The Coupling of Glucose Metabolism and Perfusion in Human Skeletal Muscle

The Potential Role of Endothelium-Derived Nitric Oxide

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Insulin-mediated glucose metabolism in skeletal muscle is associated with a commensurate increase in muscle perfusion. The link between insulin action and vasodilation may be mediated by endothelium-derived nitric oxide (EDNO). The evidence suggests that insulin causes an increase in the production of EDNO in insulin-sensitive but not insulin-resistant subjects. This defect in insulin-mediated vasodilation may contribute to *1*) enhanced pressor sensitivity and *2*) reduced rates of insulin-mediated glucose uptake. We propose that the endothelium is an insulin target tissue that exhibits an increase in the release of EDNO in response to insulin. We postulate that the insulin-resistant state of obesity is associated with insulin resistance at the level of the endothelium, reduced EDNO release, and impaired vasodilation. Thus EDNO may act as the mediator coupling glucose metabolism to vasodilation. The interaction between insulin and the endothelium to enhance EDNO release describes a novel insulin action that deserves further exploration.
***Diabetes* 45 (Suppl. 1):S105–S109, 1996**

Hyperinsulinemia in the physiological range leads to a specific increase in skeletal muscle blood flow in humans (1–3). We have previously shown that insulin's effect to vasodilate skeletal muscle vasculature is directly proportional to its ability to stimulate glucose uptake (insulin sensitivity) (2) (Fig. 1*A* and *B*). In other words, insulin sensitivity and vasodilation are linked such that the most insulin-sensitive subjects exhibit the greatest degree of vasodilation in response to insulin. Conversely, insulin-resistant subjects such as obese patients and patients with non-insulin-dependent diabetes mellitus (NIDDM) exhibit blunted vasodilatory responses to insulin.

Because insulin has a powerful stimulating effect on tissue glucose metabolism, it is reasonable to suspect that the increase in perfusion occurs in response to this metabolic need. However, evidence for this proposition is equivocal. Vollenweinder et al. (4) recently reported that the insulin concentration rather than the carbohydrate oxidation rate was the primary determinant of the degree of vasodilation. In addition, a number of reports have documented that insulin

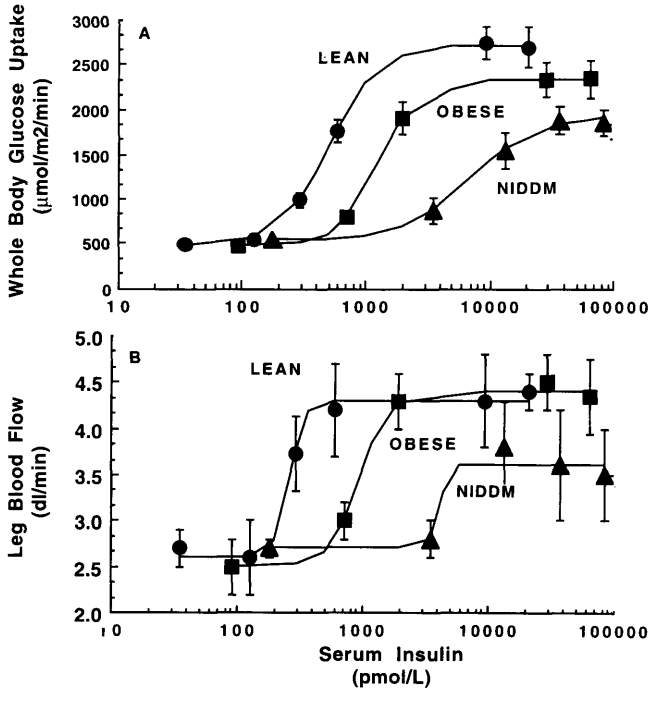

FIG. 1. Whole-body glucose uptake (*A*) and LBF (*B*) as a function of the prevailing serum insulin concentration during sequential euglycemic-hyperinsulinemic clamp studies performed in lean insulin-sensitive (●) subjects, obese insulin-resistant subjects (■), and patients with NIDDM (▲). From Laakso et al. (2).

