CORRESPONDENCE

To the Editor:

In the July 1994 issue of *Metabolism,* Metsärinne et al presented data on the effect of insulin on endothelin-1 (ET-1) secretion in human cultured endothelial cells.[1] They confirmed previous reports obtained in cultured bovine,[2] porcine,[3] human,[4] and hybrid EA.hy 926 endothelial cells,[4] which showed that insulin stimulates the release of this endothelium-derived vasoconstrictive peptide in a dose-dependent fashion.

Surprisingly, Metsärinne et al showed that hyperinsulinemia was not associated with increased plasma ET-1 levels in healthy subjects in vivo. Indeed, they infused insulin at rates of 0, 20, 60, or 400 $mU/m^2 \cdot min^{-1}$ in 23 healthy volunteers, maintaining plasma glucose concentrations of approximately 5.0, 9.0, 14.0, and 22 mmol/L, and demonstrated no significant change in plasma venous ET-1 levels after 120 minutes of each insulin infusion. Metsärinne et al claimed that their findings would need to be reconciled with the above-cited study by Hu et al,[2] who described a fourfold increase in plasma ET-1 concentration after subcutaneous abdominal implantation of an insulin pellet (Linplant, Toronto, Canada) in both healthy Sprague–Dawley male rats and Sprague–Dawley male rats made diabetic by a 50-mg/kg streptozocine injection.

The study by Metsärinne et al is an elegant piece of work, but the results could be interpreted in a different manner. Indeed, the most important reconciliation should be found with two other reports in humans, the first by Wolpert et al[5] and the second by our group,[6,7] both of which showed a significant increase in plasma venous ET-1 concentrations after a 2-hour euglycemic-hyperinsulinemic clamp in humans (20 $mU/m^2 \cdot min^{-1}$ insulin infused in 7 obese females according to an open design and 40 $mU/m^2 \cdot min^{-1}$ infused in 16 non–insulin-dependent diabetic males according to a single-blind, randomized, placebo-controlled design, respectively). To further support the role of insulin as a contributor to ET-1 regulation in vivo, Wolpert et al[5] also studied changes of plasma fasting insulin and ET-1 concentrations after weight loss due to caloric restriction. Interestingly, they showed that circulating ET-1 concentrations decrease with weight reduction in parallel with changes in insulin levels. Moreover, the reduction in basal plasma ET-1 levels with weight loss correlated strongly with the reduction in fasting insulin levels ($r = .92$, $P < .01$). Following these results, little doubt may be raised on the existence of an insulin–ET-1 relationship in vivo, and we suggest that the experimental design of the study by Metsärinne et al[1] may have revealed some other important aspects in this field.

Indeed, reading into the lines of their interesting report, it seems that only a few subjects (n = 6) received insulin under euglycemic conditions (5 mmol/L), while most subjects received insulin during mildly (n = 6) or even markedly hyperglycemic conditions (n = 11). In this context, it should be noted that D-glucose inhibits ET-1 release from cultured porcine[3] and human[8] endothelial cells. Furthermore, the inhibitory effect of D-glucose on ET-1 secretion becomes evident only for the highest molar concentrations (from 27.5/L mmol to 55 mmol/L in both studies), ie, at concentrations very close to those reached by Metsärinne et al[1] during their clamp studies. On the other hand, analyzing data obtained by the same authors during euglycemic hyperinsulinemia, it appears evident that circulating ET-1 concentrations increased in six subjects from levels of 3.8 ± 0.4 pg/mL at time 0 to 5.0 ± 0.5 pg/mL after 120 minutes of insulin infusion (20 $mU/m^2 \cdot min^{-1}$). The mean percentage insulin-induced increment of plasma ET-1 levels was of 131%, ie, very close to the 150% ET-1 increase ($P < .001$) obtained by the authors after stimulation of cultured human endothelial cells with insulin. Therefore, it does not seem arbitrary to propose that the data reported by Metsärinne et al, at least in vivo, may simply reflect the small dimension of the study population in the euglycemic-hyperinsulinemic clamp studies, as well as the opposite effects on ET-1 release (ie, stimulatory *v* inhibitory) that were exerted simultaneously by insulin and glucose during hyperglycemic-hyperinsulinemic clamp studies.

We are convinced the conclusion made by Metsärinne et al[1] at the end of their elegant work is correct, and that "the role of insulin in the regulation of ET-1 production in vivo remains unsettled." However, we think that a larger study population under euglycemic conditions will result in a evident increment of plasma ET-1 levels during insulin infusion, as suggested by the Metsärinne data, and we are convinced that data obtained by the same authors under hyperglycemic-hyperinsulinemic conditions indicate that the relationship between insulin and ET-1 in vivo needs a general rethinking under the light of the glucose–ET-1 interrelation. Obviously, we cannot exclude a more simple explanation for the discrepancies existing among the data from Metsärinne et al, Wolpert et al, and ourselves, ie, that the vascular endothelium might have different ET-1 secretory responses in patients affected by non–insulin-dependent diabetes and obesity compared with young healthy non–insulin-resistant men. This hypothesis needs confirmation by studies comparing insulin-resistant subjects with healthy subjects.

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REPLY

To the Editor:

Drs Ferri and De Mattia suggest that our finding of unchanged plasma ET-1 levels during hyperinsulinemic clamp (performed at four glucose concentrations: ~5, 9, 14, and 22 mmol/L) in 23 healthy men may be due to the small number of subjects and concomitant inhibitory effect of glucose on ET-1 release during the hyperglycemic-hyperinsulinemic clamps. As described in the Methods section,[1] the 23 volunteers were divided into four groups, which received insulin at infusion rates of 0 (n = 6), 20 (n = 6), 60 (n = 6), and 400 (n = 5) mU/m^2 for 120 minutes. Then, each of the patients participated in four studies, in which the insulin dose for a given patient was always the same, but the glucose concentration varied as specified above. Thus, under euglycemic conditions (and also during three levels of hyperglycemia), a total of 17 patients (6 + 6 + 5) received insulin as specified above. No significant effect of insulin on plasma ET-1 levels during euglycemia was found (analysis of variance followed by *t* test). The result, therefore, cannot be explained by a small study population. In addition, we measured plasma ET-1 from numerous other subjects during euglycemic-hyperinsulinemic clamp studies and did not find any stimulatory effect of insulin (unpublished results).

The question whether glucose participates in the regulation of ET-1 in vivo or in vitro is an interesting one. In vitro, both increased[2] and decreased[3] release of ET-1 have been reported. In our study, we found no effect of glucose on(1) plasma ET-1 levels in vivo and (2) ET-1 release from human umbilical cord vein endothelial cells in vitro. Neither did hyperglycemia modify the stimulatory effect of insulin on ET-1 release in vitro.

We agree with the proposition of Drs Ferri and De Mattia that the function of vascular endothelium in healthy men as compared with patients with non–insulin-dependent diabetes and obesity may be different. At least diabetes has been shown to damage endothelium. Endothelial dysfunction, which may be defined as an imbalance between endothelial production of vasodilating and vasoconstrictive agents, may involve increased production rate of ET-1, but we feel confident that insulin does not increase ET-1 concentrations in vivo.

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