Insulin Suppresses Plasma Concentration of Vascular Endothelial Growth Factor and Matrix Metalloproteinase-9

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OBJECTIVE — We recently demonstrated a potent anti-inflammatory and thus a potential antiatherogenic effect of insulin in human aortic endothelial cells and mononuclear cells at physiologically relevant concentrations. We have now further investigated the anti-inflammatory suppressive action of insulin on vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMP)-9. VEGF and MMP-9 play a central regulatory role in angiogenesis, contribute to the pathogenesis of proliferative retinopathy, and have also been found to accelerate atherosclerosis.

RESEARCH DESIGN AND METHODS — Insulin was infused (2 IU/h) in 5% dextrose (100 ml/h) and KCl (8 mmol/h) into 10 fasting, obese, nondiabetic subjects for 4 h. Subjects were also infused with 5% dextrose without insulin and with saline on two separate occasions. Blood samples were obtained at 0, 2, 4, and 6 h.

RESULTS — Plasma insulin concentrations increased from a basal level of 12.5 ± 2.2 to 28.2 ± 3.3 μU/ml at 2 h and 24.4 ± 3.7 μU/ml at 4 h after insulin infusion. VEGF concentration decreased from 307.2 ± 163.8 pg/ml (100%) at 0 h to 73.5 ± 20.9% of the basal level at 2 h and 67.1 ± 23.2% at 4h. Plasma MMP-9 concentrations decreased from 375 ± 196.3 ng/ml (100%) at 0 h to 83 ± 22% of the basal level at 2 h and to 82 ± 21% of the basal level at 4 h ($P < 0.05$). Dextrose infusion alone did not change plasma VEGF concentration. However, plasma MMP-9 concentration increased significantly at 4 h following dextrose infusion alone ($P < 0.05$). Saline infusions without insulin caused no alteration in glucose, insulin, VEGF, or MMP-9.

CONCLUSIONS — These observations may have implications for a potential antiretinopathic and antiatherosclerotic effect of insulin in the long term.

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Proliferative diabetic retinopathy is characterized by neovascularization in the retina. Although the pathogenic mechanisms underlying retinopathy are not clear, it appears that ischemia, due to capillary nonperfusion, is probably the initial event (1,2). This triggers hypoxia-induced genes to be expressed through several transcription factors, including hypoxia-inducible factor, which regulates the expression of the gene for vascular endothelial growth factor (VEGF) (3,4). VEGF induces the proliferation of endothelium at both the microvascular and macrovascular levels. It thus stimulates the formation of endothelial tubes, which develop into immature and fragile capillaries. These are highly permeable and leaky. Mature capillaries are formed through the further action of angiopoietin (5–7). The intraocular concentrations of VEGF have been shown to be markedly elevated in patients with proliferative retinopathy undergoing vitrectomy for intravitreal bleeding. Thus, VEGF may actively contribute to the pathogenesis of proliferative retinopathy.

VEGF has also been found to accelerate atherosclerosis in animal models (8) and thus may have a proinflammatory action because atherosclerosis is a chronic inflammatory process of the arterial wall (9). Neovascularization of the adventitia and the media in the arterial wall is now believed to provide blood supply to the growing atherosclerotic plaque (10). VEGF has also been shown to stimulate the expression of matrix metalloproteinases (MMPs) (11,12). MMPs cause dissolution of the extracellular matrix, which allows the endothelial cells to spread and form endothelial tubes. Furthermore, MMPs are involved in atherogenesis, vascular remodeling, and the creation and rupture of the atherosclerotic plaque (13,14). We have recently shown that insulin has an anti-inflammatory effect because it suppresses nuclear factor-κB (NF-κB), intracellular adhesion molecule (ICAM)-1, and monocyte chemoattractant protein (MCP)-1 in human aortic endothelial cells in vitro (15,16) and NF-κB, ICAM-1, MCP-1, and C-reactive protein in mononuclear cells in vivo (17). This effect is acute and potent and is exerted at physiologically relevant concentrations. We have, therefore, now explored the possibility that insulin may have an inhibitory effect on plasma concentrations of VEGF and MMP-9.