relaxes blood vessels in vitro (5) or in isolated vessel segments in vivo (6), suggesting a direct vasodilating action independent of neural inputs or tissue metabolism rate. Based on these findings, we have postulated that the insulin-mediated increase in perfusion could act also to enhance insulin's overall action to dispose of glucose by increasing tissue delivery of both insulin and glucose (7). We have recently explored the mechanism(s) responsible for linking insulin action and vasodilation; our data suggest an important role for endothelium-derived nitric oxide (EDNO) (8).

In 1980, Furchgott and Zawadski (9) discovered that arterial vasodilation induced by acetylcholine was dependent in an obligatory fashion on the presence of an intact endothelium and the release of a labile factor (10) not related to prostaglandin synthesis (11). This substance, termed endothelium-derived relaxing factor (12) (later identified as nitric oxide or a closely related compound [13]), has a half-life on the order of seconds (14) and is the most potent endogenous vasodilator. EDNO is synthesized in endothelial

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EDNO, endothelium-derived nitric oxide; LBF, leg blood flow; MCh, methacholine; NIDDM, non-insulin-dependent diabetes mellitus; L-NMMA, L-n-monomethyl arginine.

cells and immediately released; it then diffuses to the subendothelium to stimulate vascular smooth muscle guanylate cyclase (15) and may directly act on the vascular smooth muscle to increase Na^+-K^+-ATPase activity (16). A number of substances other than acetylcholine produce EDNO-dependent vasodilation (14,17), including a number of peptides (18–23). EDNO is synthesized from the terminal guanidino nitrogen atom(s) of the amino acid L-arginine in a highly specific reaction catalyzed by nitric oxide synthase (17). The arginine analogue L-NMMA is a stereospecific competitive inhibitor of EDNO synthesis that inhibits acetylcholine-mediated vasodilation (17,24) and that can be overcome by L-arginine administration (25). L-NMMA causes vasoconstriction of the arterial circulation, indicating that basal EDNO release contributes to the maintenance of vascular tone (14,26). L-NMMA has also been shown to cause hypertension in both animals and humans (17). Importantly, L-NMMA causes constriction of small-resistance arterioles, which con-trol microcirculatory flow and capillary recruitment (27,28).

The purpose of this article is to review the evidence supporting a role for EDNO in mediating insulin-induced vasodilation. The interplay between insulin and the EDNO system will be discussed and speculated upon.

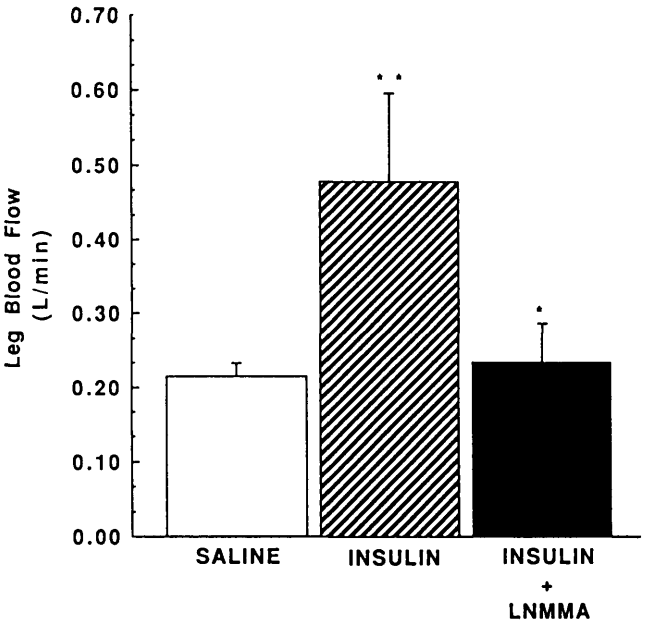

FIG. 2. Effects of intrafemoral artery infusion of 1-NMMA (16 mg/min) on LBF during insulin infusion (120 mU · m^{-2} · min^{-1}). *$P < 0.05$; **$P < 0.01$. From Steinberg et al. (8).

