

Hemodynamic actions of insulin

ALAIN D. BARON

*Division of Endocrinology and Metabolism, Department of Medicine,
Indiana University School of Medicine, Indianapolis 46202-5124;
and the Richard L. Roudebush Veterans Affairs Medical Center, Indianapolis, Indiana 46202*

Baron, Alain D. Hemodynamic actions of insulin. *Am. J. Physiol.* 267

(*Endocrinol. Metab.* 30): E187-E202, 1994.—There is accumulating evidence that insulin has a physiological role to vasodilate skeletal muscle vasculature in humans. This effect occurs in a dose-dependent fashion within a half-maximal response of ~40 μU/ml. This vasodilating action is impaired in states of insulin resistance such as obesity, non-insulin-dependent diabetes, and elevated blood pressure. The precise physiological role of insulin-mediated vasodilation is not known. Data indicate that the degree of skeletal muscle perfusion can be an important determinant of insulin-mediated glucose uptake. Therefore, it is possible that insulin-mediated vasodilation is an integral aspect of insulin's overall action to stimulate glucose uptake; thus defective vasodilation could potentially contribute to insulin resistance. In addition, insulin-mediated vasodilation may play a role in the regulation of vascular tone. Data are provided to indicate that the pressor response to systemic norepinephrine infusions is increased in obese insulin-resistant subjects. Moreover, the normal effect of insulin to shift the norepinephrine pressor dose-response curve to the right is impaired in these patients. Therefore, impaired insulin-mediated vasodilation could further contribute to the increased prevalence of hypertension observed in states of insulin resistance. Finally, data are presented to indicate that, via a yet unknown interaction with the endothelium, insulin is able to increase nitric oxide synthesis and release and through this mechanism vasodilates. It is interesting to speculate that states of insulin resistance might also be associated with a defect in insulin's action to modulate the nitric oxide system. Thus, in addition to its many other functions, insulin must also be considered a vasoactive peptide, thereby adding further complexity to this hormone's overall action. Further research into this novel area of insulin action is needed to better understand the full significance of insulin's hemodynamic effects.

vasodilation; insulin resistance; hypertension; euglycemic clamp; skeletal muscle blood flow

IT IS DIFFICULT TO THINK OF another hormone that exhibits the diversity of biological actions of insulin (98). By virtue of its actions on gene expression and activity of a large number of enzymes (85), insulin is the principal hormone responsible for metabolic fuel disposal and storage into tissues (27). In addition, insulin has major actions on cell growth and development (98), ion transport (80, 92), and sympathetic nervous system (SNS) activity (1, 98, 110). A body of research performed largely in animals has previously suggested that insulin may also have cardiovascular actions (55, 73, 76, 77). Recently a rapidly growing body of work (1, 12, 26, 45, 67, 94, 97, 110) has emerged indicating that insulin also has marked physiological and specific effects in humans to augment skeletal muscle perfusion. Insulin-mediated glucose uptake occurs principally in skeletal muscle; therefore, increased rates of glucose and insulin delivery

to that tissue could represent an important mechanism of insulin's overall action to dispose of glucose and other substrates. The purpose of this review is to critically examine the cardiovascular actions of insulin, particularly as they pertain to humans. In addition, the potential role of these hemodynamic effects to modulate *in vivo* insulin sensitivity and blood pressure will be explored.

HISTORICAL PERSPECTIVE

Historical Background

Early clinical reports (75, 87, 88) indicated that insulin administration caused hypotension in nondiabetic subjects. Page and Watkins (88) reported that diabetic patients with severe autonomic neuropathy experienced episodes of syncope after subcutaneous administration of insulin, suggesting a vasodilating or

vagal effect of insulin. However, because these episodes were associated with hypoglycemia, the effect of insulin, *per se*, could not be distinguished from those produced by counterregulatory hormone release. Recently, these findings have been confirmed in the absence of hypoglycemia (90). In 1962, Rabinowitz and Zierler (93) noted a 20% increase in forearm blood flow during intrabrachial artery insulin infusions but attributed these increases to movement of the indwelling arterial needle.

Much later in 1982, Liang and co-workers (73) published the first comprehensive study examining insulin's cardiovascular effects in dogs. Utilizing a combination of the hyperinsulinemic euglycemic clamp (30) and radiolabeled microsphere techniques, they were able to describe the effects of insulin on both global and regional hemodynamics independent of significant changes in glycemia. They noted that insulin in relatively high doses had a marked effect to increase cardiac output and skeletal muscle blood flow. Because there was no change in mean arterial pressure (MAP) the data indicated a potent effect of insulin to decrease systemic vascular resistance.

Thus much past literature pointed to a cardiovascular action of insulin; however, the data also suggested that many of these effects were merely pharmacological; therefore, this area of human investigation lay largely dormant until recently.

The Controversy

Whether insulin has physiological effects to dilate skeletal muscle vasculature in humans has been the subject of renewed interest and recent controversy; therefore, the latter deserves to be discussed in some detail. The following chronological treatise summarizes in broad strokes this recent controversy. The reader is also referred elsewhere for other perspectives (3, 27).

In 1985, Creager et al. (26) reported that pharmacological circulating insulin concentrations caused forearm vasodilation in humans. Although these investigators utilized the euglycemic clamp technique, some degree of hypoglycemia that occurred during the procedure made it difficult to distinguish β -adrenergic effects of increased circulating epinephrine concentrations from insulin effects, *per se*. In a more recent article, Gelfand and Barrett (45) demonstrated that local intrabrachial artery insulin infusion caused an ~25% rise in forearm blood flow. With the exception of these two reports the overwhelming weight of the evidence to date suggested that insulin played no significant physiological role in the modulation of skeletal muscle vascular tone in humans.

Indeed, several investigators have examined the effects of insulin on forearm and/or leg glucose uptake using the combination of the euglycemic hyperinsulinemic clamp and limb balance techniques (20, 29, 28, 54, 63, 82, 114). With this latter technique, one measures glucose concentration under steady-state conditions in both arterial and venous blood feeding and draining a limb. By simultaneously measuring the blood flow through the limb, one can calculate by applying the Fick principle the uptake or balance of a substance such as glucose; for example, glucose uptake = arteriovenous

glucose difference \times flow ($GU = AVG\Delta \times F$). Therefore, although these investigators were not primarily interested in the vascular effects of insulin, in the course of these experiments the effect of insulin on limb blood flow could be ascertained. Whether examining the forearm or the leg and regardless of the methods used to measure blood flow (dye dilution or plethysmography), clearly a large number of workers were unable to detect any significant insulin-mediated vasodilation in the physiological range of insulin concentrations (20, 29, 28, 54, 63, 82, 114).

In 1990, Baron et al. (67) reported the novel finding that intravenous insulin caused a dose-dependent increase in the rate of resting leg blood flow in humans independent of hypoglycemia. Blood flow was measured with a custom-made thermodilution catheter placed directly into the femoral vein as previously described (11). Contemporaneous and subsequent to this finding, additional groups (1, 12, 26, 45, 67, 94, 97, 110) reported an effect of insulin to increase limb (muscle) blood flow over physiological concentrations when measured by either invasive (dye dilution) or noninvasive (plethysmography) techniques.

Therefore, the controversy resides in the discrepancy between the large body of previous negative reports and the cumulative weight of more recent evidence indicating, in many cases (1, 12, 26, 45, 67, 94, 97, 110) but not all (18), a positive effect of insulin on skeletal muscle blood flow. Review of this literature clearly indicates that these divergent findings are not simply attributable to different blood flow measurement techniques or routes, length, and doses of insulin administered. Although the reasons for the different findings are not completely clear, it is reasonable to suspect that technical and procedural details, alone or more likely in combination, can account for the differences.

Investigators involved in the measurement of blood flow *in vivo* recognize the great biological variation of this parameter and the inherent difficulties and confounding factors in establishing a steady baseline measurement. Reports are only seldom specific with respect to study conditions such as room temperature and noise level. The same is true for the status of the volunteer, such as activity level before study and the degree of discomfort or bladder fullness during the study. Noninvasive techniques, although convenient, are somewhat less sensitive than invasive techniques (25, 58) and are applied and analyzed differently in various laboratories. The common practice to occlude the hand circulation so as to more specifically measure blood flow and substrate exchange in forearm muscle (20, 26, 28, 54, 114) is reasonable, but it assumes no major disturbance of the remaining forearm circulation, and this has been seriously questioned (49, 72). Finally, there is great heterogeneity of vascular responses to insulin with lesser vasodilation in the more insulin-resistant subjects (67); therefore, both subject selection and sample size are other potential confounding variables. These and other factors (3) are likely to account for the divergent findings.

With this background the remainder of the discussion will focus on the body of data supporting insulin's

physiological hemodynamic actions in humans. The terms vasodilation, blood flow, or perfusion increments are used interchangeably.

