Effects of insulin infusion on endothelium-derived vasoactive substances

K.H. Polderman¹, C.D.A. Stehouwer¹, G.J. van Kamp², L.J.G. Gooren³

¹ Department of Internal Medicine, Academisch Ziekenhuis Vrije Universiteit, Amsterdam, The Netherlands

² Department of Clinical Chemistry, Academisch Ziekenhuis Vrije Universiteit, Amsterdam, The Netherlands

³ Department of Internal Medicine, Division of Endocrinology, Academisch Ziekenhuis Vrije Universiteit, Amsterdam, The Netherlands

Summary An association between insulin resistance and hypertension has been reported in several studies. In apparent contradiction, insulin infusion in healthy volunteers is associated with vasodilatation. Furthermore, there is evidence that some insulin effects may differ between the sexes. We performed three-step hyperinsulinaemic-euglycaemic clamp studies in six men and six women to test the hypotheses that: 1) insulin might affect the release of vasoactive substances by the endothelium, and 2): this putative effect on vasoactive substances might differ between men and women. Six other women and six men served as control subjects, receiving 154 mmol/l NaCl (saline) infusion. Plasma levels of insulin, immunoreactive endothelin, L-arginine (precursor of nitric oxide), L-citrulline (by-product of nitric oxide synthesis) and cyclic GMP (second messenger of nitric oxide) were measured during infusion of insulin or 154 mmol/l NaCl (saline), respectively. We also assessed urinary excretion of 6-keto PGF-1 α (a degradation product of prostacyclin reflecting prostacyclin production). Blood pressure was monitored in all subjects throughout the experiment. In women plasma levels of immunoreactive endothelin decreased from (mean $\pm$ SD) 2.58 ± 0.96 to 1.7 ± 0.72 pmol/l during insulin infusion ($p < 0.01$), while remaining constant in female control subjects ($p < 0.02$). No changes in levels of endothelin were observed in

men during infusion of insulin or saline. In women levels of cGMP rose and levels of L-arginine decreased significantly during insulin infusion, consistent with an increase in nitric oxide production. Excretion of 6-keto PGF-1 α also increased significantly in women during insulin infusion. No such effects were observed in men, or in women during infusion of saline. Blood pressure remained constant in all subjects during hyperinsulinaemia. We conclude that sex differences exist in the effects of insulin on the endothelium. Short-term hyperinsulinaemia in women is associated with a decline in levels of immunoreactive endothelin, and possibly with a rise in production of nitric oxide and prostacyclin. In contrast, levels of vasoactive substances remained constant in men during hyperinsulinaemia. Our findings may partly explain insulin's vasodilatory effects in healthy individuals. It remains to be investigated whether these effects are lost in insulin-resistant states. Our observation that there is a sex difference in insulin effects on the endothelium may help explain why the link between hyperinsulinaemia and cardiovascular disease appears to be clearer in men than in women. [Diabetologia (1996) 39: 1284–1292]

Keywords Insulin, endothelium, endothelin, prostacyclin, nitric oxide, vasodilatation.

Received: 30 April 1996 and in revised form: 18 July 1996

Corresponding author: Dr. K.H. Polderman, Department of Internal Medicine, Free University Hospital, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands

Abbreviations: NO, Nitric oxide; HDL, high density lipoprotein; LDL, low density lipoprotein; IGF, insulin-like growth

factor; NIDDM, non-insulin-dependent diabetes mellitus; VLDL, very low density lipoprotein; EDRF, endothelium derived relaxing factor; RIA, radioimmunoassay; ANP, atrial natriuretic peptide; cGMP, cyclic 3',5'-guanosine monophosphate.

In 1988, Reaven coined the term 'syndrome X', to describe the concurrence of insulin resistance, hyperinsulinaemia, elevated levels of very-low-density lipoprotein (VLDL), decreased levels of high-density lipoprotein (HDL), and hypertension [1]. It was hypothesized that insulin resistance might play a key role in and possibly act as a cause of syndrome X. Several studies have described a link between hyperinsulinaemia and hypertension, even in otherwise healthy persons [2–5]. However, the mechanism by which hyperinsulinaemia might cause hypertension remains unclear. Indeed, although there appears to be a clear link between hyperinsulinaemia and hypertension in patients with insulin resistance, it has been conclusively demonstrated that insulin infusion in healthy volunteers is associated with vasodilatation [6, 7]; this effect seems to be, at least partly, mediated by nitric oxide (NO) [8].

Further, although there is ample evidence that hyperinsulinaemia is a cardiovascular risk factor in men, this association appears to be much less clear in women [9], thus suggesting that insulin's vascular effects may differ between the sexes. It has been shown that women are basically more insulin-sensitive than men [10], and that sex hormones can influence insulin sensitivity [11].