INSULIN-MEDIATED VASODILATION IS NITRIC OXIDE DEPENDENT

We have recently tested the hypothesis that insulin-mediated vasodilation occurs via the release of EDNO (28). To test this idea, we performed intrafemoral artery infusions of L-NMMA (an inhibitor of EDNO synthesis) in a group of seven healthy volunteers at baseline and measured leg blood flow (LBF) responses by thermodilution as previously described (1). L-NMMA was infused for 15 min at a dose (16 mg/min) previously determined to cause maximal reduction in LBF. In a separate group, L-NMMA infusions were performed after 3 h of hyperinsulinemia (~220 μU/ml) during a euglycemic clamp designed to increase LBF approximately twofold. The data indicate that, at baseline, L-NMMA caused a ~25% fall in LBF. Thus one-quarter of baseline leg vascular tone appears to be maintained by EDNO.

During hyperinsulinemia, LBF increased approximately twofold. With superimposed intrafemoral artery infusion of L-NMMA, LBF fell by ~50% back to basal levels (Fig. 2). Both saline and D-NMMA control infusions were performed with no effect on LBF. Therefore these data strongly indicate that insulin-mediated vasodilation is largely (if not exclusively) EDNO dependent.

Insulin could potentially cause EDNO-dependent vasodilation by either *1*) stimulating the synthesis/release of EDNO or *2*) sensitizing the vascular smooth muscle to EDNO. Thus, to test for these possibilities, we performed other studies to examine the effect of insulin on the dose-response curves for methacholine (MCh), which increases EDNO production, and sodium nitroprusside, a nitric oxide donor that acts directly upon vascular smooth muscle to increase LBF (Fig. 3*A* and *B*). The data indicate that low-dose insulin infusion (insulin levels ~25 μU/ml) leads to a significant shift to the left in the MCh but not the sodium nitroprusside dose-response curve ($P < 0.01$, analysis of variance) (8). Because MCh causes the synthesis and release of EDNO, the data are consistent with the idea that insulin mediates EDNO-dependent vasodilation by modulating the synthesis/release of EDNO rather than enhancing EDNO action at the level of the vascular smooth muscle. Others have recently confirmed a role for insulin in regulating EDNO in control and hypertensive subjects (30,31). Together these data point to a novel and physiological interaction between insulin and the endothelium to modulate the EDNO system. A hypothetical model of this interaction is shown in Fig. 4.

IMPAIRED INSULIN-MEDIATED VASODILATION IN INSULIN-RESISTANT STATES

Given that insulin-resistant subjects exhibit impaired vasodilation, it is interesting to consider whether EDNO release in response to insulin is impaired in these subjects. We have begun preliminary studies (32) exploring insulin-mediated EDNO-dependent vasodilation in obese insulin-resistant humans. For this purpose, we have performed MCh dose-response curves in lean (<26% body fat, $n = 5$) and obese (>26% body fat, $n = 5$) healthy subjects at baseline and dur-ing euglycemic hyperinsulinemia (15 and 40 mU · m^{-2} · min^{-1} in lean and obese subjects, respectively). The data indicate that insulin-resistant obese (normotensive and normocholesterolemic) subjects exhibit reduced MCh-induced vasodilation under basal and insulin-stimulated conditions. In addition, small insulin concentrations (25 μU/ml) shift the MCh dose-response curve to the left in lean subjects, but insulin levels as high as 65 μU/ml appear to have no modulating effect upon EDNO synthesis/release in insulin-resistant obese subjects. This occurred despite the fact that obese subjects exhibited normal vasodilatory responses to sodium nitroprusside. Therefore the preliminary data suggest that obese subjects have impaired endothelium-dependent vasorelaxation and are resistant to insulin's physiological action to modulate EDNO release.