ACTIONS OF INSULIN ON SKELETAL MUSCLE BLOOD FLOW

Dose-Response and Time Course Characteristics of Insulin-Mediated Vasodilation

By combining the hyperinsulinemic euglycemic clamp with the leg balance technique, Baron et al. (67, 68) have constructed the insulin dose-response curve for insulin's effect to increase leg (or for all intents and purposes

skeletal muscle) blood flow. In lean insulin-sensitive subjects, insulin caused a twofold rise in resting leg blood flow with an effective dose to produce a half-maximal response (EC_{50}) of $\sim 40 \mu\text{U}/\text{ml}$, indicating a potent and highly physiological hemodynamic effect (Fig. 1). In this regard, it is noteworthy that the EC_{50} for insulin's effect to stimulate whole body glucose uptake in the studies by Baron et al. (65) was $\sim 60 \mu\text{U}/\text{ml}$ and has been reported to be as high as $130 \mu\text{U}/\text{ml}$. Insulin's vasodilatory effect was highly variable as some individuals exhibited maximal vasodilation at the level of the EC_{50} , whereas others required insulin concentrations of $> 100 \mu\text{U}/\text{ml}$. Although other studies have not looked at

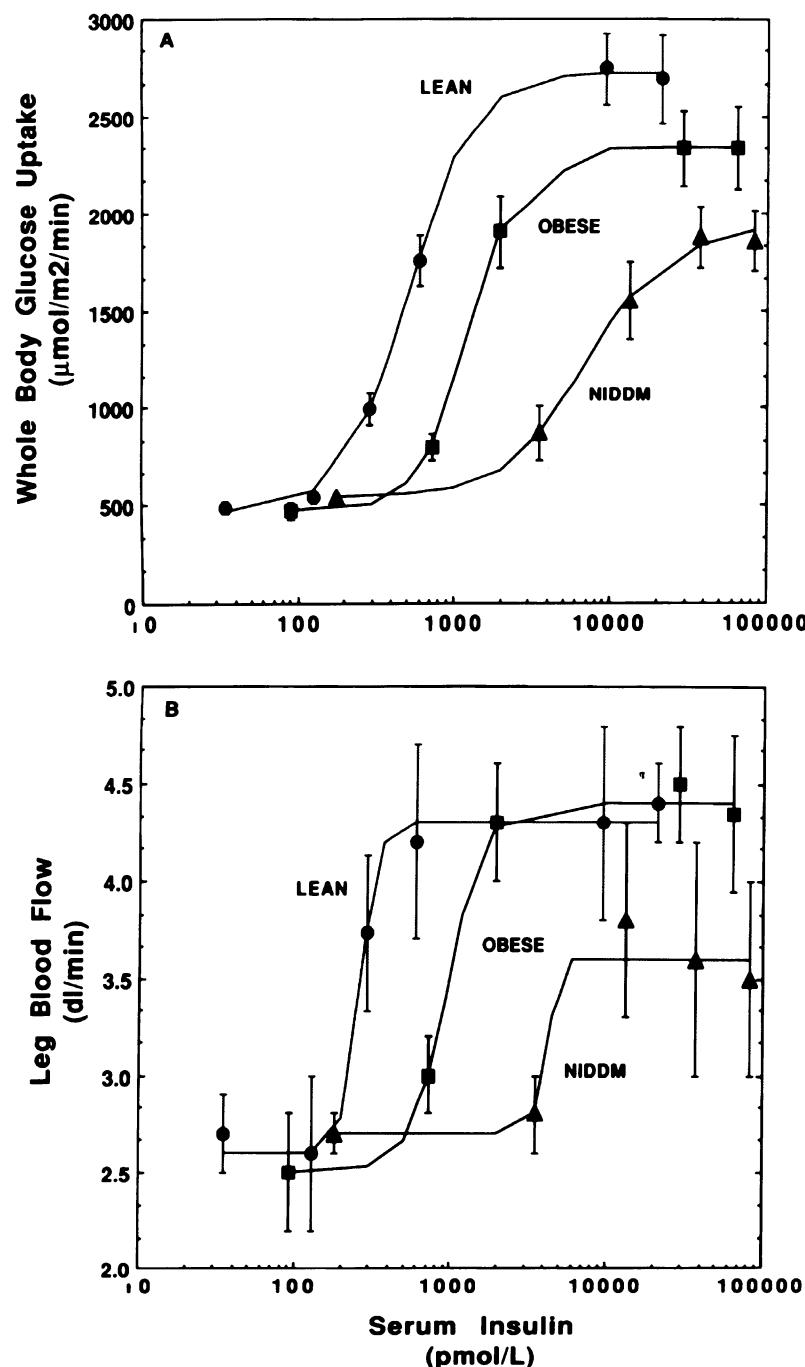


Fig. 1. A: rates of whole body insulin-mediated glucose uptake (expressed in $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) determined during euglycemic clamp studies over wide range of steady-state serum insulin concentrations in lean and obese nondiabetic subjects and patients with non-insulin-dependent diabetes mellitus (NIDDM). Note log scale on abscissa. To convert insulin concentrations from pmol/l to $\mu\text{U}/\text{ml}$, divide by 7.175. B: rates of leg blood flow determined during euglycemic clamp studies over wide range of steady-state serum insulin concentrations in lean and obese nondiabetic subjects and patients with NIDDM. Note log scale on abscissa. To convert insulin concentrations from pmol/l to $\mu\text{U}/\text{ml}$, divide by 7.175.

dose-response effects, many reports utilizing a variety of techniques to measure flow have confirmed the findings of Baron et al. With the use of strain-gauge plethysmography, Anderson et al. (1), Bennett et al. (12), and Neahring et al. (84) have documented $\sim 40\text{--}80\%$ increments in forearm blood flow and Vollenweider and co-workers (110) an $\sim 60\%$ increment in calf blood flow during physiological euglycemic hyperinsulinemia. Richter et al. (97) have recently reported similar effects on thigh blood flow using thermodilution. Finally, I have recently confirmed my own findings in the leg with the dye dilution technique and $0^{15}\text{-H}_2\text{O}$ using positron emission tomography (unpublished data). Therefore, insulin's hemodynamic action to increase limb blood flow has now been widely reported using a large variety of commonly employed techniques.

Few reports have documented the time course of insulin's action to dilate skeletal muscle vasculature. Most studies (1, 67, 110) have employed an insulin infusion rate of $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or $40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ to achieve circulating insulin concentrations of $\sim 70\text{--}100 \mu\text{U/ml}$ and have found that the time to reach half-maximal rates of blood flow was $\sim 40\text{--}60 \text{ min}$, which is roughly similar to the time course of whole body insulin-mediated glucose uptake (91). On the basis of the observation that the time course of insulin action to stimulate glucose uptake is accelerated at higher insulin concentrations (91), it is reasonable to suspect that the rate of rise of skeletal muscle blood flow would also be greater at higher insulin infusion rates. However, this idea has yet to be formally studied. In summary, both the dose-response and time course characteristics of insulin's dilatory action on skeletal muscle vasculature are highly physiological.

Insulin-Mediated Vasodilation in Insulin-Resistant Humans

Insulin-mediated vasodilation in obesity. In 1990, Baron and co-workers (67) reported that the dose-response curve for insulin's action to increase leg blood flow in insulin-resistant obese humans was markedly right shifted, exhibiting an EC_{50} threefold ($\sim 120 \mu\text{U/ml}$) that for lean insulin-sensitive subjects (Fig. 1). This increase in EC_{50} is comparable to the right shift in the dose-response curve for insulin's action to stimulate glucose uptake (Fig. 1; Refs. 65, 67). Impaired insulin-mediated skeletal muscle vasodilation in obese humans has recently been confirmed by Vollenweider et al. (109) but not by Neahring et al. (84). No studies have systematically assessed insulin-mediated vascular responses in subjects with central vs. peripheral obesity (15, 64). My anecdotal impression based on data from my own laboratory would suggest that central obesity is associated with more severe impairment of insulin-mediated vasodilation. This impression clearly requires formal confirmation.

Insulin-mediated vasodilation in diabetes. In a group of obese patients with non-insulin-dependent diabetes mellitus (NIDDM) the dose response for insulin vasodilation is virtually flat (68), thus exhibiting large reductions in insulin responsiveness (Fig. 1). Therefore, paral-

eling the dose-response characteristics for glucose uptake, insulin-mediated vasodilation is reduced in subjects with obesity and NIDDM. Because NIDDM subjects exhibited a similar degree of obesity than the obese nondiabetic group, it follows that the diabetes state, per se, contributes in an independent and additive fashion to the impaired insulin-mediated vasodilation. In this respect, in a group of insulin-resistant poorly controlled long-standing insulin-dependent diabetics (IDDM), Baron et al. (9) found that insulin-mediated vasodilation is markedly reduced, thus strongly supporting a role for the diabetic state, per se, in the impaired vasodilation.

Insulin-mediated vasodilation in hypertension. Ferrannini et al. (35) and others (19, 83, 89) have presented compelling evidence that essential hypertension independent of obesity is an insulin-resistant state. These authors have documented an inverse relationship between the height of the resting blood pressure and insulin sensitivity. Because established essential hypertension is characterized by elevated vascular resistance, it is reasonable to suspect that insulin's ability to vasodilate could be reduced in this population. Barib et al. (8) have recently reported an inverse relationship between the height of the resting blood pressure and both the vasodilatory response and the rate of glucose uptake achieved at maximally effective insulin concentrations across a cohort of normotensive subjects. These data suggest a nondiscreet or continuous relationship between resting blood pressure and both insulin responsiveness and vasodilation (Fig. 2, A and B). Recently, Feldman and Bierbrier (34) have reported that insulin vasodilation of dorsal hand veins was impaired in patients with hypertension (34), further supporting the idea that states of elevated vascular resistance such as hypertension may be associated with impaired insulin-mediated vasodilation.

Thus there is strong evidence that insulin's ability to dilate skeletal muscle vasculature is impaired across a wide variety of insulin-resistant states.

EFFECTS OF INSULIN ON CARDIAC OUTPUT

In 1987, Fisher et al. (39) reported rises in cardiac output in humans within minutes after intravenous insulin administration before any significant decreases in blood glucose could be detected. Avasthi et al. (2) reported greater increments in cardiac output after ingestion of mixed meals with a predominant carbohydrate-based caloric content, demonstrating that, in the physiological context of a meal (when insulin concentrations are above the EC_{50} for insulin's vasodilating action), cardiac output increases. In addition, a number of reports have documented a rise in heart rate during euglycemic hyperinsulinemia. None of these studies, however, support a cause-and-effect relationship between insulin and cardiac output.

