briefly, delivering vessels from blood components, EC were detached by incubation with 0.05% collagenase A (Roche, Basel, Switzerland), washed several times with 10 mM Hepes (Sigma, St. Louis, Missouri, USA), and cultivated in endothelial growth medium (EGM-kit; Cat.Nr. C-22010, Promocell, Heidelberg, Germany) containing 30% pooled human serum (CMS, cell culture medium with serum). Confluent cultures were dissociated and transferred to 0.1% gelatine-coated tissue culture surfaces (Merck, Darmstadt, Germany). EC stock cultures were stored in liquid N_2 (in CMS containing 10% DMSO) until usage. EC were characterized by their cobblestone structure monitored by phase contrast microscopy (Leica DMRBE, Bensheim, Germany) and by flow cytometric analysis using anti-human CD31 antibody staining.

Experimental set-up. HSVEC in passage 3 (4000 cells/cm^2) were seeded in 96-well microtiter-plates with 150 µl CMS and cultured under standard culture conditions. On day 3 the medium was renewed for an additional 3 days.

For growth kinetic analysis, cell counts were determined (in quadruplicate) before, 24, 48 and 72 h after medium exchange using CASY1 (Schärfe Systems, Reutlingen, Germany). For each time point, supernatant was removed, adherent cells were washed with prewarmed PBS and detached with 50 µl collagenase for 5 min at 37 °C. After addition of 100 µl CMS on ice an aliquot of the cell suspension was used to determine cell count per well.

Cellular surface expression of adhesion molecules (E-selectin, VCAM-1, ICAM-1) was detected using cellular ELISA as described in our previous study [15]. In short, at each time point during exponential (24 and 48 h) and stationary growth kinetic (72 h) EC were fixed with acetone-methanol (1:1; −20 °C, 10 min), thawed and rehydrated with PBS. For celluar ELISA, EC were blocked with 1% BSA for one hour and incubated with mouse monoclonal anti-human ICAM-1 and VCAM-1 (DAKO, Glostrup, Denmark), and anti-human E-selectin (Ancell, Bayport, MN, USA) antibodies (diluted 1:1000 in PBS; 1 h, 37 °C). Biotinylated anti-mouse antibody (Vector Laboratories, Peterborough, UK) was diluted 1:1000 for ICAM-1 and VCAM-1, 1:500 for E-selectin, and incubated for another 30 min at 37 °C. Alkaline phosphatase streptavidine (Vector) was added as a conjugate (diluted 1:1000; 30 min, 37 °C). After each incubation step cells were washed twice with PBS. After addition of *p*-nitrophenylphosphate (Sigma; 1 mg/mL in 0.1 M diethanolamine, pH 10) the absorption at 405 nm was measured with microplate-reader (Molecular Devices, Union City, CA, USA). At each time point the cellular absorption was defined as the ratio of the absorption per well and the respective cell count per well (see above).

The quantitative detection of soluble ICAM-1, VCAM-1, E-selectin and IL-6, IL-8 and MCP-1 in HSVEC cultured supernatants was performed using commercially available ELISA-kits (Biosource, Nivelles, Belgium; R&D Systems, Wiesbaden; Beckman-Coulter, Krefeld; PromoCell, Heidelberg; Germany), according to the manufacturer's instructions.

Statistical analysis. Data are represented as median including 25% and 75% percentile. Mann–Whitney Rank Sum Test was used to statistically verify differences between both cell groups. A *p*-value $\leqslant 0.05$ was considered to be statistically significant.