RESEARCH DESIGN AND METHODS

— Ten obese nondiabetic subjects (age range 29–64 years; mean 48.3 ± 10.9 years), all with BMI >37 kg/m^2, (mean BMI 42.6 ± 9.1 kg/m^2) were included in this study. All patients had a fasting venous plasma glucose <100 mg/dl. All had impaired glucose tolerance with a 2-h postchallenge glucose ranging between 145 and 180 mg/dl. None of the obese subjects were on vitamin E or C or

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Abbreviations: ICAM, intracellular adhesion molecule; MCP, monocyte chemoattractant protein; MMP, matrix metalloproteinase; NF-κB, nuclear factor-κB; VEGF, vascular endothelial growth factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Sequential glucose and insulin concentrations following insulin + glucose, glucose, and saline infusions*

	0 h	2 h	4 h	6 h
Insulin infusion				
Insulin (μIU/ml)	13 ± 2	28 ± 3*	24 ± 4*	11 ± 3
Glucose (mg/dl)	73 ± 2	76 ± 5	73 ± 2	71 ± 11
Dextrose infusion				
Insulin (μIU/ml)	16 ± 10	17 ± 9	14 ± 6	9 ± 5
Glucose (mg/dl)	76 ± 11	82 ± 14	76 ± 14	69 ± 5
Saline infusion				
Insulin (μIU/ml)	14 ± 3	12 ± 1	11 ± 2	13 ± 2
Glucose (mg/dl)	80 ± 2	77 ± 3	74 ± 2	70 ± 3

Data are means ± SE. Fasting insulin concentrations in normal subjects in our laboratory are 4 ± 2 μU/ml (<8 μU/ml). *Significantly different from 0 h at $P < 0.05$.

any other antioxidant therapy. There were eight female and two male subjects. Insulin was infused into subjects (2–2.5 IU/h), along with 5% dextrose (100 ml/h) and 10 mmol KCl for 4 h. The rate of insulin infusion was titrated such that glucose concentrations were maintained as close to the basal levels as possible. Blood glucose concentrations were measured every 15 min from superpricks (Hemocue) to ensure that they did not deviate significantly. The rate of insulin infusion was altered accordingly as necessary. Minimal alterations were necessary at the low rates of infusion of both glucose and insulin. Blood samples for detailed analysis were collected at baseline, 2, 4, and 6 h and were collected in Na–EDTA as the anti-coagulant. Subjects returned after 2–3 weeks on two occasions and were infused with 5% dextrose (100 ml/h), 10 mmol KCl, and normal physiological saline (100 ml/h and 10 mmol KCl/h) for control data. The Institutional Review Board of State University of New York at Buffalo based at Millard Fillmore Hospital approved the study. Written informed consent was obtained from all subjects.

Plasma insulin and glucose measurements

Insulin was measured from fasting plasma samples using an enzyme-linked immunosorbent assay kit from Diagnostic Systems Laboratories (Webster, TX). Glucose was measured in whole blood by a Hemocue glucose analyzer (Hemocue, Mission Viejo, CA).

Plasma VEGF and MMP-9 measurement

Plasma VEGF and MMP-9 were assayed with enzyme-linked immunosorbent assay kits from R&D Systems (Minneapolis, MN). The minimum detectable dose of VEGF assay was 5 pg/ml, with an intra-assay variation of 4.5% and inter-assay variation of 7%. The minimum detectable dose of MMP-9 assay was 0.156 ng/ml, with an intra-assay variation of 2% and interassay variation of 8%. The variability in fasting MMP-9 concentrations is in the range of 10%.

Statistical analysis

Statistical analysis was performed using SigmaStat software (Jandel Scientific, San Rafael, CA). All data on VEGF and MMP-9 were normalized to a baseline of 100% in view of the interindividual variability and are expressed accordingly as the percentage of the basal level. One-way ANOVA for repeated measures was used to compare all of the indexes measured in this study. The results are expressed as mean ± SE.

RESULTS — Plasma glucose concentration remained steady during insulin infusion. Glucose levels were 73 ± 2 mg/dl at baseline, 76 ± 5 mg/dl at 2 h, and 73 ± 2 mg/dl at 4 h. Plasma glucose concentration also remained steady following dextrose or saline infusion. The concentrations of glucose were similar to those after glucose-insulin infusion. Plasma insulin concentrations increased from a basal level of 13 ± 2 to 28 ± 3 μU/ml at 2 h, and 24 ± 4 μU/ml at 4 h after insulin infusion (Table 1). Insulin levels decreased after the cessation of insulin infusion and returned to the basal level (11 ± 3 μU/ml) at 6 h. Dextrose infusion caused a slight increase in glucose levels at 2 h. There was no significant increase in insulin in the glucose-infused group.