Baron and Brechtel (5) recently established that, in lean insulin-sensitive humans, insulin causes a maximal 25% rise in cardiac output in a dose-dependent fashion with an EC_{50} of $\sim 70 \mu\text{U/ml}$ (Fig. 3). At this physiological insulin concentration the cardiac output is $\sim 15\%$ higher than at baseline, secondary to $\sim 10\%$ increments

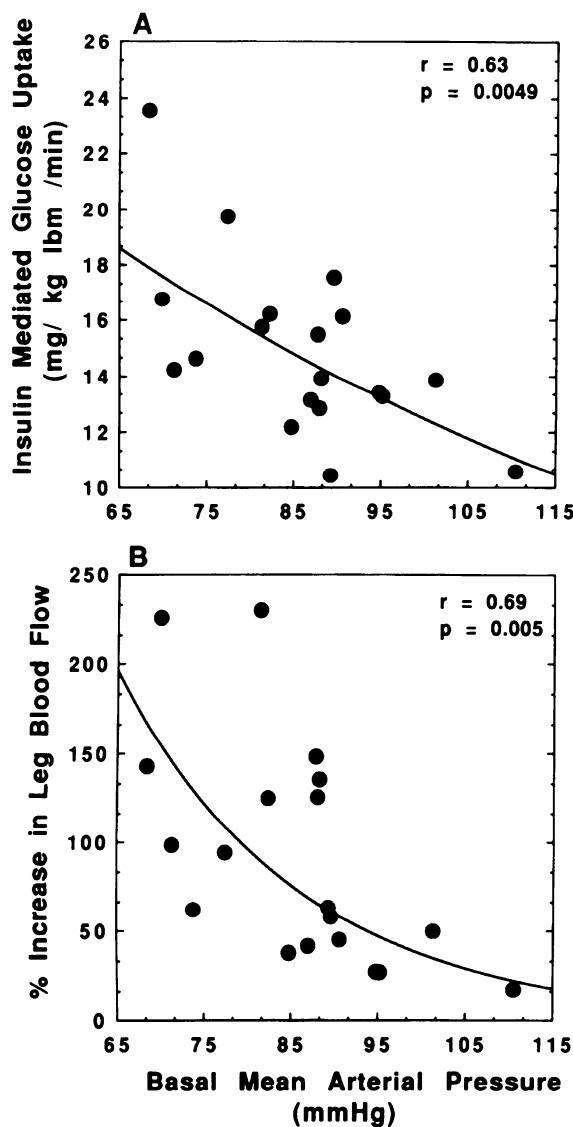


Fig. 2. A: scatterplot of relationship between basal mean arterial pressure (MAP) and rates of whole body insulin-mediated glucose uptake [expressed in mg/kg lean body mass $^{-1}$ (lbm^{-1}) $\cdot \text{min}^{-1}$] measured during hyperinsulinemic ($600 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) euglycemic clamp studies. B: scatterplot of relationship between resting MAP and relative increment above baseline (%increment) in leg (muscle) blood flow during hyperinsulinemic ($600 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$) euglycemic clamp studies.

in both heart rate and stroke volume. In contrast, similar prevailing insulin concentrations in obese insulin-resistant subjects had no effect on cardiac output (Fig. 3). Baron and Brechtel also found a significant correlation ($r = 0.70$, $P < 0.0001$) between the rise in cardiac output (measured by dye dilution) and the rate of leg blood flow (measured by thermodilution), indicating that changes in peripheral hemodynamics are accompanied by similar proportional changes in cardiac output.

Baron et al. (10) have also examined the cardiac output response to an oral glucose load in lean and obese subjects. Lean subjects exhibited an ~25% rise in cardiac output (measured by the Doppler/Echo technique) at ~60 min after glucose ingestion, which was coincident with the ~40% maximum rise in leg blood

flow and peak insulin concentrations. In contradistinction, obese subjects failed to show any rise in cardiac output despite prevailing insulin concentrations that were threefold higher.

Thus it is apparent that physiological insulin concentrations under both steady-state and postprandial conditions lead to significant increments in cardiac output, and this effect is impaired in insulin-resistant obese humans.

EFFECTS OF INSULIN ON BLOOD PRESSURE AND VASCULAR RESISTANCE

The magnitude of changes in cardiac output observed in response to physiological hyperinsulinemia would be expected to cause significant increments in MAP if not accompanied by commensurate decrements in peripheral vascular resistance. Several groups have monitored blood pressure during euglycemic clamp studies, and most (67, 82, 97, 110), but not all (99), have documented essentially no change in blood pressure over this acute period of hyperinsulinemia. Baron and Brechtel (5) have recently conducted studies over a range of euglycemic hyperinsulinemia combining intra-arterial pressure monitoring with measurements of both cardiac output and leg (muscle) blood flow. Careful blood pressure monitoring revealed, on average, an ~3–5% significant fall in MAP during infusions, achieving both physiological and pharmacological insulin concentrations. During these studies, at no time did acute hyperinsulinemia result in a rise in blood pressure in either insulin-sensitive or insulin-resistant groups of either obese, diabetic, or hypertensive subjects (unpublished data).

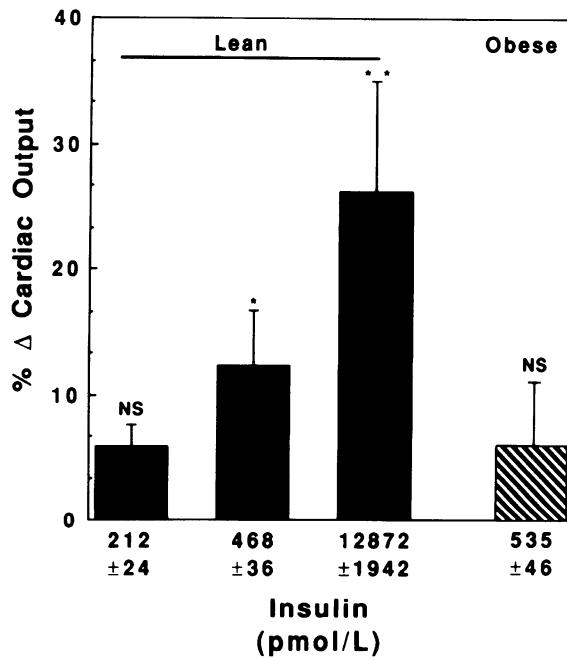


Fig. 3. Percent change (% Δ) from baseline in cardiac output (CO) measured in lean subjects during hyperinsulinemic (20 , 40 , and $600 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ in lean subjects and $40 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$ in obese subjects) euglycemic clamp studies in lean and obese subjects. * $P < 0.05$, ** $P < 0.01$ and not significant (NS) vs. baseline. To convert insulin concentrations from pmol/l to $\mu\text{U}/\text{ml}$, divide by 7.175 .

With the hemodynamic data in hand, Baron and Brechtel (5) were able to calculate changes in both systemic and leg muscle vascular resistance (vascular resistance = MAP/cardiac output or leg blood flow) in response to insulin (5). Across all insulin concentrations, leg muscle vascular resistance fell to a markedly greater degree than systemic vascular resistance. For example, at physiological insulin concentrations of $\sim 70 \mu\text{U}/\text{ml}$, vascular resistance in muscle fell $>40\%$, whereas at the systemic level vascular resistance fell only $<20\%$ (Fig. 4). Thus it follows that insulin regulates vascular resistance in a differential fashion and that it preferentially causes dilatation of skeletal muscle vasculature.

Insulin's potency to reduce muscle vascular resistance is all the more impressive given that the fall in systemic vascular resistance is actually overestimated, as the latter includes the decrement in muscle vascular resistance. Moreover, a number of groups have recently reported that insulin markedly stimulates muscle SNS activity (MSNA), yet muscle vascular resistance is either reduced or unchanged (1, 71, 110), thus further emphasizing the specific and potent effect of insulin to reduce skeletal muscle vascular tone. Via this selective fall in muscle vascular resistance, insulin in effect directs a greater proportion of cardiac output to skeletal muscle and thus engenders the increase in blood flow. If one assumes that one leg represents 20% of whole body skeletal muscle, it is apparent that physiological insulin concentrations lead to an $\sim 16\%$ rise in the proportion of cardiac output directed to skeletal muscle ($P < 0.01$). In obese insulin-resistant subjects, physiological insulin concentrations fail to lower leg muscle vascular resistance to a greater extent than systemic resistance, and

thus these subjects display no significant increase in muscle blood flow (Fig. 4).

EFFECTS OF SKELETAL MUSCLE PERfusion ON INSULIN ACTION

I have thus far presented compelling evidence that insulin has integrated physiological actions on the cardiovascular system that result in a net increase in skeletal muscle perfusion. These data beg the probably more important question as to what, if any, physiological consequences this hemodynamic action may have. Stated teleologically, what is the purpose of the insulin-mediated increase in skeletal muscle perfusion? Conversely, what are the consequences of impaired insulin-mediated vasodilation?

Insulin is characteristically known for its action to stimulate the uptake of glucose and that of other substrates into tissues. Given that insulin stimulates glucose uptake principally into skeletal muscle, it is reasonable and obvious to hypothesize that augmentation of perfusion to that tissue may play an instrumental role in insulin's overall action to augment glucose clearance.

Empirical inspection of the limb glucose balance equation ($\text{GU} = \text{AVG}\Delta \times F$) suggests that glucose uptake could theoretically be enhanced by an augmentation of glucose extraction ($\text{AVG}\Delta$), flow, or both. Full appreciation of the significance of the balance equation (116) requires understanding of the physiology of solute exchange, which will be dealt with in detail under SOLUTE EXCHANGE ACROSS CAPILLARY MEMBRANES. Regardless of any theoretical considerations, one can ask the simple and empirical question whether changes in skeletal muscle perfusion can independently modulate insulin-mediated glucose uptake in that tissue.

Is Skeletal Muscle Perfusion a Determinant of Glucose Uptake?