Results

Cell donors and growth of isolated EC

Demographic data of both groups of cell donors were comparable (non-diabetic patients: 1 female/7 male, 67 ± 8 years, high 170 ± 7 cm, weight 84 ± 13 kg, BMI 28.0 ± 1.9 kg/m^2; diabetic patients: 2 female/6 male, 64 ± 10 years, high 173 ± 7 cm, weight 77 ± 14 kg, BMI 27.7 ± 3.7 kg/m^2). Diabetic patients were adjusted to a mean HbA1 c of 6.3 ± 1.4%. No differences with respect to hemodynamics and number of risk factors (excluding diabetes mellitus) for coronary artery disease were observed between both groups of cell donors. HSVEC from normal and diabetic adults were isolated and successfully maintained in culture. The content of contaminating fibroblasts or smooth muscle cells was <1%. For immunohistological evaluation, HSVEC of both sources were characterized by their specific cobblestone-like morphology, incorporation of Dil-Ac-LDL, and positive staining for CD31 and von Willebrand Factor.

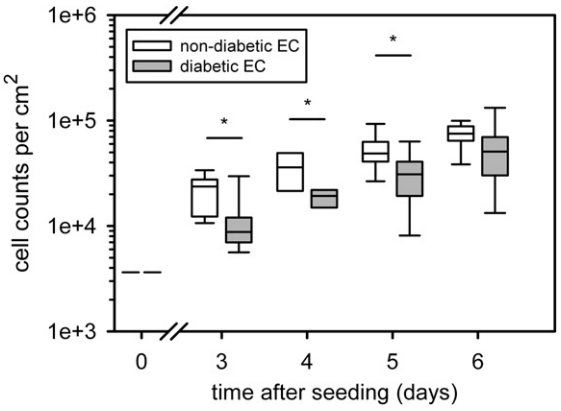

Fig. 1. Growth curves of non-diabetic and diabetic HSVEC. EC ($n = 8$ of each cell type) were seeded with a density of 4000 cells/cm^2 in 96-well microtiter plates and cultured for 6 days under identical culture conditions. Differences in the cell density were analyzed on day 3 to 6 ($^*p \leqslant 0.05$).

For growth kinetic analysis, EC of both groups were seeded with a density of 4000 cells/cm^2 and cultured under identical culture conditions for 6 days. Growth curves are shown in Fig. 1. After 3 days in culture, cell counts of non-diabetic and diabetic EC increased by a factor of 6.5 (3.4/7.3) ($p \leqslant 0.001$) and 2.4 (1.9/3.1) ($p \leqslant 0.001$), respectively. The reduced increase in cell density of diabetic cells differed significantly from the other cell group ($p \leqslant 0.05$). During the next 24 h, the number of cells increased by a factor of 2.0 (1.8/2.4) and 2.1 (2.0/2.3) for non-diabetic and diabetic cells, respectively. The doubling was significant ($p \leqslant 0.05$). The difference in cell density between non-diabetic and diabetic cells was maintained from day 3 to 5. After 6 days in culture, both cell groups reached confluence with cell densities of 75,200 (66,300/85,900) and 50,800 (42,700/61,000) EC per cm^2 (not significant) for non-diabetic and diabetic EC, respectively.

Expression of adhesion molecules

To describe the basic state of both cell groups, we analyzed the cellular expression of surface-bound adhesion molecules under non-stimulating conditions during cell growth and in the steady state of a confluent monolayer (day 6). The basal expression of adhesion molecules of non-diabetic EC was not affected over 6 days (Fig. 2). In contrast, diabetic EC showed a growth-dependent increase