Recently, much attention has been focused on the role of the vascular endothelium in the pathogenesis of hypertension and atherosclerosis. Endothelial cells synthesize many active substances that regulate local blood pressure and maintain the fluidity of blood and the patency of blood vessels (reviewed by Vane et al. [12]). These include vasodilators such as endothelium-derived relaxing factor (EDRF) or NO, and prostacyclin, both of which also inhibit platelet adhesion and aggregation, thus playing an important role in the prevention of thrombus formation. By contrast, the endothelium also synthesizes vasoconstrictors such as endothelin. The release of these substances affects the local environment; endothelin may also have a systemic function [12]. There is evidence that endothelin may be involved in the pathogenesis of hypertension [12, 13] and atherosclerosis [14]. As is the case with insulin sensitivity, there appear to be sex differences in the secretion of vasoactive substances, and these differences appear to be modulated by sex hormones [15].

There is evidence that the secretion of these vasoactive substances is altered in patients with diabetes, even when there is no evidence of vascular disease. High levels of endothelin have been found in patients with diabetes [16, 17], and there have been reports suggesting that prostacyclin generation by vessel walls taken from patients with diabetes is low [18–20]. Several authors have found that production and receptor binding of endothelin increase when endothelial cells are incubated with high concentrations of insulin [21–24], and two studies have reported an

increase in endothelin levels during insulin infusion in obese (insulin-resistant) subjects [25, 26].

Thus, changes in endothelial function may occur early in diabetes, and these changes may be relevant to the pathogenesis of atherosclerosis and hypertension. Results from *in vitro* and clinical studies suggest that hyperinsulinaemia (as is found in insulin resistance) may be important; its role in modulating the secretion of vasoactive substances by the endothelium, however, has not yet been examined thoroughly. Further, it is unclear whether there are differences between men and women in the effects of insulin on vasoactive substances, and whether such differences could explain previous observations suggesting that the association between insulin resistance and atherosclerotic vascular disease is clearer in men than in women [9].

These considerations prompted us to investigate the effects of short-term hyperinsulinaemia on plasma levels and urinary excretion of endothelium-derived vasoactive substances in healthy young men and women.

Subjects and methods

Study design. Three-step hyperinsulinaemic euglycaemic clamp studies were performed in six healthy men aged 17–38 (mean: 33.8) years, and six healthy women aged 18–29 (mean: 25.1) years. Insulin was infused sequentially at 450, 1350 and 4500 pmol/kg lean body mass/h during 120 min for each period. The euglycaemic clamp technique has been described by De Fronzo et al. [27]. For logistical reasons we were unable to study the same persons twice. We therefore studied six women aged 18–30 (mean: 25.3) years and six men aged 23–34 (mean: 27.6) years as control subjects. Although this may increase variability due to between-subject differences, any statistically significant differences in outcome observed are likely to be valid. All subjects were within 10% of their ideal body weight (Metropolitan Life insurance tables 1959). None had a personal or family history of diabetes or hypertension, and none had used any medication in the 6 months prior to the test. Tests were performed in women on days 3–5 of their menstrual cycle. Informed consent was obtained from all subjects, and the study was approved by the hospital ethics committee. After an overnight fast, studies were begun between 08.30 and 09.30 hours. Two intravenous cannulae were inserted in an antecubital vein of each arm. Baseline blood samples were drawn 30 min after insertion of the cannulae; insulin and 154 mmol/l NaCl (saline) infusion commenced immediately thereafter. Glucose (1.11 mol/l) was administered using an IMED variable rate volumetric infusion device (Pharmacia, Knowhill, UK). The volume infused varied with the amount of glucose required to maintain euglycaemia; saline was infused simultaneously, so that the total volume infused during the experiment was approximately 1100 ml. In control subjects, the same procedure was followed; however, only saline was infused. Thus, the same volume of fluid (1100 ml) was infused in all groups. In all subjects, blood pressure was determined every 5 min using an automatic device (Nippon Colin sphygmomanometer, Hyashi, Komaki-city, Japan); the subjects remained at rest in a supine position during the entire test period. Patients were requested to pass urine at 30-min intervals starting 30 min before

the start of the infusion. On average, 11.2 samples were obtained from each subject in the 6-h period of the test (maximum: 13, range: 10–13 samples). Baseline values were calculated from the first sample collected during the test, and from a sample which the patients had been asked to collect at home (overnight portion; amount of time taken to produce this portion was noted by the subjects). 6-keto PGF-1 α excretion was calculated from the concentration in each portion of urine, the volume of each urine sample, and the elapsed time between production of each sample.