Impaired EDNO release in insulin-resistant obese humans may be the result of a reduction in EDNO production capacity (for example, reduced activity of nitric oxide synthase). Alternatively, if the endothelium is an insulin target tissue, insulin resistance occurring at the level of the endo-

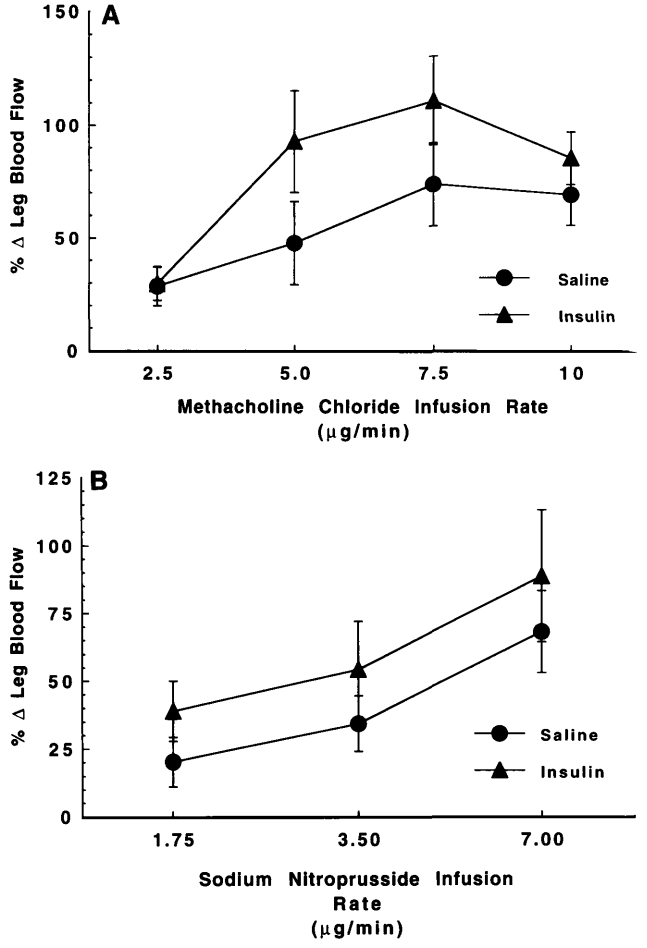

FIG. 3. *A:* **Percentage increment in LBF above baseline in response to graded intrafemoral artery infusion of MCh (2.5–10 μg/min) during saline and during euglycemic hyperinsulinemia (15–40 mU · m^{-2} · min^{-1}).** *B:* **Percentage increment in LBF above baseline in response to graded intrafemoral infusion of sodium nitroprusside (1.75–7.0 μg/min) during saline and during euglycemic hyperinsulinemia (15–20 mU · m^{-2} · min^{-1}). From Steinberg et al. (8).**

thelium would be predicted to result in impaired EDNO production, perhaps as a result of a defect in insulin signal transduction. Regardless of the mechanism, given the overall cardiovascular protective role of EDNO (17), impaired endothelium-dependent vasorelaxation in obese humans may be instrumental in the pathogenesis of atherosclerosis and hypertension, which occur with a greater incidence in these subjects. Indeed, EDNO not only is the most potent endogenous vasodilator but also has potent effects to reduce or inhibit *1*) platelet adhesiveness and aggregation, *2*) smooth muscle cell proliferation, and *3*) lipoprotein peroxidation (33), all critical mechanisms contributing to atherosclerosis. A hypothetical model summarizing the behavior of the insulin-EDNO interaction in insulin-resistant states is presented in Fig. 5.

IS THE ENDOTHELIUM THE SITE FOR CROSS TALK BETWEEN TISSUES AND BLOOD VESSELS?