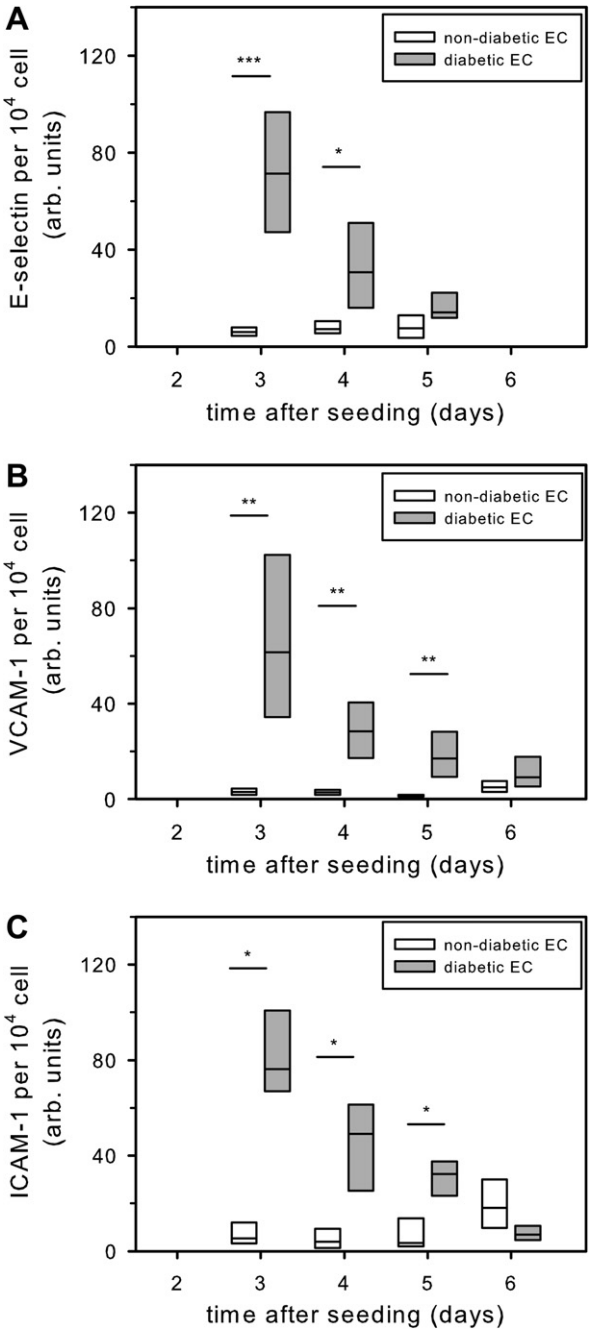

Fig. 2. Expression of surface-bound adhesion molecules during cell growth. The basal expression of E-selectin (A), VCAM-1 (B) and ICAM-1 (C) was determined during exponential growth and under steady-state conditions (as shown in Fig. 1) using a cellular ELISA. Statistical analysis compares expression on non-diabetic and diabetic EC at different time points (***$p \leqslant 0.001$; **$p \leqslant 0.01$; *$p \leqslant 0.05$).

in the expression of each adhesion molecule under non-stimulating conditions (Fig. 2). This means that proliferating diabetic EC (day 3) expressed by a factor of 12.8 (8.1/15.1), 15.0 (6.3/24.9) and 23.8 (9.6/47.3) significantly more E-selectin, VCAM-1 and ICAM-1 than non-diabetic cells, respectively. Under steady state conditions the basal expression of each adhesion molecule of both cell groups was rearranged.

Table 1
Expression of cellular adhesion molecules of TNF-stimulated non-diabetic ($n = 8$) and diabetic EC ($n = 8$) on day 6 in culture

	Activation factor		
	Non-diabetic EC	Diabetic EC	p value
E-selectin	14.7 (11.4/18.1)	15.6 (7.2/22.9)	n.s.
VCAM-1	16.6 (13.6/22.4)	11.7 (9.5/14.8)	n.s.
ICAM-1	8.9 (6.8/11.5)	5.3 (4.1/6.5)	0.04

Median (25%/75% percentile); p value compares activation factor (= ratio of TNF-stimulated vs respective unstimulated expression of adhesion molecules) of diabetic vs. non-diabetic endothelial cells (EC).