Assays. Venous blood samples were taken at 30-min intervals; samples were immediately placed on ice. Plasma was separated within 1 h and then stored at -20°C until assayed. Plasma insulin was measured in duplicate by radioimmunoassay (RIA) according to instructions by the manufacturer (Sorin Biomedica, Sallugia, Italy). Plasma immunoreactive endothelin was measured by RIA (Nichols Institute, formerly ITS, Wijnchen, The Netherlands) after extraction on 'Sep-Pak C₁₈' cartridges (Waters, Milford, Mass., USA) according to the manufacturer's instructions, as described previously [28]. Recovery rate for this assay is 92.4%. Intraassay and interassay coefficients of variation according to the manufacturer are 2.4 and 4.2%, respectively (we found these values to be 3.8 and 5.0%, respectively). Sensitivity of the assay is 1 pg/ml; cross-reactivity with endothelin-2 is 52%, with endothelin-3, 96% and with 'big' endothelin 7%. Prostacyclin synthesis was assessed by measuring the urinary excretion of its stable hydrolysis product 6-keto PGF-1a, using a procedure described by Zijlstra et al. [29]; sensitivity of this assay is 0.05 pmol/ml.

NO itself cannot be measured directly because of its short half-life. It is synthesised from L-arginine by the enzyme NO synthase, in the so-called L-arginine/NO pathway. The amino acid L-citrulline is generated as a by-product of NO synthesis. Cyclic 3',5'-guanosine monophosphate (cGMP) is the 'second messenger' of NO. Therefore, we used changes in levels of cGMP, L-arginine and citrulline to estimate changes in NO levels. We determined plasma amino acid levels by high-performance liquid chromatography (HPLC) after pre-column derivatization with ortho-phthalaldehyde, as previously described [30]. Plasma levels of cGMP were measured by RIA (Immuno Biological Laboratories, Hamburg, Germany) after extraction of plasma proteins by trichloroacetic acid. Intraassay and interassay coefficients of variation were 4.3 and 8.2%, respectively; sensitivity of the assay is 0.05 pmol/ml.

Cyclic GMP is the second messenger of NO, but also of atrial natriuretic peptide (ANP). To exclude confounding effects of ANP on levels of cGMP, ANP was also measured by RIA (Inctar Corporation, Stillwater, Minn., USA). Sensitivity of this assay is 0.5 pmol/ml. Intraassay and interassay coefficients of variation were 6.5 and 11.8%, respectively.

Statistical analysis

Results are expressed as mean $\pm$ SD. Analysis of variance for repeated measurements was used for comparison of baseline and infusion-related plasma or urine concentrations of the various hormones within each group. Student's two-tailed unpaired *t*-test was used to compare the effects of insulin vs saline infusion on hormone concentrations in men and women, respectively. The same test was used to compare the effects of insulin infusion between men and women. Statistical significance was accepted at *p* less than 0.05.

Fig. 1 a, b. Endothelin levels in women (a) and men (b) during infusion of insulin (*) and saline (■). Levels of endothelin decreased during insulin infusion in women ($p < 0.01$) but not in men ($p < 0.02$ for men vs women) while remaining constant during infusion of saline ($p < 0.02$ for insulin vs saline).

Results

Insulin levels. Insulin levels were measured at 30-min intervals. Mean plasma insulin levels reached during the last 60-min of each step of the three-step clamp were calculated from the last three measurements of each 2 h period. Mean plasma insulin levels reached during the last 60 min of each step of the clamp were: for women 373 ± 129 , 880 ± 230 and 5486 ± 660 pmol/l, and for men 344 ± 136 , 970 ± 337 and 6280 ± 838 pmol/l, respectively. The differences between men and women were not significant. Thus, the values attained in the first step were in the physiological (postprandial) range, and those reached in the second step in the range were found in severe insulin resistance. Values reached in the third step were supraphysiological.

Endothelin (Figs. 1 a and b). Baseline plasma concentrations of immunoreactive endothelin were slightly higher in men than in women (2.65 vs 2.45 pmol/l, $p = 0.36$). Levels of endothelin decreased during insulin infusion in women from 2.58 ± 0.96 to 1.70 ± 0.72 pmol/l (Fig. 1 a) while remaining constant during infusion of saline (2.33 ± 0.92 vs 2.28 ± 0.84 pmol/l,

Fig. 2a, b. Urinary excretion of 6-keto PGF-1α in women (a) and men (b) during infusion of insulin (*) and saline (■). Excretion of 6-keto PGF-1α increased during infusion of insulin in women ($p < 0.01$), but not in men ($p < 0.02$ for men vs women). No increase was observed in women during infusion of saline ($p < 0.01$ for insulin vs saline)

Fig. 3a, b. Levels of cyclic GMP in women (a) and men (b) during infusion of insulin (*) and saline (■). Levels of cGMP increased during insulin infusion in women ($p < 0.01$) but not in men ($p < 0.02$ for men vs women). No increase was observed in women during infusion of saline ($p < 0.02$ for insulin vs saline)

Fig. 1 a). The decrease vs baseline at $t = 120$ min (i.e. at high physiological insulin levels) was also significant ($p < 0.05$). The difference in endothelin levels between women infused with insulin and those infused with saline was significant ($p < 0.02$). In men, no significant changes in levels of endothelin were observed during infusion of insulin or saline (Fig. 1b). The difference between male and female subjects was significant ($p < 0.02$).