Insulin-mediated vasodilation is EDNO dependent, and the magnitude of vasodilation is proportional to the degree of insulin sensitivity; therefore it follows that the rates of glucose metabolism and EDNO release are coupled in some fashion. At least two separate theoretical mechanisms could explain how the endothelium may be coupled to tissue

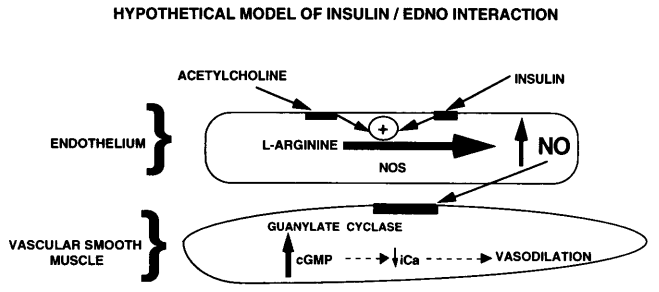

FIG. 4. Hypothetical model of the interaction between insulin and EDNO in healthy insulin-sensitive humans. Insulin via interaction with its receptor results in a signal that increases the production of EDNO. EDNO then diffuses to the subendothelium, where it causes vasodilation by activating guanylate cyclase and subsequent generation of cGMP. EDNO may also stimulate Na^+-K^+-ATPase activity, leading to vascular smooth muscle hyperpolarization (33,43). Increases in both cGMP and Na^+-K^+-ATPase activity result in reduced intracellular calcium, leading to vasodilation. NO, nitric oxide; NOS, nitric oxide synthase.

meta-bolic response. The first proposes that insulin acts directly upon the endothelium. In this case, the interaction could, for example, involve insulin activation of nitric oxide synthase. Alternatively, insulin could stimulate glucose metabolism in endothelium (perhaps to a similar degree as in tissues) and activate EDNO production via metabolic coupling. In this scheme, insulin resistance in tissues would be reflected in endothelium, and insulin's ability to modulate EDNO release would be impaired to a degree commensurate with the magnitude of insulin resistance. In a second proposal, insulin-mediated metabolism in tissues could generate a biochemical signal, which could diffuse to the endothelium to cause enhanced EDNO synthesis/release. The evidence indicating a role for insulin in dilating blood vessels in vitro supports a direct effect of insulin upon the endothelium and/or the vascular smooth muscle. However, one cannot exclude that both mechanisms may be operative in vivo.

It is interesting to note that other peptides such as insulin-like growth factor I (34) and epidermal growth factor (35,36), which like insulin are ligands of tyrosine kinase receptors, appear to have a direct action upon the endothelium to stimulate EDNO production. Moreover, insulin receptors are abundant in small arteriolar and capillary endothelium (37,38). Thus there is a body of circumstantial evidence supporting a potential interaction between insulin and the endothelium to stimulate EDNO production. If this were the case, any defect in insulin signal transduction or downstream effector system would result in resistance to insulin's action to stimulate EDNO production.

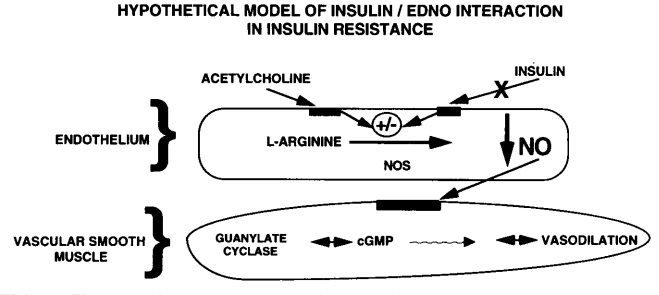

FIG. 5. Hypothetical model of the interaction between insulin and EDNO in insulin-resistant humans. Insulin's physiological ability to stimulate EDNO production is impaired, resulting in diminished cGMP production and blunted vasodilation. Resistance to insulin's ability to stimulate glucose uptake into tissues is reflected at the level of the endothelium as a diminished effect of insulin to modulate EDNO production.