Finally, the induction of these adhesion molecules after TNF stimulation was analyzed for both groups. Cells in a confluent monolayer (day 6) were treated with TNF (10 ng/mL) for 4 h (E-selectin) and 24 h (VCAM-1, ICAM-1). The activation factor was defined as the ratio of the expression after TNF stimulation vs. basal expression (Table 1). Stimulation with TNF resulted in a significant increase in the expression of each adhesion molecule ($p \leqslant 0.001$), independent of the origin of the cells. In Table 1 the activation factors of non-diabetic and diabetic EC were opposed. Only the expression of ICAM-1 of diabetic EC was significantly lower after TNF induction. In addition, a comparison of the response to increasing doses of TNF for non-diabetic and diabetic EC is shown in Fig. 3. There was no difference in the degree of up-regulation of VCAM-1, ICAM-1 and E-selectin. The concentration of TNF where half of the maximal available adhesion molecules were expressed (EC_{50}) was identical for all adhesion molecules on all EC of both groups. The EC_{50} ranged between 0.02 and 0.06 ng/mL TNF.

Release of proinflammatory molecules

Endothelial activation was not only characterized by the surface expression of adhesion molecules, but also the release of their soluble forms and of proinflammatory cytokines/chemokines into the supernatants illustrates the activation status of EC. As shown in Fig. 4, diabetic EC released under non-stimulating conditions significantly higher amounts of soluble E-selectin, VCAM-1, MCP-1 and IL-6 than non-diabetic EC. In contrast, the concentrations of soluble ICAM-1 and IL-8 in the supernatants of diabetic and non-diabetic EC were comparable.

After TNF induction both cell types produced significantly higher amounts of IL-6, IL-8 and MCP-1 ($p \leqslant 0.001$). The activation factor was defined as the ratio of the TNF-induced and basal concentration in the supernatant (Table 2). There was no difference in the TNF-induced release of IL-6 and IL-8 for non-diabetic and diabetic EC. However, the increase in MCP-1 production was significantly reduced for diabetic EC. After incubation with TNF (24 h), diabetic EC produced 1437 (1170/1836) fg/mL and non-diabetic EC produced 1189 (1075/1270) fg/mL MCP-1 per cell (not significant).