6-keto PGF-1α (Figs. 2a and b). In women, excretion of 6-keto PGF-1α increased from 70 ± 20 pmol/h to 190 ± 36 pmol/h during hyperinsulinaemia ($p < 0.01$) while remaining constant in control subjects during infusion of saline (Fig. 2a). The increase vs baseline at $t = 120$ min (i.e. at high physiological insulin levels) was also significant ($p < 0.01$). 6-keto PGF-1α excretion in men did not increase during infusion of insulin or saline. The differences between female subjects and control subjects, and between male and female subjects were significant ($p < 0.01$ and $p < 0.02$, respectively).

Cyclic GMP (Figs. 3a and b). For technical reasons, plasma levels of cyclic GMP could not be measured

in two female subjects during infusion of insulin or in two female control subjects. Therefore, data were obtained in only four female subjects during infusion of insulin and in four female control subjects during infusion of saline. Levels of cGMP increased gradually during insulin infusion in all four subjects, from 3.4 ± 1.0 to 4.8 ± 1.2 pmol/ml. The increase from baseline levels was statistically significant ($p < 0.01$). The increase from baseline at $t = 120$ min (i.e. at high physiological insulin levels) was also significant ($p < 0.02$). No such increase was observed during infusion of saline (3.9 ± 1.3 vs 4.1 ± 1.4 pmol/ml). Levels of cGMP did not change in male subjects during infusion of either insulin or saline. The difference between men and women was significant ($p < 0.02$).

Atrial natriuretic peptide (ANP) (Figs. 4a and b). Levels of ANP remained unchanged during infusion of insulin or saline in both male and female subjects.

Levels of L-arginine (Figs. 5a and b) and L-citrulline (Fig. 6). During insulin infusion, overall levels of amino acids declined by 20% in women, from 2748 ± 658

Fig. 4a, b. Levels of ANP in women (a) and men (b) during infusion of insulin (*) and saline (■). Levels of ANP did not change significantly during infusion of insulin or saline in either men or women

to 2194 ± 542 $\mu\text{mol/l}$ ($p < 0.01$), and by 16% in men, from 2880 ± 754 to 2422 ± 632 $\mu\text{mol/l}$ ($p < 0.01$). In women, the decrease in levels of L-arginine was larger than the overall decrease in amino acid levels: from 94 ± 36 to 46 ± 18 $\mu\text{mol/l}$, a decrease of 51% ($p < 0.01$; $p < 0.05$ vs other amino acids), whereas the decrease in L-citrulline levels was below average (12%, from 26 ± 8 to 23 ± 6 $\mu\text{mol/l}$; $p = 0.12$ vs other amino acids). In contrast, the decrease in men was in keeping with the overall decrease: 18% for L-arginine and 17% for L-citrulline, respectively. The decrease of L-arginine levels in women was larger than the decrease in men ($p < 0.02$). Levels of amino acids remained unchanged during infusion of saline (2422 $\mu\text{mol/l}$ at $t = 0$ and 2354 $\mu\text{mol/l}$ at $t = 360$ min in women; 2670 $\mu\text{mol/l}$ at $t = 0$ and 2598 $\mu\text{mol/l}$ at $t = 360$ min in men).

Blood pressure and heart rate. No significant changes in blood pressure were observed in any group during infusion of insulin or saline (Fig. 7). An increase in heart rate was observed during infusion of insulin in women (from 71.8 ± 8 to 75.6 ± 12 ; $p < 0.01$) and in men (from 72.2 ± 11 to 74.4 ± 14 ; $p < 0.01$). The

Fig. 5a, b. Levels of L-arginine and overall levels of amino acids in women (a) and men (b) during infusion of insulin (*) and saline (■). Overall levels of amino acids decreased in both men and women during infusion of insulin. Infusion of saline did not affect levels of amino acids ($p < 0.01$ for insulin vs saline in both men and women). The decrease in amino acid levels during insulin infusion was similar in women and men (20 vs 16%; $p = \text{NS}$). Levels of L-arginine also decreased during insulin infusion in both women and men. However, the decrease in women was significantly greater than in men (51 vs 18%, $p < 0.02$). In women, the decrease in levels of L-arginine was significantly greater than the overall decrease (51 vs 20%; $p < 0.05$). This was not the case in men (18 vs 16%; $p = \text{NS}$)

increases from baseline at $t = 120$ min were significant in both women and men ($p < 0.02$). No increase was observed during infusion of saline in either group (p values for insulin vs saline: women, $p < 0.01$; men, $p < 0.01$). The difference in effect of insulin infusion between men and women was not significant ($p = 0.21$).