WHAT IS THE PHYSIOLOGICAL ROLE OF INSULIN-MEDIATED VASODILATION?

Although it is clear that insulin dilates skeletal muscle vasculature, it has not yet been established what physiological role(s) this insulin action plays. The two most immediately evident roles are *1*) modulation of vascular resistance and blood pressure and *2*) modulation of fuel disposal by increasing substrate and hormone delivery.

Effect of insulin on vascular tone. We have recently extensively reviewed this question (39). In brief, physiological hyperinsulinemia is able to shift the norepinephrine pressor dose-response curve to the right in insulin-sensitive subjects. In contrast, insulin-resistant obese subjects exhibited a greatly diminished effect of insulin to mitigate the pressor response and an increased sensitivity to the pressor effects of norepinephrine (40). Others have recently reported an effect of insulin to reduce the increase in forearm vascular resistance associated with lower body negative pressure (41). Thus insulin action appears to have modulating effects upon vascular tone and pressor responsiveness. In contrast, insulin resistance appears to be associated with enhanced pressor sensitivity.

Effect of insulin-mediated vasodilation on glucose uptake. To quantitate the contribution of insulin-mediated vasodilation to insulin sensitivity, we recently performed high-dose hyperinsulinemic (120 $mU \cdot m^{-2} \cdot min^{-1}$) euglycemic clamp studies in 10 nondiabetic glucose-tolerant subjects. Leg glucose uptake was measured at 200–240 min of the clamp, when near–steady-state insulin action is achieved, and remeasured during a 30-min intrafemoral infusion of L-NMMA at 240–270 min. L-NMMA infusion caused a complete abrogation of the twofold rise in the insulin-induced vasodilation, returning rates of leg blood flow back to baseline values. Accompanying the reduction in LBF, glucose extraction rose ~45%; however, this rise in extraction was not sufficient to overcome the fall in perfusion, and leg glucose uptake decreased by ~23% ($P < 0.001$) (42).

Together, these data strongly support a role for insulin-mediated increments in skeletal muscle perfusion to contribute to or amplify insulin's overall action to stimulate glucose uptake. Because insulin concentrations achieved during these studies were maximally effective, it follows that muscle perfusion contributes significantly to insulin responsiveness. Whether insulin has similar effects upon other substrates such as lipids and amino acids has not yet been established.

CONCLUSION

The evidence presented suggests that vasodilation accompanying insulin stimulation is not merely a passive response to metabolic need but rather an active process instrumental in amplifying insulin's action to stimulate glucose uptake. The signal for vasodilation may, however, be in response to the initial stimulation of glucose metabolism. In other words, insulin first stimulates cellular glucose metabolism (perhaps in endothelium), which, upon reaching a critical threshold, produces a vasodilatory response. In turn, this vasodilation increases the delivery of substrate and thus further amplifies glucose metabolism. The evidence presented strongly supports a critical role for EDNO to act as the vasodilatory signal. The coupling between glucose metabolism and EDNO release is still obscure, but the circumstantial evidence supports the notion that the endothelium may be an important site for this interaction. Further research is necessary to better understand the role of circulating hormones such as insulin upon endothelial biology and insulin's role in the regulation of vascular tone and microcirculatory flow. Finally, given the effect of EDNO to reduce thrombosis, platelet adhesiveness, and cellular proliferation, it is reasonable to postulate that reduced EDNO production in human obesity may contribute to the elevated rates of atherosclerosis that these patients exhibit.

ACKNOWLEDGMENTS

This work was supported by Research Grants DK-38765 and DK-42469 from the National Institutes of Health, by the Merit Review program of the Department of Veterans Affairs, and by the American Heart Association.

The author thanks Shirley Kendrick and Ginger Brechtel-Hook for their invaluable assistance in the preparation of the manuscript.