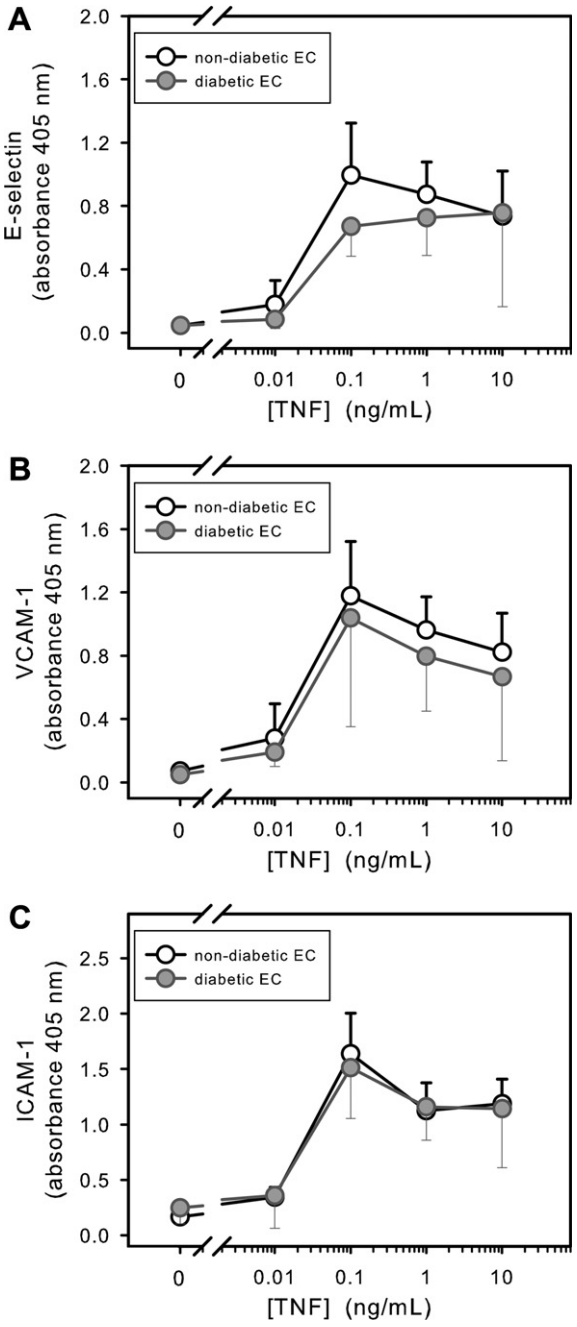

Fig. 3. Dose-dependent expression of surface-bound adhesion molecules on non-diabetic and diabetic EC. HSVEC of different origin in a confluent monolayer (day 6) were incubated with increasing doses of TNF (20 h) and the dose-dependent expression of E-selectin (A), VCAM-1 (B) and ICAM-1 (C) was determined with a cellular ELISA.

Discussion

Previous studies have associated an increased concentration of circulating adhesion molecules and cytokines in diabetic patients with the degree of metabolic control and the development of diabetic complications [9–13], suggesting that long-term chronic hyperglycemia may induce the development of complications. The present study shows for the first time that EC isolated from saphenous veins of diabetic patients remember their dysfunction also *in vitro*.

Saphenous veins from diabetic patients with coronary heart disease are frequently unusable for an aortocoronary bypass graft due to atherosclerotic plaques. To understand the underlying mechanisms of the dysfunction of diabetic endothelium and, furthermore, to verify therapeutic regimens in vascular diseases, the isolation and characterization of these cells was documented in the present study. Most scientific groups used human umbilical vein EC (HUVEC) for analysis of effects of high glucose concentrations [6–8], advanced glycation end products (AGES) [16,17], and oral antidiabetic drugs [18] on endothelial function. HUVEC have been the major source of primary EC, mainly because umbilical cords are readily available and preparation is easy [14] with high efficacy and purity. However, HUVEC are derived from immune-privileged fetal tissue [19], and show significant functional differences from adult vascular endothelium [20]. The availability of HSVEC was high in many cardio-thoracic hospitals. In our group we used all available vessel material no longer necessary for coronary bypass grafting to isolate HSVEC. Independent of accompanying diseases. about 95% of our preparations were successfully cultivated with purities of >99% EC in the cultures. One limitation for isolation of diabetic HSVEC in sufficiently large quantities for detailed experimentation was that only one third of our patients had non-insulin-dependent diabetes mellitus.

To verify our hypothesis that HSVEC isolated from vessels of diabetic patients remember their endothelial dysfunction also *in vitro*, we analyzed the growth characteristics and the inflammatory status of non-diabetic and diabetic EC. Since there was no difference other than the diabetic disease in the demographic data of both groups of respective cell donors, a direct comparison between normal and diabetic EC was performed. Our diabetic EC showed delayed growth kinetics. Therefore we speculate that a successful regeneration of denuded areas in the endothelial layer of an atherosclerotic stressed diabetic vessel may be time-retarded and incomplete. In addition, our data also provide evidence for the wound healing problems of diabetic patients.

Diabetic EC may have been subject to prolonged exposure to hyperglycaemia, AGES, and other stimuli, potentially resulting in an altered phenotype [16]. Thus, diabetic subjects expressed P-selectin and increased levels of ICAM-1 on retinal vessels [21], and E-selectin in glomeruli of stretozotocin-induced diabetic mice [22]. In addition, from several *in vitro* studies we know that high concentrations of glucose and AGES induce expression of ICAM-1 and VCAM-1 [6–8] in HUVEC. Our data clearly show that HSVEC isolated from vessels of diabetic patients were characterized by an activated basal level. Diabetic EC expressed significantly higher amounts of adhesion molecules and produced more IL-6 and MCP-1 than non-diabetic EC. In addition, proliferating cells expressed by a factor of 10–25 more membrane-bound