Discussion

We have studied the effects of insulin infusion on levels of vasoactive substances secreted by the endothelium. Our observations strongly suggest that hyperinsulinaemia may influence endothelial mediator production. Further, we found a sex difference in the response of these mediators to insulin infusion; this finding may help explain the observation that the

Fig. 6. Levels of L-citrulline in women (----) and men (—) during infusion of insulin (*) or saline (■). Levels of L-citrulline decreased in both men and women during infusion of insulin; infusion of saline did not affect levels of amino acids ($p < 0.05$ for insulin vs saline in men, $p < 0.01$ for insulin vs saline in women). The decrease in levels of L-citrulline in women was smaller than in men (12 vs 17%, $p = 0.11$). The decrease in women was smaller than the overall decrease (12 vs 20%, $p = 0.12$)

link between hyperinsulinaemia and cardiovascular disease is less clear in women than in men [9].

Data regarding the effect of insulin on the vascular endothelium are conflicting. On the one hand, a link between hyperinsulinaemia and hypertension has been clearly established in several clinical surveys [2–5]. On the other hand, it has been shown that insulin has vasodilating properties in normal subjects [6, 7, 31–35], an effect that is at least partly NO-dependent [33]. In *in vitro* experiments, local hyperinsulinaemia has been shown to accelerate formation of atherosclerosis in the perfused artery [36]. *In vitro* production of prostacyclin and endothelin by endothelial cells is influenced by insulin [18, 19, 21–24]; these findings might be explained either by a direct effect of insulin on the endothelium or by (excessive) glucose uptake by endothelial cells induced by insulin. In this regard, it is noteworthy that specific binding sites for insulin and for insulin-like growth factors 1 and 2 have been demonstrated on endothelial cells, in isolated microvessels, in perfused organs and in intact animals [32–40]. *In vivo*, it has been shown that baseline levels of endothelin are higher in obese and/or insulin-resistant patients than in normal subjects [25, 26, 41]. In obese patients endothelin levels increase significantly during infusion of insulin [25, 26]. In contrast, we found that short-term insulin infusion in young women was associated with a decrease in levels of immunoreactive endothelin (Fig. 1a). We also observed an increase in urinary excretion of 6-keto PGF $_{1\alpha}$ (Fig. 2a) indicating an increase in systemic (i.e. vascular) and/or renal prostaglandin production. NO cannot be measured directly. To assess NO production, we therefore measured levels of

Fig. 7. Blood pressure during infusion of insulin. Solid lines indicate blood pressure in women, dotted lines indicate blood pressure in men. No significant changes were observed during infusion of insulin or saline

cyclic GMP, which is the second messenger of NO, levels of L-arginine, a precursor of NO, and levels of L-citrulline, a by-product of NO production. The results (a rise in levels of cGMP coupled with a large decrease in levels of L-arginine and a smaller-than-expected decline in levels of L-citrulline) are consistent with an increase in endothelial production of NO. (Insulin also affected other amino acids to a lesser degree, a finding which we interpret as an anabolic effect of insulin).

To explain these apparently contradictory findings we propose the following hypothesis. Two mechanisms exist through which insulin can influence endothelial mediators in physiological situations. Firstly, insulin can directly stimulate the production of endothelin by endothelial cells. This concept is supported by several clinical observations and *in vitro* studies [16, 21–26]. Secondly, insulin can increase the production of NO by the intact vessel wall. This view is supported by data from a study by Steinberg et al. [33], who demonstrated that insulin's vasodilating properties can be blocked by inhibiting NO-synthase, thereby suppressing production of NO. Kourembanas et al. [42] have shown that NO is one of the regulators of endothelin production, and that an increase in levels of NO induces a decrease in levels of endothelin. Hence, insulin can simultaneously affect endothelin levels in two ways: directly, by increasing endothelin production by endothelial cells; and indirectly, by increasing production of NO and thereby indirectly decreasing production of endothelin. Thus, insulin infusion could either increase or decrease levels of endothelin, depending on which of these mechanisms predominates. We speculate that our finding that endothelin levels in women decrease during infusion of insulin may have been secondary to an insulin-induced increase in levels of NO.

Insulin resistance induced by obesity may alter the response of the endothelium to insulin infusion.