In 1977, Schultz et al. (100, 101) and Grubb and Snarr (47) reported that modulating the rate of substrate delivery (perfusion) in an isolated rat hindlimb preparation resulted in an augmentation of glucose uptake when both insulin and glucose concentrations were kept fixed. Unfortunately, these findings were not pursued. Baron and Brechtel (5) have recently addressed this issue in humans using a direct experimental approach. After establishment of steady-state rates of leg glucose uptake after 3 h of systemic euglycemic hyperinsulinemia, an intrafemoral artery infusion of methacholine hydrochloride (Mch) was begun and was designed to raise leg blood flow approximately threefold above baseline. Thus, with this study design, muscle perfusion is pharmacologically "dialed up" while prevailing insulin and glucose concentrations are kept unchanged. Therefore, assuming that Mch has no intrinsic properties to enhance cellular glucose permeability (vide infra), any rise in leg glucose uptake can be attributed to the augmentation of skeletal muscle perfusion and a net increase in capillary exchange of glucose. As illustrated in Fig. 5, steady-state rates of leg glucose uptake achieved

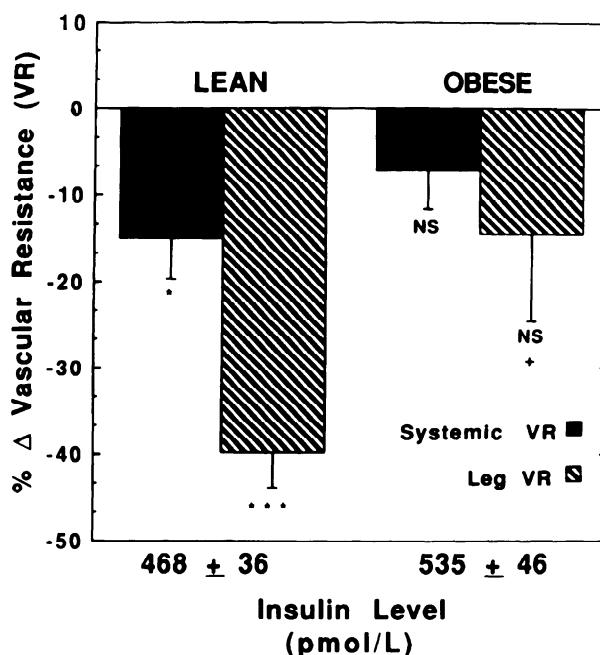


Fig. 4. Percent fall (% Δ) in systemic (filled bar) and leg (hatched bar) vascular resistance during hyperinsulinemic ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) euglycemic clamp studies in both lean and obese subjects. * $P < 0.05$, ** $P < 0.001$ vs. baseline, + $P < 0.05$ vs. lean and not significant (NS) vs. baseline. To convert insulin concentrations from pmol/l to $\mu\text{U}/\text{ml}$, divide by 7.175.

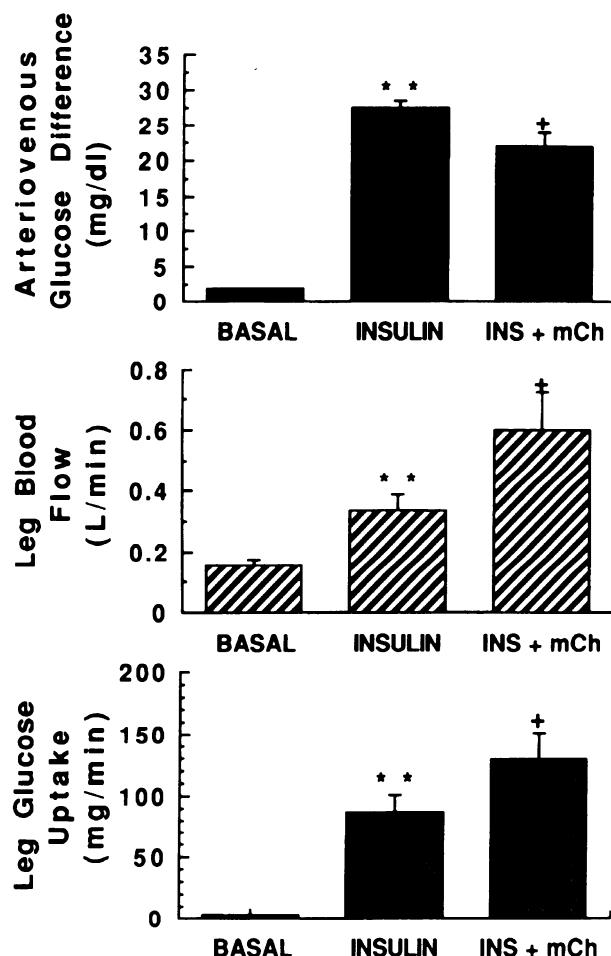


Fig. 5. Femoral arteriovenous glucose difference, leg blood flow, and leg glucose uptake at baseline, during steady-state hyperinsulinemia ($300 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$), and hyperinsulinemia with superimposed intrafemoral artery infusion of methacholine hydrochloride (Mch; $5 \mu\text{g}/\text{min}$). $**P < 0.01$ vs. basal, $+P < 0.05$ vs. steady-state hyperinsulinemia. Ins, insulin.

at maximally effective insulin concentrations were ~ 30 -fold that of basal, as reflected by a large widening of the $\text{AVG}\Delta$ and a twofold rise in leg blood flow. With superimposed intra-arterial infusion of Mch, leg glucose extraction fell by 20%; however, the 112% rise in leg blood flow more than offset the fall in extraction for a net increase in leg glucose uptake of $\sim 50\%$ ($P < 0.05$). Thus augmentation of skeletal muscle perfusion was able to increase rates of leg glucose uptake above those already achieved by maximally effective insulin concentrations. To verify that Mch had no intrinsic activity on cellular permeability, in collaboration with Dr. Steve Marshall (Glaxo), I carried out experiments examining the effects of Mch on glucose uptake in isolated rat soleus strips and found no effect of Mch to modulate either basal or insulin-stimulated $\text{D}-[^{14}\text{C}]$ glucose incorporation into glycogen (unpublished data). Therefore, Mch caused an increment in glucose uptake by virtue of its hemodynamic (vasodilating) effect and not via any effect on cellular permeability to glucose. Baron et al.'s laboratory (69) and that of others (18) have produced perhaps less direct but equally convincing data that skeletal muscle perfusion can determine the rate of glucose

uptake. Baron et al. (69) have previously reported that epinephrine infusion during euglycemic hyperinsulinemic clamps caused a reduction in leg glucose uptake, as reflected by a reduction in leg glucose extraction at submaximally effective insulin concentrations. However, by virtue of epinephrine's effect to augment skeletal muscle blood flow, it caused no net change in glucose uptake at maximally effective insulin concentrations (69). More recently, Buchanan et al. (18) have reported that systemic infusion of angiotensin II sufficient to cause a significant rise in arterial blood pressure also resulted in an increase in rates of insulin-mediated glucose uptake in whole body and leg by virtue of a rise in skeletal muscle blood flow with only a small reduction in leg glucose extraction. Baron et al. (7, 6) also observed the same effect with systemic infusions of norepinephrine (NE). Therefore, taken together, these data strongly support a role for perfusion, per se, as an independent determinant of glucose uptake into skeletal muscle.

Is Insulin-Mediated Vasodilation a Determinant of Glucose Uptake?

Although it is clear that pharmacological modulation of skeletal muscle blood flow can influence glucose uptake, it is not yet established whether insulin's action to augment blood flow has an instrumental modulating effect on skeletal muscle glucose uptake. Definitive experiments proving this point should ideally demonstrate that specific blunting of insulin-mediated vasodilation results in reduced rates of insulin-mediated glucose uptake. Studies to address this issue are currently being conducted in my laboratory (and probably that of others), and preliminary evidence is supportive. Nevertheless, short of this definitive evidence, the currently available body of data strongly supports the notion that insulin vasodilation is instrumental in modulating insulin's action to stimulate glucose uptake.

For example, simple calculations using data from a previous report by Baron et al. (8) can help to make this point. At maximally effective circulating insulin concentrations, the rate of glucose disappearance in a typical lean 70-kg man rises from a basal rate of ~ 2.0 to $12.0 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Given that about three-fourths of basal glucose uptake ($1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) occurs mostly in nonmuscle tissues such as brain (53) and splanchnic organs (29), it follows that skeletal muscle uptake actually rises from ~ 0.5 to $10.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ($12-1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) or ~ 21 -fold over baseline. Therefore, if skeletal muscle glucose uptake were solely determined by insulin's ability to increase cellular permeability to glucose, one would expect glucose extraction ($\text{AVG}\Delta$) to increase ~ 21 -fold. In lean normal subjects the mean basal femoral blood $\text{AVG}\Delta$ has been determined to be $\sim 1.75 \text{ mg}/\text{dl}$ and rises with insulin stimulation to $\sim 23.6 \text{ mg}/\text{dl}$ or 13.5 -fold (8). Therefore, it follows that the rise in $\text{AVG}\Delta$ alone is not sufficient to account for the rise in whole body skeletal muscle glucose uptake. However, if one considers the typical insulin-mediated 80% rise in leg blood flow from ~ 0.30 to $\sim 0.54 \text{ l}/\text{min}$, which occurs under these experimental conditions, it follows logically that the product of $\text{AVG}\Delta$ and flow can

readily account for the overall degree of rise in glucose uptake.

Another interesting analysis is to examine whether a positive correlation exists between skeletal muscle extraction ($\text{AVG}\Delta$) and insulin responsiveness, as measured by the euglycemic hyperinsulinemic clamp technique. Indeed, if the rate of glucose uptake was largely determined by the ability of tissues to extract glucose, one would expect to find a positive relationship between the individual femoral $\text{AVG}\Delta$ and the rate of insulin-stimulated glucose uptake. This would not be unreasonable given that, within the same individual, insulin causes a dose-dependent increase in glucose uptake and widening of the $\text{AVG}\Delta$ (67). Notwithstanding, Baron et al. (8) previously reported the complete lack of such correlation in a population of lean controls studied at maximally effective insulin concentrations. In fact, as is illustrated in Fig. 6, for a given rate of glucose disposal of $\sim 14 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, individuals exhibit femoral $\text{AVG}\Delta$ values ranging from 12.5 to 34 mg/dl (i.e., 2.7-fold range). Given that there is no correlation between body mass and $\text{AVG}\Delta$, it follows logically that insulin's effect to increase glucose extraction alone cannot account for the overall increase in rates of glucose uptake observed at maximally effective insulin concentrations, although this may not be true at submaximally effective insulin concentrations (22).