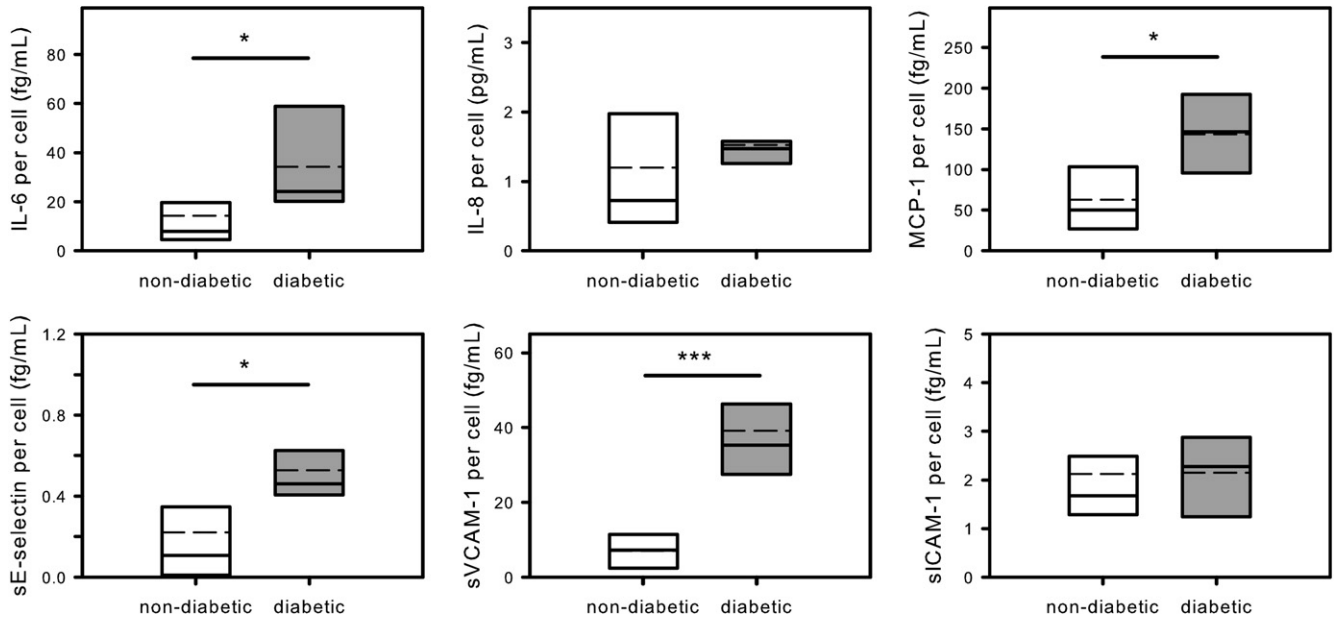

Fig. 4. Release of soluble adhesion molecules and proinflammatory cytokines in the supernatant of non-diabetic and diabetic EC under non-stimulating conditions. HSVEC were incubated with endothelial growth medium for 48 h. The content of L-6, IL-8, MCP-1 and soluble adhesion molecules was determined in the supernatants using commercially available immunoassays ($^{***}p \leqslant 0.001$; $^{*}p \leqslant 0.05$).

Table 2
TNF-induced release of IL-6, IL-8 and MCP-1 of non-diabetic ($n = 8$) and diabetic EC ($n = 8$)

	Activation factor		
	Non-diabetic EC	Diabetic EC	p value
IL-6	5.7 (3.1/8.2)	3.8 (2.4/4.0)	n.s.
IL-8	3.2 (2.1/6.5)	2.9 (2.5/4.0)	n.s.
MCP-1	25.6 (16.1/36.8)	11.0 (8.3/12.9)	0.04

Median (25%/75% percentile); p value compares the activation factor of diabetic vs. non-diabetic endothelial cells (EC).