This view is corroborated by the observation that endothelin levels decrease in obese patients during a low-calorie diet [25, 41]. There is strong evidence that endothelium-dependent relaxation may be reduced in diabetes and in hyperinsulinaemic states, and a decrease in production of NO may be one of the mechanisms underlying this phenomenon [7, 17, 43, 44]. It has been shown that insulin's vasodilating effects are decreased in two classic insulin-resistant states, i.e. obesity and NIDDM [7]. Consequently, there may be differences in the effects of insulin infusion between normal subjects and subjects with insulin resistance. We speculate that, in physiological situations in healthy young subjects, insulin increases the production of NO, thereby causing a decrease in endothelin levels and inducing vasodilation. When insulin resistance is present, this normal effect of insulin could be (partly) lost; then, the direct stimulatory effect of insulin on endothelin production would predominate, and endothelin levels would rise in response to insulin infusion. Whether these mechanisms play a role in the association between insulin resistance and hypertension requires further study.

Remarkably, we found no effects of short-term hyperinsulinaemia on levels of vascular mediators in men. This difference between the sexes may provide an explanation for the observation that hyperinsulinaemia appears to be a cardiovascular risk factor in men, but not in women [9]. There is evidence from various studies suggesting that there are indeed sex-associated differences in the synthesis of vasoactive substances [45–47]. It has been shown that the release of NO from aortic rings is greater in female rabbits than in male rabbits, and that oophorectomy in female rabbits reduces the NO levels to those found in male rabbits [45]. Weiner et al. [46] found that oestrogens can induce the enzyme NO synthase and increase the amount of mRNA for this enzyme. In vitro, human endothelial cells taken from the umbilical cords of female infants appear to produce more prostacyclin (measured as 6-keto PGF-1 α) than do cells from the umbilical cord of male infants [47]. Administration of anabolic steroids has been shown to reduce the activity of the NO pathway [48, 49]. We have previously reported that plasma levels of endothelin are higher in healthy men than in healthy age-matched women [15]. Endothelin levels appear to decrease during pregnancy; administration of testosterone causes endothelin levels to rise, while ethinyloestradiol administration is associated with lower endothelin levels [15]. The results of the present study suggest that there is also a sex-associated difference in the response of the vascular wall to insulin, possibly on the basis of differences in levels of the respective sex steroids.

One earlier study has reported on the effect of insulin infusion on endothelin levels in vivo in

normal subjects [26]. In this study by Piatti et al. [26], a small peak in endothelin levels occurred approximately 10 min after the start of insulin infusion; after this time, endothelin levels decreased toward baseline. We did not observe an increase in endothelin levels in any of our subjects; however, endothelin levels were measured at 30-min intervals, so that an early 'peak' in endothelin levels might not have been detected. Further, it is not clear whether the effects observed by Piatti et al. [26] were present only in men or in both men and women, as the data were not analysed separately for men and women.

In spite of the effects of insulin on endothelial mediators observed in women in our study we did not find changes in blood pressure during infusion of insulin. This may imply that short-term changes in these mediators do not affect vascular resistance, or that blood pressure is an insensitive measure of vasodilatation, or both. It has been shown that short-term intravenous administration of endothelin induces a rise in blood pressure only when endothelin levels are raised four- to tenfold [50]. Accordingly, the changes in levels of endothelin or other vasoactive substances observed in our patients may have been too small to affect systemic vascular resistance and blood pressure in the short term. Alternatively, very small changes in blood pressure may not have been detected by the method used in our study. Another possibility is that effects on blood pressure may have been offset by counterregulatory forces. In support of the latter possibility, we observed a small but significant increase in heart rate in our subjects during insulin infusion.

In conclusion, we examined the effect of short-term hyperinsulinaemia on the endothelium. Levels of endothelin decreased in women during hyperinsulinaemia, and there was evidence consistent with increased production of NO and prostacyclin. Such changes in endothelial mediator production may partly explain insulin's vasodilating properties. We speculate that these physiologic effects of insulin on the endothelium may be lost when insulin resistance occurs, and that this may partly explain the previously reported link between hyperinsulinaemia and hypertension. We also found a sex difference in the response of vasoactive mediators to insulin infusion; this finding may help explain why the link between hyperinsulinaemia and cardiovascular disease appears to be clearer in men than in women. Finally, studies that investigate insulin's vascular effects should differentiate between men and women.

Acknowledgements. We are indebted to J.A.J. Megens for assistance in the logistics of this study, and to T. Teerlink for technical assistance. Dr. Stehouwer is supported by a fellowship from the Diabetes Fonds Nederland and the Netherlands Organisation for Scientific Research (NWO).