REFERENCES

1. 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* 85:1844–1852, 1990
2. Laakso M, Edelman SV, Brechtel G, Baron AD: Impaired insulin mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
3. Baron AD, Brechtel G: Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol* 265:E61–E67, 1993
4. Vollenweinder P, Tappy L, Randin D, Schneiter PE, Nicod P, Scherrer U: Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J Clin Invest* 92:147–154, 1993
5. Yagi S, Takata S, Kiyokawa H, Yamamoto M, Noto Y, Ikeda T, Hattori N: Effects of insulin on vasoconstrictive responses to norepinephrine and angiotensin II in rabbit femoral artery and vein. *Diabetes* 37:1064–1067, 1988
6. Feldman RD, Bierbrier GS: Insulin mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 342:707–709, 1993
7. Baron, AD: Cardiovascular actions of insulin in humans: implications for insulin sensitivity and vascular tone. In *Clinical Endocrinology and Metabolism*. Ferrannini E, Ed. London, Bailliere-Tindal, 1993, p. 961–986
8. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: 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, 1994
9. Furchgott RF, Zawadski JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288:373–376, 1980
10. Griffith TM, Edwards DH, Lewis MJ, Newby AC, Henderson AH: The nature of endothelium derived vascular relaxant factor. *Nature* 308:645–647, 1984
11. Milner PG Jr, Izzo NJ, Saye J, Loeb AL, Johns RA, Peach MJ: Endothelium dependent relaxation is independent of arachidonic acid release. *J Clin Invest* 81:1795–1803, 1988
12. Myers PR, Minor RL Jr, Guerra R Jr, Bates JN, Harrison DG: Vasorelaxant properties of the endothelium derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. *Nature* 345:161–163, 1990
13. Greenberg SS, Wilcox DE, Rubanyi GM: Endothelium derived relaxing factor released from canine femoral artery by acetylcholine cannot be identified as free nitric oxide by electron paramagnetic resonance spectroscopy. *Circ Res* 67:1446–1452, 1990
14. Moncada S, Palmer RMJ, Higgs EA: The discovery of nitric oxide as the endogenous nitrovasodilator. *Hypertension* 12:365–372, 1988
15. Rapaport RM, Murad F: Agonist induced endothelium dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ Res* 52:352–357, 1983
16. Gupta S, McArthur C, Grady C, Ruderman NB: Stimulation of vascular Na(+)-K(+)-ATPase activity by nitric oxide: a cGMP-independent effect. *Am J Physiol* 266:H2146–H2151, 1994
17. Moncada S, Higgs A: Mechanisms of disease: The L-arginine-nitric oxide pathway. *N Engl J Med* 329:2002–2012, 1993
18. De Mey JG, Claeys M, Vanhoutte PM: Endothelium dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the canine femoral artery. *J Pharmacol Exp Ther* 222:166–173, 1982
19. Cherry PD, Furchgott RF, Zawadski JV, Jothianandan D: Role of endothelial cells in relaxation of isolated arteries by bradykinin. *Proc Natl Acad Sci USA* 2106–2110, 1982
20. Van de Voorde J, Leusen I: Role of endothelium in the vasodilator