E-selectin, VCAM-1 and ICAM-1 than confluent cultures. Respective effects were described in retinal EC (HREC) of diabetic vs. non-diabetic origin in response to epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Diabetic HREC secreted higher concentrations of plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) [23,24]. Analogous to our data for the expression of adhesion molecules, the increase in PA from HREC of diabetic origin was dependent on the state of confluency, with only proliferating diabetic cells showing an increase in protein expression [24]. The HREC model was also used to support an enhanced insulin-like growth factor (IGF-I) action in diabetic EC as an important cellular event in diabetic retinopathy [25]. Otherwise, the use of a diabetic EC model for detection of mechanisms in vascular dysfunction failed.

Despite an activated basal level of diabetic HSVEC, the response to exogenous stimuli such as TNF was largely not affected. Only the TNF-induced expression of ICAM-1 was lower in diabetic than in non-diabetic EC. At the moment, we are unable to explain this isolated effect.

With our *in vitro* model of diabetic EC we could verify the endothelial dysfunction of patients with NIDDM. Impaired cell growth and a proinflammatory basic state of the diabetic cell indicates imbalanced wound healing and increased endothelial activation.

Acknowledgments

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References

[1] N.D. Wong, Screening and risk stratification of patients with the metabolic syndrome and diabetes, Expert. Rev. Cardiovasc. Ther. 4 (2) (2006) 181–190.
[2] G.R. De Meyer, A.G. Herman, Vascular endothelial dysfunction, Prog. Cardiovasc. Dis. 39 (4) (1997) 325–342.
[3] C. Rask-Madsen, G.L. King, Mechanisms of disease: endothelial dysfunction in insulin resistance and diabetes, Nat. Clin. Pract. Endocrinol. Metab. 3 (1) (2007) 46–56.
[4] M.M. Hartge, U. Kintscher, T. Unger, Endothelial dysfunction and its role in diabetic vascular disease, Endocrinol. Metab. Clin. North Am. 35 (3) (2006) 551–560, viii–ix.
[5] M.F. Lopes-Virella, G. Virella, The role of immune and inflammatory processes in the development of macrovascular disease in diabetes, Front Biosci. 8 (2003) s750–s768.
[6] S. Takami, S. Yamashita, S. Kihara, K. Kameda-Takemura, Y. Matsuzawa, High concentration of glucose induces the expression of intercellular adhesion molecule-1 in human umbilical vein endothelial cells, Atherosclerosis. 138 (1) (1998) 35–41.
[7] T.S. Altannavch, K. Roubalova, P. Kucera, M. Andel, Effect of high glucose concentrations on expression of ELAM-1, VCAM-1 and