VEGF concentrations decreased significantly following insulin infusion ($P <$

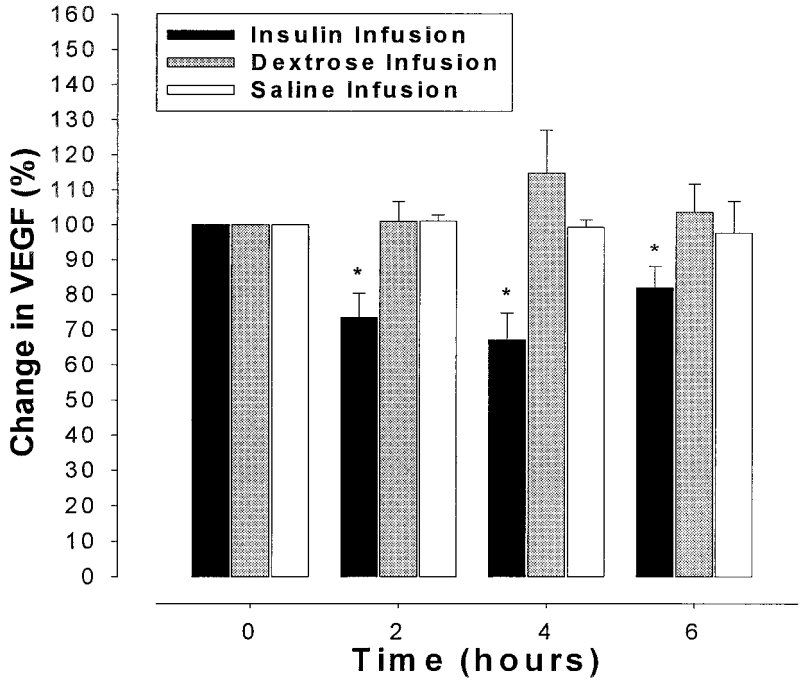

**Figure 1—*Percent change in plasma VEGF concentrations following insulin, dextrose, or saline infusions. Note that plasma VEGF decreased significantly following insulin infusion and continued to be inhibited significantly after 2 h from the cessation of insulin infusion (*$P < 0.05$ *when compared with baseline). Dextrose and saline infusions caused no change in VEGF concentration.*

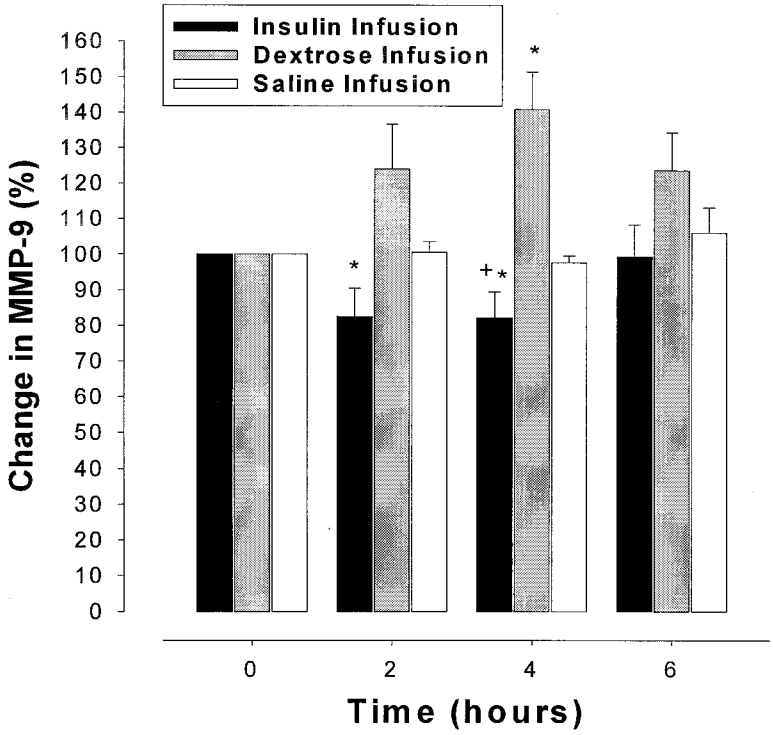

Figure 2—*Percent change in plasma MMP-9 concentrations following insulin or dextrose or saline infusions (*P < 0.05 when compared with baseline; +P < 0.05 when compared with glucose-infused group). Note the significant fall in MMP-9 following insulin infusion and the increase after dextrose infusion. Saline infusion caused no change.*

0.001) (Fig. 1) and continued to be inhibited at 6 h, even after the cessation of insulin infusion. Basal plasma VEGF concentration was 307.2 ± 163.8 pg/ml. Levels decreased to 73.5 ± 20.9% of the basal level at 2 h, 67.1 ± 23.2% at 4 h, and 82.0 ± 18.5% at 6 h. Dextrose infusion alone did not change plasma VEGF concentrations significantly. Plasma MMP-9 concentrations decreased from 375.1 ± 196.3 ng/ml (100%) to 83 ± 7.9% of the basal level at 2 h, 82 ± 7.4% of the basal level at 4 h, and reverted to 99 ± 9.0% at 6 h ($P < 0.05$). Plasma MMP-9 concentrations increased significantly following dextrose infusion alone ($P < 0.05$), as shown in Fig. 2. Thus, the fall in MMP-9 concentration with insulin was even more impressive (by 40%) when compared with glucose-infused control subjects. Saline infusion caused no change in glucose, insulin, VEGF, or MMP-9 concentrations.