References

- Reaven GM (1988) Banting lecture 1988: Role of insulin resistance in human disease. *Diabetes* 37: 1595–607
- Ferrannini E, Buzzigoli G, Bonnadona R et al. (1987) Insulin resistance in essential hypertension. *N Engl J Med* 317: 350–357
- Foster DW (1989) Insulin resistance: A secret killer? *N Engl J Med* 320: 733–734
- Swislocki AL, Hoffman BB, Reaven GM (1989) Insulin resistance, glucose intolerance and hyperinsulinemia in patients with hypertension. *Am J Hypertension* 2: 419–423
- Zavaroni I, Bonora E, Pagliara M et al. (1989) Risk factors for coronary artery disease in healthy persons with hyperinsulinemia and normal glucose tolerance. *N Engl J Med* 320: 702–706
- Anderson EA, Hoffman RP, Balon TW, Sinkey CA, Mark AL (1991) Hyperinsulinemia produces both sympathetic neural activation and vasodilatation in normal humans. *J Clin Invest* 87: 2246–2252
- Laakso M, Edelman SV, Brechtel G, Baron AD (1992) Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41: 1076–1083
- Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod O (1994) Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94: 2511–2515
- Modan M, Karesik A, Drory Y, Fuchs Z, Lusky A, Chertit A, Halkin H (1991) Hyperinsulinemia, sex, and risk of atherosclerotic cardiovascular disease. *Circulation* 84: 1165–1175 [related editorial comment:1442–1444]
- Nuutila P, Knuuti MJ, Mäki M et al. (1995). Gender and insulin sensitivity in the heart and in skeletal muscles. *Diabetes* 44: 31–36
- Polderman KH, Gooren LJG, Asscheman H, Bakker A, Heine RJ (1994) Induction of insulin resistance by androgens and estrogens. *J Clin Endocrinol Metab* 79: 265–271
- Vane JR, Ånggård EE, Botting RM (1990) Mechanisms of disease: regulatory functions of the vascular endothelium. *N Engl J Med* 323: 27–36
- Kohno M, Yasunari K, Murakawa K, Yokokawa K, Horio T, Fukui T, Takeda T (1990) Plasma immunoreactive endothelin in essential hypertension. *Am J Med* 88: 614–618
- Lerman A, Edwards BS, Hallet JW, Heublein DM, Sandberg SM, Burnett JC (1991) Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. *N Engl J Med* 325: 997–1001
- Polderman KH, Stehouwer CDA, van Kamp GJ, Dekker GA, Verheugt FWA, Gooren LJG (1993) Influence of sex hormones on plasma levels of endothelin. *Ann Intern Med* 118: 428–432
- Takahashi K, Gbatei MA, Lam HC, O'Halloran DJ, Bloom SR (1990) Elevated plasma endothelin in patients with diabetes mellitus. *Diabetologia* 33: 306–310
- Collier A, Leach JP, McLellan A, Jardine A, Morton JJ, Small M (1992) Plasma endothelin-like immunoreactivity levels in IDDM patients with microalbuminuria. *Diabetes Care* 15: 1038–1040
- Johnson M, Harrison HE, Raftery AT, Elder JB (1976) Vascular prostacyclin may be reduced in diabetes in man. *Lancet* 1: 325–326
- Silberbauer K, Schernthaner G, Sinzinger H, Katzer HP, Winter M (1979) Decreased vascular prostacyclin in juvenile onset diabetes. *N Engl J Med* 300: 366–367
- Grén K, Vesterqvist O, Grill V (1988) Urinary metabolites of thromboxane and prostacyclin in diabetes mellitus. *Acta Endocrinol (Copenh)* 118: 301–305
- Anfossi G, Cavalot F, Massucco P, Mattiello L, Mularoni E, Hahn A, Trovati M (1993) Insulin influences immunoreactive endothelin release by human vascular smooth muscle cells. *Metabolism* 42: 1081–1083
- Frank HJ, Levin ER, Hu RM, Pedram A (1993) Insulin stimulates endothelin binding and action on cultured vascular smooth muscle cells. *Endocrinology* 133: 1092–1097
- Hu RM, Levin ER, Pedram A, Frank HJ (1993) Insulin stimulates production and secretion of endothelin from bovine endothelial cells. *Diabetes* 42: 351–358
- Hattori Y, Kasai K, Nakamura T, Emoto T, Shimoda S (1991) Effect of glucose and insulin on immunoreactive endothelin-1 release from cultured porcine aortic endothelial cells. *Metabolism* 40: 165–169
- Wolpert HA, Steen SN, Istfan NW, Simonson DC (1993) Insulin modulates circulating endothelin-1 levels in humans. *Metabolism* 42: 1027–1030
- Piatti P, Monti LD, Conti M et al. (1996). Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. *Diabetes* 45: 316–321
- DeFronzo RA, Tobin JD, Andes R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223
- Voerman HJ, Stehouwer CDA, van Kamp GJ, Strack van Schijndel RJM, Groeneveld ABJ, Thijs LG (1992) Plasma endothelin levels are increased during septic shock. *Crit Care Med* 20: 1097–1101
- Zijlstra FJ, Vincent JE (1984) Determination of leukotrienes and prostaglandins in [¹⁴C] arachidonic acid labelled human lung tissue by high-performance liquid chromatography. *J Chromat* 311: 39–50
- Teerlink T, Leeuwen P and Houdijk A (1994) Plasma amino acids determined by liquid chromatography within 17 minutes. *Clin Chem* 40: 245–249
- Natali A, Buzzigoli G, Taddei S, Santori D, Cerri M, Pedrini R, Ferrannini E (1990) Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes* 39: 490–500
- Anderson EA, Hoffmann RP, Balon TW, Sinkey CA, Mark AL (1990) Hyperinsulinemia in humans increases muscle sympathetic nerve activity, but reduces forearm vascular resistance. *Clin Res* 20: 219–225
- Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD (1994) Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent – a novel action of insulin to increase nitric oxide release. *J Clin Invest* 94: 1172–1179
- Scott AR, Bennet T, Macdonald IA (1988) Effects of hyperinsulinaemia on the cardiovascular responses to graded hypovolaemia in normal and diabetic subjects. *Clin Sci* 75: 85–92
- Gans ROB, vd Toorn L, Bilo HJG, Nauta JJP, Heine RJ, Donker AJM (1991) Renal and cardiovascular effects of exogenous insulin in healthy volunteers. *Clin Sci* 80: 219–225
- Stolar MW (1988) Atherosclerosis in diabetes: the role of hyperinsulinemia. *Metabolism* 37 [Suppl 1]:1–9
- van Houten M, Posner BI (1979) Insulin binds to brain blood vessels in vivo. *Nature* 282: 623–625
- Frank HJL, Partridge WM (1981) A direct in vitro demonstration of insulin binding to isolated brain microvessels. *Diabetes* 30: 757–761
- Bar RS, Hoack JC, Peacock ML (1978) Insulin receptors in human endothelial cells: Identification and characterization. *J Clin Endocrinol Metab* 47: 699–702
- Bar RS, Boes M (1984) Disinct receptors for IGF-I, IGF-II, and insulin are present on bovine capillary endothelial cells