ICAM-1 in HUVEC with and without cytokine activation, Physiol Res. 53 (1) (2004) 77–82.
[8] L. Quagliaro, L. Piconi, R. Assaloni, R. Da Ros, A. Maier, G. Zuodar, A. Ceriello, Intermittent high glucose enhances ICAM-1, VCAM-1 and E-selectin expression in human umbilical vein endothelial cells in culture: the distinct role of protein kinase C and mitochondrial superoxide production, Atherosclerosis. 183 (2) (2005) 259–267.
[9] M. Koga, M. Otsuki, M. Kubo, J. Hashimoto, S. Kasayama, Relationship between circulating vascular cell adhesion molecule-1 and microvascular complications in type 2 diabetes mellitus, Diabet. Med. 15 (8) (1998) 661–667.
[10] K. Matsumoto, Y. Sera, H. Nakamura, Y. Ueki, S. Miyake, Serum concentrations of soluble adhesion molecules are related to degree of hyperglycemia and insulin resistance in patients with type 2 diabetes mellitus, Diabetes Res. Clin. Pract. 55 (2) (2002) 131–138.
[11] M. Bluher, R. Unger, F. Rassoul, V. Richter, R. Paschke, Relation between glycaemic control, hyperinsulinaemia and plasma concentrations of soluble adhesion molecules in patients with impaired glucose tolerance or Type II diabetes, Diabetologia 45 (2) (2002) 210–216.
[12] S. Muller, S. Martin, W. Koenig, P. Hanifi-Moghaddam, W. Rathmann, B. Haastert, G. Giani, T. Illig, B. Thorand, H. Kolb, Impaired glucose tolerance is associated with increased serum concentrations of interleukin 6 and co-regulated acute-phase proteins but not TNF-alpha or its receptors, Diabetologia 45 (6) (2002) 805–812.
[13] M.S. Boulbou, G.N. Koukoulis, E.D. Makri, E.A. Petinaki, K.I. Gourgoulianis, A.E. Germenis, Circulating adhesion molecules levels in type 2 diabetes mellitus and hypertension, Int. J. Cardiol. 98 (1) (2005) 39–44.
[14] E.A. Jaffe, R.L. Nachman, C.G. Becker, C.R. Minick, Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria, J. Clin. Invest. 52 (1973) 2745–2756.
[15] K. Lehle, J. Buttstaedt, D.E. Birnbaum, Expression of adhesion molecules and cytokines in vitro by endothelial cells seeded on various polymer surfaces coated with titaniumcarboxonitride, J. Biomed. Mater. Res. A 65 (3) (2003) 393–401.
[16] G. Basta, A.M. Schmidt, R. De Caterina, Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes, Cardiovasc. Res. 63 (4) (2004) 582–592.
[17] P. Ehlermann, K. Eggers, A. Bierhaus, P. Most, D. Weichenhan, J. Greten, P.P. Nawroth, H.A. Katus, A. Remppis, Increased proinflammatory endothelial response to S100A8/A9 after preactivation through advanced glycation end products, Cardiovasc Diabetol. 5 (2006) 6.
[18] M. Itoh, H. Omi, M. Okouchi, K. Imaeda, M. Shimizu, T. Fukutomi, N. Okayama, The mechanisms of inhibitory actions of gliclazide on neutrophils-endothelial cells adhesion and surface expression of endothelial adhesion molecules mediated by a high glucose concentration, J. Diabetes Complications 17 (1) (2003) 22–26.
[19] L.E. French, M. Hahne, I. Viard, G. Radlgruber, R. Zanone, K. Becker, C. Muller, J. Tschopp, Fas and Fas ligand in embryos and adult mice: ligand expression in several immune-privileged tissues and coexpression in adult tissues characterized by apoptotic cell turnover, J. Cell. Biol. 133 (2) (1996) 335–343.
[20] P.H. Tan, C. Chan, S.A. Xue, R. Dong, B. Ananthesayanan, M. Manunta, C. Kerouedan, N.J. Cheshire, J.H. Wolfe, D.O. Haskard, K.M. Taylor, A.J. George, Phenotypic and functional differences between human saphenous vein (HSVEC) and umbilical vein (HUVEC) endothelial cells, Atherosclerosis. 173 (2) (2004) 171–183.
[21] D.S. McLeod, D.J. Lefer, C. Merges, G.A. Lutty, Enhanced expression of intracellular adhesion molecule-1 and P-selectin in the diabetic human retina and choroid, Am. J. Pathol. 147 (3) (1995) 642–653.
[22] S. Narumi, M.L. Onozato, A. Tojo, S. Sakamoto, T. Tamatani, Tissue-specific induction of E-selectin in glomeruli is augmented following diabetes mellitus, Nephron 89 (2) (2001) 161–171.
[23] I.D. Munjal, N.V. McLean, M.B. Grant, D.A. Blake, Differences in the synthesis of secreted proteins in human retinal endothelial cells of diabetic and nondiabetic origin, Curr. Eye Res. 13 (4) (1994) 303–310.
[24] M.B. Grant, C. Guay, Plasminogen activator production by human retinal endothelial cells of nondiabetic and diabetic origin, Invest. Ophthalmol. Vis. Sci. 32 (1) (1991) 53–64.
[25] P.E. Spoerri, E.A. Ellis, R.W. Tarnuzzer, M.B. Grant, Insulin-like growth factor: receptor and binding proteins in human retinal endothelial cell cultures of diabetic and non-diabetic origin, Growth Horm. IGF Res. 8 (2) (1998) 125–132.