response of rat thoracic aorta to histamine. *Eur J Pharmacol* 87:113–120, 1983
21. Cohen RA, Sheperd JT, Vanhoutte PM: 5-Hydroxytryptamine can mediate endothelium dependent relaxation of coronary arteries. *Am J Physiol* 245:H1077–H1080, 1983
22. Bevan JA, Joyce EH, Wellman GC: Flow dependent dilation in a resistance artery still occurs after endothelium removal. *Circ Res* 63:980–985, 1988
23. D'Orleans-Juste P, Dion S, Mizrahi J, Regoli D: Effects of peptides and non-peptides on isolated arterial smooth muscles: role of endothelium. *Eur J Pharmacol* 114:9–21, 1985
24. Vanhoutte PM, Miller VM: Heterogeneity of endothelium dependent responses in mammalian blood vessels. *J Cardiovasc Pharmacol* 7 (Suppl. 3):S12–S23, 1985
25. Angus JA, Campbell GR, Cocks TM, Manderson JA: Vasodilation by acetylcholine is endothelium dependent: a study by sonomicrometry in canine femoral artery in vivo. *J Physiol* 344:209–222, 1983
26. Vallance P, Collier J, Moncada S: Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* ii:997–1000, 1989
27. Yamamoto H, Bossaller C, Cartwright J Jr, Henry PD: Videomicroscopic demonstration of defective cholinergic arteriolar vasodilation in atherosclerotic rabbit. *J Clin Invest* 81:1752–1758, 1988
28. Kaley G, Rodenburg JM, Messina EJ, Wolin MS: Endothelium associated vasodilators in rat skeletal muscle microcirculation. *Am J Physiol* 256:H720–H725, 1989
30. Moreau P, Yamaguchi N, De Champlain J: Increased activity in the nitric oxide pathway during chronic euglycemic hyperinsulinemia in the rat. *J Hypertens* 11 (Suppl. 5):S270–S271, 1993
31. Virdis A, Taddei S, Mattei P, Natali A, Ferrannini E, Salvetti A: Insulin potentiates forearm vasodilation to acetylcholine by hyperpolarization in essential hypertension (Abstract). *Hypertension* 24:379, 1994
32. Steinberg H, Brechtel G, Johnson A, Baron A: Insulin mediated endothelium dependent vasodilation is impaired in obesity (Abstract). *Diabetes* 43:315, 1994
33. Galle J, Bengen J, Schollmeyer P, Wanner C: Oxidized lipoprotein(a) inhibits endothelium-dependent dilation: prevention by high density lipoprotein. *Eur J Pharmacol* 265:111–115, 1994
34. Galle J, Bengen J, Schollmeyer P, Wanner C: Oxidized lipoprotein(a) inhibits endothelium-dependent dilation: prevention by high density lipoprotein. *Eur J Pharmacol* 265:111–115, 1994
35. Namiki A, Akatsuka N: Vascular smooth muscle relaxation induced by epidermal growth factor is endothelium dependent. *Eur J Pharmacol* 180:247–254, 1990
36. Gan BS, MacCannell KL, Hollenberg MD: Epidermal growth factor urogastrone causes vasodilation in the anesthetized dog. *J Clin Invest* 80:199–206, 1987
37. Bar RS, Boes M, Yorek M: Processing of insulin-like growth factor I and II by capillary and large vessel endothelial cells. *Endocrinology* 118: 1072–1080, 1986
38. Bar RS, Boes M, Dake BL, Booth BA, Henley SA, Sandra A: Insulin, insulin-like growth factors, and vascular endothelium. *Am J Med* 85 (Suppl. 5A):59–70, 1988
39. Baron AD: Hemodynamic actions of insulin. *Am J Physiol* 267:E187–E202, 1994
40. Baron AD, Brechtel G, Johnson A, Fineberg N, Henry DP, Steinberg HO: Interactions between insulin and norepinephrine on blood pressure and insulin sensitivity: studies in lean and obese men. *J Clin Invest* 93:2453–2462, 1994
41. Lembo J, Rendina, V, Iaccarino, G, Lamenza, F, Volpe, M, Trimarco, B: Insulin reduces reflex forearm sympathetic vasoconstriction in healthy humans. *Hypertension* 21:1015–1019, 1993
42. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 96:786–792, 1995
43. Gupta G, Susman I, McArthur C, Tornheim K, Cohen R, Ruderman N: Endothelium-dependent inhibition of Na+-K+ ATPase activity in rabbit aorta by hyperglycemia: possible role of endothelium-derived nitric oxide. *J Clin Invest* 90:727–732, 1992