These data strongly suggest that differences in insulin responsiveness between lean nondiabetic individuals appear to be in large part determined by differences in the hemodynamic response to insulin rather than insulin's effect to increase muscle glucose extraction.

SOLUTE EXCHANGE ACROSS CAPILLARY MEMBRANES

Theoretical Considerations

I have presented convincing data indicating that skeletal muscle blood flow can act as an independent modulator of glucose uptake. To understand how an increment in skeletal muscle perfusion can lead to an increase in glucose uptake, it is critical to consider the

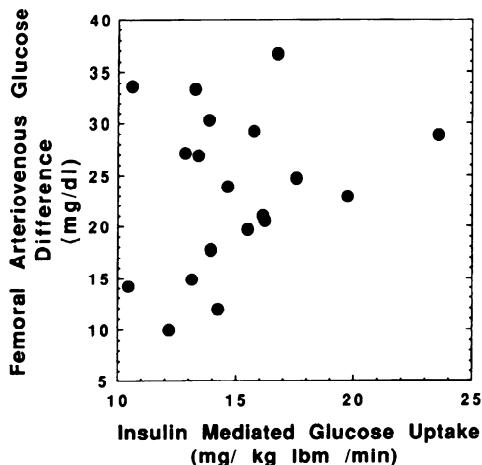


Fig. 6. Scatterplot of correlation between rates of whole body insulin-mediated glucose uptake (expressed in $\text{mg} \cdot \text{kg}^{-1} \cdot \text{l bm}^{-1} \cdot \text{min}^{-1}$) with femoral arteriovenous glucose difference measured during hyperinsulinemic ($600 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) euglycemic clamp studies.

nature of solute (in this case glucose) exchange across a capillary bed.

Fixed capillary network model. If one considers a capillary network as a fixed array of tubes, the capillary exchange surface area is therefore fixed, and a single capillary will behave like all the others. In this model, Renkin (96) has proposed that glucose exchange across the capillary endothelium is best described as intermediate between two extreme situations, namely 1) flow-limited exchange and 2) permeability-limited exchange.

FLOW-LIMITED EXCHANGE. In this situation, glucose is freely diffusible, and thus permeability of glucose through the capillary wall to the interstitial and intracellular spaces is very rapid and unimpeded. In this scenario, all of the glucose delivered will be taken up by tissues, irrespective of the rate of delivery (blood flow) within a physiological range. This is illustrated in Fig. 7 in which extraction of glucose (arterial [glucose] – venous [glucose]/arterial [glucose], where brackets indicate concentration) is equal to 100% (i.e., no permeability barrier).

PERMEABILITY-LIMITED EXCHANGE. In this situation, permeability of glucose through the capillary wall to the interstitial and intracellular spaces is severely restricted (extraction near 0). In this condition, increments in capillary perfusion or glucose delivery have essentially no effect to increase uptake since perfusion cannot overcome the severe permeability barrier.

The data of Baron et al. (8, 67) indicate that, in normal humans under conditions of maximal insulin stimulation, glucose extraction is consistently $<40\%$ and in most cases $<30\%$. Therefore, the true physiological situation is somewhere intermediate in the two extremes described above. Figure 7 illustrates the effect of an increase in capillary perfusion (blood flow) on extraction along the distance of a single capillary when overall extraction is fixed at 40%. Under the assumption that there is an exponential decline in glucose concentration from the arterial to the venous circulation, it is apparent that an increase in perfusion rate would have a relatively minor effect to increase the glucose concentration along the length of the capillary and thus would have an equally minor effect on glucose uptake. In actuality, extraction is not fixed and will vary; therefore, the relationship between permeability, extraction, and flow is more complicated. This point is perhaps better appreciated by inspecting the equation proposed by Renkin (96) relating solute (in this case glucose) exchange, perfusion rate, and glucose concentration

$$J_g = C_a Q (1 - e^{-PS/Q})$$

where J_g is net flux of glucose from blood to tissue, C_a is arterial glucose concentration, Q is blood flow, e is a base of natural logarithms, P is permeability of membranes to glucose, and S is capillary endothelial surface area exposed to blood perfusion.

In a capillary system with fixed PS , a large PS relative to Q (small Q -to- PS ratio) allows glucose clearance (J_g/C_a) to approach Q as a limit, and thus flow becomes rate limiting to glucose exchange. Conversely, as Q/PS increases J_g/C_a also increases, approaching the PS as the limit for glucose exchange. Thus, in a model with

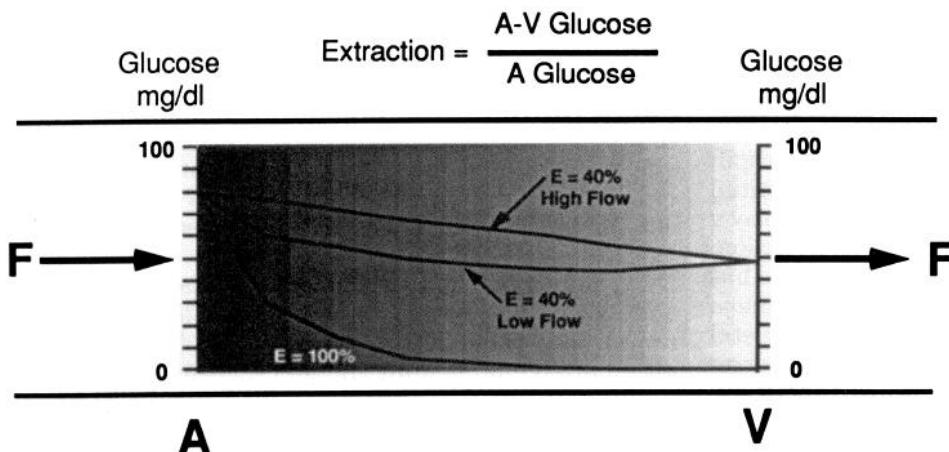


Fig. 7. Model of glucose exchange along single capillary. Glucose concentration gradient from artery to vein is assumed to be exponential. When glucose extraction (E) is complete (100%), rate of glucose delivery (blood flow, F) is limiting to exchange. If glucose extraction is fixed at 40%, augmenting blood flow or delivery exposes capillary to relatively minor increase in glucose concentration, resulting in only small (<10%) increase in glucose extraction. A, arterial; V, venous.

PS/Q approximately one, it follows that perfusion can have only a modest modulating effect on uptake. The reason for the lack of an effect of perfusion to increase glucose uptake in a capillary system with fixed PS is related to the effect of Q on glucose extraction.

According to Fick's principle, J_g (glucose uptake) = $Q(C_a - C_v)$, where C_v is venous glucose concentration. Viewed in simplistic terms, the C_a minus C_v (AVG Δ) represents tissue glucose extraction and reflects to a large degree tissue permeability and Q , or blood flow reflects the rate of delivery of glucose. However, the Fick equation holds that, for an increase in Q , there will be a reciprocal fall in AVG Δ . This is because the mean transit time (t) of glucose through a capillary network is inversely proportional to the rate of flow (Q) and directly proportional to the volume of distribution (V_d) of glucose $t = V_d/Q$. Thus, if one considers the fixed capillary surface area and permeability model (fixed PS), no increase in V_d is possible; therefore, an increase in Q would lead to an increase in velocity of blood and, consequently, to a decrease in transit time of glucose molecules through the capillary bed. The net effect of a reduction in transit time for glucose is to diminish the residence time of glucose in the capillary, thus reducing its ability to diffuse out of the vascular space and leading to a narrowing of the AVG Δ or reduced extraction. Thus, in the fixed capillary space model, one would predict that an increment in flow rate would result in only a trivial net increase in uptake because, according to Fick's principle, the augmentation of glucose delivery is offset by the reduction in extraction (116).

Variable capillary network. The data presented in *Is Skeletal Muscle Perfusion a Determinant of Glucose Uptake?* indicating a potent effect of perfusion on glucose uptake suggests that the fixed capillary network model discussed above does not accurately reflect the actual physiology of insulin-stimulated glucose exchange in the microcirculation.

From what is known of microcirculatory dynamics, it is clear that the fixed capillary surface exchange area (fixed PS) model is a gross oversimplification (21, 96). Indeed, it is known that, under control of the feeding arterioles or precapillary sphincters, capillaries undergo vasomotion (52) so that at any one time capillaries are

all neither closed nor open, and thus there is great heterogeneity of flow volume and velocity through each capillary (21, 96). Moreover, it is well documented that, in response to certain stimuli (such as exercise and hypoxia), skeletal muscle exhibits a great capacity for capillary recruitment so that the number of open capillaries at any moment and the integrated time that capillaries remain open is increased (21, 96). In turn, because capillary recruitment and/or increased homogeneity of capillary flow leads to greater tissue exposure to glucose, it is likely to be accompanied by an increase in the V_d for glucose. Thus, if one assumes that, in response to insulin, commensurate increments in glucose V_d accompany the increased flow, it follows that the transit time for glucose will not be altered. Under these circumstances, glucose extraction will not fall in response to increased perfusion, and glucose uptake will increase. Current available data strongly support this formulation and further suggest that insulin causes a maximally efficient coupling of PS and flow.

Effect of Insulin on Glucose V_d

Ferrannini and DeFronzo (38) have elegantly documented that, when given systemically, insulin causes a substantial increase in whole body V_d for glucose. More recently, Bonadonna and colleagues (16) demonstrated that high-dose systemic insulin administration was accompanied by a 25% rise in forearm blood flow and, importantly, an ~30% increase in forearm extracellular V_d for glucose. This increase in V_d was highly correlated with the rate of forearm glucose uptake. Therefore, these workers concluded that insulin (at least in supraphysiological doses) recruits previously unperfused skeletal muscle, thereby enhancing glucose uptake. These data also suggest that the increase in glucose V_d observed previously at the whole body level may in large part be due to expansion of the glucose V_d in skeletal muscle. As previously discussed, insulin preferentially reduces skeletal muscle vascular resistance. Because precapillary arteriolar tone is responsible for setting both vascular resistance and capillary flow (21, 96), it follows that increased glucose access to previously underperfused muscle tissue is in all likelihood secondary to insulin-induced capillary recruitment.