- and large vessel endothelial cells. *Biochem Biophys Res Commun* 124: 203–209
41. Ferri C, Bellini C, Desideri G, Di Francesco L, Baldoncini R, Santucci A, De Mattia G (1995) Plasma endothelin-1 levels in obese hypertensive and normotensive men. *Diabetes* 44: 431–436
 42. Kourembanas S, McQuillan LP, Leung GK, Faller DV (1993) Nitric oxide regulates the expression of vasoconstrictors and growth factors by vascular endothelium under both normoxia and hypoxia. *J Clin Invest* 92: 99–104
 43. Baron AD (1996) The coupling of glucose metabolism and perfusion in human skeletal muscle. The potential role of endothelium-derived nitric oxide. *Diabetes* 45 [Suppl 1]:S105–S109
 44. McVeigh GE, Brennan GM, Johnston GD et al. (1992) Impaired endothelium-dependent and -independent vasodilation in patients with type 2 (non-insulin dependent) diabetes mellitus. *Diabetologia* 35: 771–776
 45. Hayashi T, Fukutu JM, Ignarro LJ, Chaudhuri G (1992) Basal release of nitric oxide from aortic rings is greater in female rabbits than in male rabbits: implications for atherosclerosis. *Proc Natl Acad Sci* 89: 11259–11263
 46. Weiner CP, Lizasoain I, Baylis SA, Knowles RG, Charles IG, Moncada S (1994) Induction of calcium-dependent nitric oxide synthases by sex hormones. *Proc Natl Acad Sci USA* 91: 5212–5216
 47. Nordø, Berge L, Svensson B, Nordfy A (1992) A gender difference in the endothelial cells? The response to exogenous female sex hormones. *Proceedings of the 12th International Congress on Thrombosis*, Florence, May 1992. *Thromb Res* 65 Suppl 1: 106
 48. Belfagón G, Ferrer M, Encabo A, Regadera J, Marín J (1992) Changes in reactivity of rabbit aorta induced by chronic treatment with the anabolic steroid nandrolone. In: Moncada S, Marletta MA, Hibbs JB Jr, Higgs EA (eds) *The biology of nitric oxide: physiological and clinical aspects*, Vol 1. Portland Press, London, pp 118–122
 49. Green DJ, Cable NT (1993) Anabolic steroids and vascular responses. *Lancet* 342: 863 [Letter]
 50. Vierhapper H, Wagner O, Nowotny P, Waldhäusl W (1990) Effect of endothelin-1 in man. *Circulation* 81: 1415–1418