CONCLUSIONS — Our data demonstrate clearly that insulin acutely causes a lowering of plasma VEGF and MMP-9 concentrations. The magnitude of this fall was 35% for VEGF and 40% for MMP-9 when compared with glucose-infused control subjects. The fall was also significant when compared with the baseline of saline-infused control subjects. These data are consistent with our previous observations (17,18) that insulin inhibits the expression of NF-κB and early growth response-1, the two proinflammatory transcription factors that modulate the inflammation-inducing genes such as ICAM-1, MCP-1, C-reactive protein, p47phox, plasminogen activator inhibitor 1, and tissue factor. This inhibitory effect lasted for 4 h, the duration of insulin infusion. Because NF-κB is a modulator of VEGF expression (19), it is possible that NF-κB suppression by insulin causes the suppression of VEGF concentrations. It is of interest that VEGF also induces NF-κB activity (20).

This observation is relevant to the pathogenesis of retinopathy in that the absence of insulin and of adequate insulin action, as in type 2 diabetes, may result in an increase in VEGF expression/secretion. This will promote neovascularization at the microvasculature level and from adventitia into the media and intima to supply blood to the growing atherosclerotic plaque (8). Hyperglycemia also increases the expression and secretion of VEGF at the cellular level (21) and may thus contribute to the endothelial abnormalities leading to local inflammatory changes, which trigger leukocytic adhesion to endothelium. Leukocytic adhesion to retinal capillaries mediated by the adhesion molecule, ICAM-1 (22,23), can cause plugging of the capillaries, capillary nonperfusion, retinal ischemia, and the changes that trigger increased VEGF expression locally. We have previously demonstrated that insulin suppresses ICAM-1 expression by endothelial cells and therefore lowers the concentration of ICAM-1 in plasma. Our current observations indicate that insulin lowers VEGF concentration rapidly, suggesting that it may have a direct effect on the expression of VEGF at the cellular level. MMP-9 implicated in the lysis of the matrix proteins, especially collagen, was also suppressed by insulin. MMP action on the extracellular matrix allows the endothelial tubes to be formed from the proliferation of endothelial cells under the action of VEGF. In addition, MMPs are responsible for the rupture of the atherosclerotic plaque through the lysis of the fibrous cap of the plaque. The increase in MMP-9 concentration by glucose is intriguing but is consistent with a proinflammatory effect of glucose. Saline-infused control subjects showed no changes in MMP-9.

Although VEGF is usually secreted under hypoxic conditions, inflammatory stimuli may also cause it to be expressed and secreted. Thus, hypoxia-inducing factor, the transcription factor that regulates VEGF gene expression, may be induced through NF-κB activation by interleukin-1 and tumor necrosis factor-α (24).

There is one observation showing an increase in the expression of VEGF retinal pigment endothelial cells following incubation with insulin in vitro. However, these increases were observed with pharmacological concentrations of insulin (5 nmol/l ≅ 800 μU/ml) (25). The same authors showed that a similarly high concentration of insulin injected into the eye also induced an increased expression of VEGF in the retina.

It is also noteworthy that there is a lack of correlation between plasma concentrations of VEGF and the prevalence

or severity of retinopathy. This is probably due to the fact that the local concentrations of VEGF in any tissue determine the action of VEGF and thus the neovascularization in that tissue. Therefore, local ischemia in the retina determines the expression of VEGF in the retina; this then triggers MMPs and neovascularization. Although insulin may have a general tonic inhibitory effect on the expression of VEGF, ischemia may turn out to be the major determinant locally. On the other hand, the lack of adequate insulin bioavailability and action in diabetes may trigger VEGF release and neovascularization. Indeed, intensive insulin treatment in patients with diabetes over prolonged periods has been shown to markedly reduce the incidence of background and proliferative retinopathy in many studies: the Diabetes Control and Complications Trial (26), the Kumamoto Study (27), and the Oslo study (28). Although a reduction in glucose concentrations may have largely induced this improved outcome, an increase in the bioavailablity of insulin at appropriate times during intensive insulin therapy may have also contributed to this effect. It should also be appreciated that there may be factors other than VEGF that determine endothelial proliferation and neovascularization, e.g., fibroblast growth factor and angiopoietin. This requires further investigation.

In conclusion, insulin acutely suppresses the concentration of VEGF and MMP-9 in plasma. This has implications for the pathogenesis of proliferative retinopathy of diabetes and for atherogenesis and plaque rupture in patients with diabetes and insulin resistance.

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