Role of the Insulin Gradient from Plasma to Interstitium and Capillary Density

To access its specific target cell surface receptor, insulin, which is secreted in the portal vein, must 1) travel through the vascular space, 2) undergo transcapillary transport, and 3) diffuse through the interstitial space. Thus microvascular effects of insulin could play a significant role in insulin's integrated metabolic action.

The delivery and access of insulin to target cells has recently been the subject of intensified attention. Lillijoja et al. (74) reported that capillary density of skeletal muscle obtained from Pima Indians was directly correlated to the rate of insulin-mediated glucose uptake or insulin sensitivity measured during euglycemic clamp studies. Based on these findings, they hypothesized that the diffusion distance from the feeding capillary to the interstitium may be limiting to insulin action. More recently, Bergman and colleagues (13, 113) have reported data indicating that the insulin concentration in lymph fluid (a surrogate for interstitial fluid) is markedly lower (~33% lower) than in plasma, suggesting a transendothelial insulin barrier giving rise to both insulin concentration and action gradients proportional to the distance away from the feeding capillary, as proposed by Krogh (66) for oxygen. Moreover, their data indicate that the time course of insulin's action to stimulate glucose uptake more closely parallels insulin appearance in lymph than in plasma, suggesting that lymph insulin is the more proximal determinant of insulin action and a good surrogate of interstitial insulin. Under the assumption of no significant interstitial insulin degradation, the plasma-to-interstitium insulin gradient could be the result of 1) a transendothelial barrier to insulin transport/diffusion, 2) constitutively low capillary density as in Pima Indians (74) and established hypertension, which is associated with capil-

lary rarefaction (46, 59, 108), or 3) reduced capillary recruitment (low functional capillary density) or more likely a combination of the above. Therefore, another mechanism whereby insulin-mediated vasodilation/capillary recruitment could enhance glucose uptake is via the enhanced delivery of insulin and consequent reduction of the plasma-to-interstitium insulin gradient. In this regard, recent data obtained in rats from Holmang and co-workers (50) support a relationship between capillary density and insulin uptake into muscle and blood flow (personal communication). However, recent data from Castillo et al. (22) indicate that the plasma-to-lymph insulin gradient and interstitial insulin concentrations are normal in obese insulin-resistant subjects. Thus the potential role for diminished interstitial insulin concentrations in insulin resistance remains controversial.

PROPOSED MECHANISM FOR HEMODYNAMIC MODULATION OF INSULIN ACTION

An Integrated View

At this juncture, it is appropriate to integrate the information presented above into a coherent sequence of events depicting a theoretical scheme for insulin-mediated vasodilation to amplify insulin's action to stimulate glucose uptake. This scheme is illustrated in Fig. 8. 1) In response to a meal, circulating insulin concentrations rise and reach the capillary bed. Once at the capillary bed, with some delay, insulin begins to be transported across the endothelium out of the vascular space into the interstitial space to bind to cellular insulin receptors and initiate the insulin action cascade, leading to increased cellular facilitative glucose diffusion. 2) At this early stage, functional capillary density is low, and because there is a biophysical barrier for transendothelial insulin transport, insulin and glucose

Fig. 8. Hypothetical model for flow/perfusion-modulated glucose uptake. *Top:* in basal state, feeding arteriole is relatively constricted, and muscle blood flow/perfusion, and thus glucose and insulin delivery, is low. Both functional capillary density and capillary perfusion are reduced. Both insulin and glucose exhibit large gradient from capillary to interstitium. *Bottom:* in insulinized state, arteriolar resistance is reduced, and muscle blood flow is augmented. Both capillary flow and functional capillary density are increased. Insulin and glucose gradient between plasma and interstitium is reduced, and greater mass of muscle is marshalled to participate in metabolism.

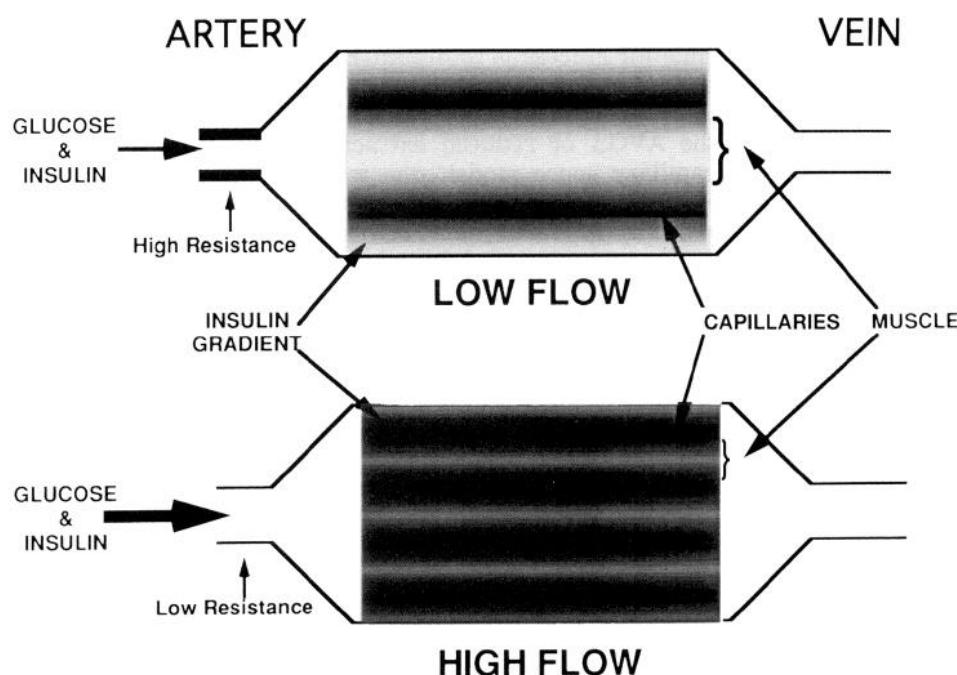


exhibit a large concentration gradient from the plasma to the interstitium, leaving some skeletal muscle fibers understimulated (this concentration gradient is presumably highest when circulating insulin levels are rising and lowest as they fall in the context of a meal). 3) At this stage, insulin causes precapillary arteriolar vasorelaxation either via a direct interaction with the endothelium (*vide infra*) or perhaps via signals resulting from the stimulation of glucose metabolism in endothelium or skeletal muscle. 4) As a result of the vasodilation, skeletal muscle vascular tone is reduced, and a greater proportion of cardiac output is directed to skeletal muscle. 5) As a result of the consequent increased arteriolar flow, capillary recruitment is initiated and capillary perfusion augmented, leading to greater insulin and glucose delivery. 6) With capillary recruitment and consequent increase in functional capillary density the plasma-to-interstitium insulin concentration gradient is reduced, and more muscle tissue is marshalled to participate in insulin-stimulated metabolism. Thus insulin's overall action is amplified.

Role of Skeletal Muscle Perfusion in Insulin Resistance

An obvious and central question is how much overall insulin-mediated glucose uptake can be accounted for by insulin's hemodynamic action and the corollary, namely, how much insulin resistance can be accounted for by impaired insulin-mediated vasodilation? As stated above, the answer to former questions requires physiological manipulations that abrogate insulin-mediated vasodilation (specifically capillary recruitment) in normal subjects, and the answer to the latter requires physiological manipulations that normalize skeletal muscle perfusion (capillary recruitment) in insulin-resistant subjects. Although definitive answers to these questions are not yet in, it is possible to arrive at a reasonable estimate of the contribution of perfusion to insulin sensitivity from the data currently in hand.

Figure 6 indicates that variation in rates of glucose uptake at maximally effective insulin concentrations between individuals are largely accounted for by differences in skeletal muscle perfusion rate and not in glucose extraction. From these data, one can estimate that ~20–30% of insulin responsiveness is dependent upon insulin-mediated vasodilation. Therefore, defective vasodilation could account for ~20–30% of the decrement in insulin action (insulin resistance). It is important to understand that, while at maximally effective insulin concentrations (when glucose uptake is maximally stimulated), the rate of glucose delivery may become rate limiting, and this may not be true at submaximal insulin concentrations. Indeed, at physiological insulin concentrations, perfusion modulation may merely shift the insulin dose-response curve to the left (86). Thus, in this scenario, defective insulin vasodilation may merely contribute to decrements in insulin sensitivity and compensatory hyperinsulinemia (95) but not actually be associated with reduced rates of insulin-mediated glucose uptake. Clearly, much more work has yet to be performed before the answers to these questions are obtained.

MECHANISM OF INSULIN-MEDIATED VASODILATION

Proposed Mechanisms of Insulin-Mediated Vasodilation

The mechanism by which insulin vasodilates is still not completely known, but new knowledge has shed light on this issue. Insulin-mediated skeletal muscle vasodilation could occur by 1) a direct effect of insulin to relax vascular smooth muscle, 2) an indirect effect via the release of an endothelial or vascular wall mediator, 3) an indirect effect coupled to metabolic activity such as oxygen consumption, or 4) a combination of these mechanisms.

Yagi et al. (112) reported that insulin caused a shift to the right in the pressor dose-response curve for angiotensin II and NE in rabbit vascular rings, suggesting a direct effect of insulin on vascular smooth muscle cells (VSMC). Insulin is known to stimulate $\text{Na}^+ \text{-K}^+$ -ATPase activity (92). Thus insulin could vasodilate by hyperpolarizing VSMC, reducing calcium influx. Kahn et al. (62) have provided evidence for this mechanism. Regardless of the actual signaling pathway(s), because VSMC ultimately contract in response to an increase in intracellular calcium (Ca_i^{2+}), it follows that insulin must directly or indirectly reduce the Ca_i^{2+} . Reduction of Ca_i^{2+} can occur as a result of a diminution of cellular influx of Ca^{2+} , augmentation of the efflux of Ca_i^{2+} , or both. Some investigators (102, 103, 115) have suggested that Ca^{2+} -ATPase gene expression is reduced in insulin-resistant obese Zucker rats. Because Ca^{2+} -ATPase is the major system responsible for Ca_i^{2+} efflux, dysfunction of Ca^{2+} -ATPase could lead to elevated Ca_i^{2+} and enhanced VSMC reactivity.

Alternatively, insulin could vasodilate by modulating the release of locally active compounds such as endothelial factors. Steinberg et al. (104) have very recently explored the possibility that insulin vasodilation is dependent on the release of endothelium-derived nitric oxide (EDNO). For this purpose, Steinberg et al. (104) performed intrafemoral artery infusions of the nitric oxide synthase inhibitor N^{G} -monomethyl-L-arginine (L-NMMA) as described by Moncada and Higgs (79). I have found that, under basal resting conditions, ~20% (~60 ml/min) of leg blood flow is EDNO dependent (Fig. 9). In contrast, when leg blood flow was increased during euglycemic hyperinsulinemia ~40% of leg blood flow was EDNO dependent (213 ml/min). Importantly, L-NMMA was able to nearly completely abrogate the insulin-induced vasodilation (104). Thus the data strongly indicate a major role for EDNO in the insulin-induced vasodilation. EDNO vasodilates by diffusing to the VSMC where it stimulates guanylate cyclase and thus generates guanosine 3',5'-cyclic monophosphate, which, through yet to be clarified mechanisms, reduces Ca_i (79). Therefore, although EDNO may be the principal vasodilating mechanism, it does not preclude the other mechanisms capable of regulating VSMC Ca_i discussed above to have potential additive, synergistic, or facilitative properties. Whether abnormalities of the EDNO system exist in insulin-resistant states to ac-

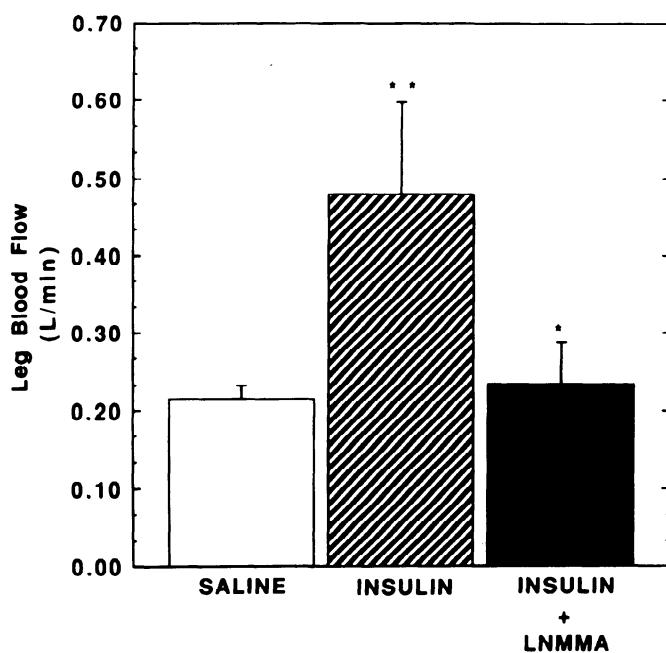


Fig. 9. Effect of intrafemoral artery infusion of N^G -monomethyl-L-arginine (L-NMMA; 16 mg/min) on leg blood flow during hyperinsulinemic ($120 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) euglycemic clamp studies. * $P < 0.05$, ** $P < 0.01$.

count for the impaired insulin-mediated vasodilation is not known and deserves further attention.

Of course, one cannot rule that redundant systems may be at play so that other mechanisms should not be ruled out. For example, insulin could suppress endothelial production of potent vasoconstrictors such as endothelins and thromboxanes or, conversely, stimulate the production of other relaxing factors (107). It is also reasonable to suspect that blood flow (and capillary recruitment) could be regulated through a mechanism linked to metabolic demand. For example, in exercising muscle, metabolic demands are met with a 5- to 10-fold increase in skeletal muscle flow (58) and enhanced capillary recruitment (51, 52). I (3) have previously made the observation that, for a given rate of glucose uptake, rates of leg blood flow are equivalent in lean and obese subjects. On the other hand, this was not the case in IDDM and NIDDM patients (68). Baron et al. (68) and Felber et al. (33) have also noted that the EC₅₀ for insulin's effect to increase leg blood flow is similar to that reported for insulin stimulation of glucose oxidation. Therefore, it is possible that a product or a consequence of glucose metabolism could be responsible for vasodilation. One could propose a parsimonious hypothesis that insulin-mediated endothelial glucose metabolism could regulate in some fashion EDNO synthesis/release and thus provide a coupling mechanism between metabolism and vasodilation. Vollenweider et al. (110) have explored the relationship between carbohydrate oxidation and vasodilation. They found that, for equivalent rates of carbohydrate oxidation achieved with infusion of fructose and dextrose, vasodilation was markedly greater with glucose infusion (when insulin levels were high) than with fructose when insulin levels were relatively low. Thus they concluded that insulin

rather than glucose oxidation, per se, was responsible for the vasodilation. However, because it is possible that a significant proportion of fructose oxidation occurred in tissues other than skeletal muscle, this issue is still unresolved.

Finally, it is possible that the SNS plays a role in the modulation of skeletal muscle blood flow in response to insulin. Insulin is now well known to stimulate MSNA (1, 110), which would be expected to cause vasoconstriction. However, animal data suggest that there are vasodilatory sympathetic fibers (81); therefore, it is possible that insulin could vasodilate via this mechanism. I recently explored this possibility and found no relationship between the magnitude or time course of MSNA and vasodilation in response to euglycemic hyperinsulinemia (unpublished data), casting serious doubt on this possible mechanism. Moreover, in a recent report, Randin et al. (94) found that the infusion of either α - or β -blockers had no effect to diminish insulin-mediated vasodilation, thus making it unlikely that an adrenergic mechanism is mediating insulin vasodilation.

IMPLICATIONS FOR THE REGULATION OF VASCULAR TONE

In 1981, Rowe et al. (99) reported that hyperinsulinemia, independent of changes in glycemia, caused a 30% elevation in circulating NE concentrations and a concomitant increase in MAP. Subsequently, there has been a surge of epidemiological reports relating insulin resistance and hyperinsulinemia with hypertension (17, 23, 31, 57, 76). The timely occurrence of these reports

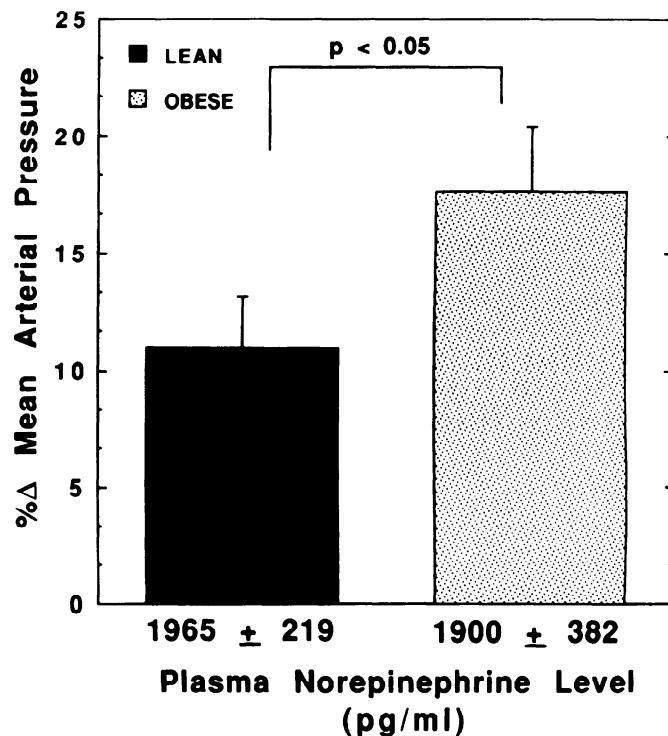


Fig. 10. Percent increment in MAP during norepinephrine infusion. Percent changes are calculated relative to MAP values observed after 3-h hyperinsulinemic ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$) euglycemic clamp study in lean and obese subjects.

led many authors (14, 36, 60, 70, 106) to propose that hyperinsulinemia via its activation of SNS activity may be instrumental in the pathogenesis of hypertension. Although logical, clinical, and direct experimental evidence for this hypothesis is lacking (56), clinical experience suggests that severe hyperinsulinemia in patients with extreme forms of insulin resistance (61) or with insulinomas (40, 105) is not associated with a higher incidence of hypertension. Intensive insulin therapy that creates a chronic hyperinsulinemic state does not raise blood pressure. Acute systemic (1, 7, 5, 43) or local (82) infusions of insulin produce either no change or a fall in MAP. In the dog, Hall and co-workers (48) reported that chronic hyperinsulinemia produced both SNS activation and renal sodium retention but did not raise the blood pressure and actually produced a fall in systemic vascular resistance. Finally and most importantly, Anderson et al. (1) and subsequently others (110) elegantly demonstrated in humans that systemic hyperinsulinemia produced a dramatic increase in muscle sympathetic nerve firing rate, indicating SNS activation with a simultaneous reduction of forearm vascular resistance and a small fall in MAP. Thus, paradoxically, insulin activates the SNS but also reduces muscle vascular resistance and produces no net change in MAP. Therefore, taken together the data suggest that it is highly unlikely (but still possible) that hyperinsulinemia, per se, is instrumental in the pathogenesis of hypertension via activation of the SNS.

Notwithstanding the above discussion, it is, however, plausible that insulin resistance rather than hyperinsulinemia with its attendant impaired insulin-mediated vasodilation could predispose to increased vascular tone and hypertension. This follows logically from the fact that vascular tone is set by the dynamic balance of both pressor and depressor vascular forces. Thus a loss of vasodepression associated with insulin resistance could diminish vasodilatory reserve and thus "sensitize" the vasculature to pressor forces. Baron et al. (6) have recently tested this idea by examining the pressor response to NE in lean insulin-sensitive and obese insulin-resistant humans at baseline and during euglycemic hyperinsulinemia. The data indicate that insulin causes a rightward shift in the NE pressor dose-response curve in lean but not in obese subjects. More importantly, NE had a greater pressor effect in obese than in lean subjects (Fig. 10). Therefore, it appears that, while insulin resistance alone does not necessarily lead to increased vascular resistance and hypertension, it is possible that it makes the vascular wall more sensitive to pressors and as such may predispose to the development of hypertension.

CONCLUSIONS AND SPECULATIONS

Thus I have presented a body of data which strongly indicates that insulin has physiological actions at the level of the cardiovascular system. The specific action of insulin to vasodilate skeletal muscle vasculature may be integral to its overall action to dispose of substrate. As such, insulin-mediated vasodilation could act as an amplifier of insulin action in peripheral tissues. Insulin-

mediated vasodilation is impaired across a number of insulin-resistant states, where it may further reduce insulin sensitivity and predispose to the development of vascular tone elevation.

Insulin-mediated vasodilation appears to be largely nitric oxide (endothelium) dependent. Given the role of nitric oxide in the regulation of vascular tone, platelet aggregation (111), and cellular proliferation (44), the interaction of insulin with the nitric oxide system could also have important consequences on the progression of atherosclerosis and vascular thrombosis (24). Last, via capillary recruitment, insulin could enhance the delivery of substrate to endothelial-based proteins such as lipoprotein lipase and thus play a role in the clearance of lipoproteins (32). Taken together, microcirculatory dysfunction associated with insulin resistance could contribute to the recently described insulin resistance syndrome (37, 42, 57, 78, 95) characterized by insulin resistance, dyslipidemia, and hypertension.

Therefore, in addition to its many other functions, insulin must also be considered a vasoactive peptide, thus adding further complexity to this hormone's overall actions. Clearly, a significant amount of research into this novel area of insulin action is in progress, and more is needed to better understand the full significance of insulin's hemodynamic effects. Finally, this discussion reminds us that metabolism should not be considered divorced from hemodynamics and vice versa.

I thank Ginger Brechtel, Ann Johnson, and Drs. Markku Laakso, Steven Edelman, and Helmut Steinberg for invaluable contributions, Kate Petrey for the preparation of the manuscript, and Drs. Scott Denne, Edward Leichty, and Timothy Garvey for constructive criticism.

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-42469, a Veterans Affairs Merit Review Award, and a Grant-In-Aid from the American Heart Association.

Address for reprint requests: A. D. Baron, Div. of Endocrinology and Metabolism, Dept. of Medicine, 545 Barnhill Dr., Emerson Hall, Rm. 421, Indianapolis, IN 46202-5124.

REFERENCES

- Anderson, E. A., R. P. Hoffman, T. W. Balon, C. A. Sinkey, and A. L. Mark. Hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J. Clin. Invest.* 87: 2246-2252, 1991.
- Avasthi, P. S., F. R. Greene, and W. F. Voyles. Non-invasive doppler assessment of human postprandial blood flow and cardiac output. *Am. J. Physiol.* 252 (*Renal Fluid Electrolyte Physiol.* 21): F1167-F1194, 1983.
- Baron, A. D. *Baillière's Clinical Endocrinology and Metabolism: Insulin Resistance and Disease*. London: Baillière, Tindall, & Cox, 1993, p. 961-987.
- Baron, A. D., and G. Brechtel. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am. J. Physiol.* 265 (*Endocrinol. Metab.* 28): E61-E67, 1993.
- Baron, A. D., G. Brechtel, A. Johnson, N. Fineberg, D. Henry, and H. Steinberg. Interactions between insulin and norepinephrine on blood pressure and insulin sensitivity. *J. Clin. Invest.* 93: 2453-2462, 1994.
- Baron, A. D., G. Brechtel, A. Johnson, and D. Henry. Insulin (I) attenuates norepinephrine's (NE) pressor effect (Abstract). *Diabetes.* 41, Suppl. 1: 125A, 1992.
- Baron, A. D., G. Brechtel-Hook, A. Johnson, and D. Hardin. Skeletal muscle blood flow. A possible link between insulin resistance and blood pressure. *Hypertension Dallas* 21: 129-135, 1993.

9. **Baron, A. D., M. Laakso, G. Brechtel, and S. V. Edelman.** Mechanism of insulin resistance in insulin dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. *J. Clin. Endocrinol. Metab.* 73: 637–643, 1991.
10. **Baron, A. D., M. Laakso, G. Brechtel, B. Hoitt, C. Watt, and S. V. Edelman.** Reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *J. Clin. Endocrinol. Metab.* 70: 1525–1533, 1990.
11. **Baron, A. D., P. Wallace, G. Brechtel, and S. V. Edelman.** Rates and tissue sites of non-insulin-mediated and insulin-mediated glucose uptake in man. *Am. J. Physiol.* 255 (*Endocrinol. Metab.* 18): E769–E771, 1988.
12. **Bennett, W. M., A. A. Connacher, C. M. Scrimgeour, R. T. Jung, and M. J. Rennie.** Euglycemic hyperinsulinemic augments amino acid uptake by human leg tissues during hyperaminoacidemia. *Am. J. Physiol.* 259 (*Endocrinol. Metab.* 22): E185–E194, 1990.
13. **Bergman, R. N., D. C. Bradley, and M. Ader.** *Advances in Experimental Medicine and Biology. New Concepts in the Pathogenesis of NIDDM.* New York: Plenum, 1993, p. 181–198.
14. **Berne, C.** Insulin resistance in hypertension—a relationship with consequences? *J. Int. Med.* 229, Suppl. 2: 65–73, 1991.
15. **Björntorp, P.** Metabolic implications of body fat distribution. *Diabetes Care* 14: 1132–1143, 1991.
16. **Bonadonna, R. C., M. P. Saccomani, and C. Cobelli.** Effects of systemic hyperinsulinemia on muscle perfusion and glucose metabolism in man (Abstract). *Diabetes* 41, Suppl. 1: 659, 1992.
17. **Bonora, E., I. Zavaroni, O. Alpi, A. Pezzarossa, F. Bruschi, E. Dall'Aglio, L. Guerra, C. Coscelli, and U. Butturini.** Relationship between blood pressure and plasma insulin in non-obese and obese non-diabetic subjects. *Diabetologia* 30: 719–723, 1987.
18. **Buchanan, T. A., H. Thawani, W. Kades, J. G. Modrall, F. A. Weaver, C. Laurel, R. Poppili, A. Xiang, and W. Hsueh.** Angiotensin II increases glucose utilization during acute hyperinsulinemia via a hemodynamic mechanism. *J. Clin. Invest.* 92: 720–726, 1993.
19. **Capaldo, B., G. Lembo, R. Napoli, V. Rendina, G. Albano, Saccà, and B. Trimarco.** Skeletal muscle is a primary site of insulin resistance in essential hypertension. *Metab. Clin. Exp.* 40: 1320–1322, 1991.
20. **Capaldo, B., R. Napoli, R., P. DiBonito, G. Albano, and L. Saccà.** Glucose and gluconeogenic substrate exchange by the forearm skeletal muscle in hyperglycemia and insulin treated type II diabetic patients. *J. Clin. Endocrinol. Metab.* 71: 1220–1223, 1990.
21. **Caro, G. G., T. J. Pedley, R. C. Schroter, and W. A. Seed.** *The Mechanics of the Circulation.* New York: Oxford Univ. Press, 1978, p. 418–422.
22. **Castillo, C., C. Bogardus, R. Bergman, P. Thuillez, and S. Lillioja.** Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. *J. Clin. Invest.* 93: 10–16, 1994.
23. **Christlieb, A. R., A. S. Krolewski, J. H. Warram, and J. S. Soeldner.** Is there a link between hypertension and obesity? *Hypertension Dallas* 7: I154–I157, 1985.
24. **Cooke, J. P., and P. Tsao.** Cellular mechanisms of atherogenesis and the effects of nitric oxide. *Curr. Opin. Cardiol.* 7: 799–804, 1992.
25. **Corbally, M. T., and M. F. Brennan.** Non-invasive measurement of regional blood flow in man. *Am. J. Surg.* 160: 313–321, 1990.
26. **Creager, M. S., C.-S. Liang, and J. D. Coffman.** Beta adrenergic mediated vasodilator response to insulin in the human forearm. *J. Pharmacol. Exper. Ther.* 235: 709–714, 1985.
27. **DeFronzo, R. A., R. C. Bonadonna, and E. Ferrannini.** Pathogenesis of NIDDM—a balanced overview. *Diabetes Care* 15: 318–368, 1992.
28. **DeFronzo, R. A., R. Gunnarson, O. Björkman, M. Olson, and J. Wahsen.** Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin dependent (type II) diabetes mellitus. *J. Clin. Invest.* 76: 149–155, 1985.
29. **DeFronzo, R. A., E. Jacot, E. Jequier, E. Maeder, J. Wahren, and J. P. Felber.** The effect of insulin on the disposal of intravenous glucose: results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 30: 1000–1007, 1981.
30. **DeFronzo, R. A., J. D. Tobin, and R. Andres.** Glucose clamp technique. A method for quantifying insulin secretion and resistance. *Am. J. Physiol.* 237 (*Endocrinol. Metab. Gastrointest. Physiol.* 6): E214–E223, 1979.
31. **Donahue, R. P., J. S. Skyler, N. Schneiderman, and R. J. Prineas.** Hyperinsulinemia and elevated blood pressure: cause, confounder or coincidence? *Am. J. Epidemiol.* 132: 827–836, 1990.
32. **Eckel, R. H.** Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. *N. Engl. J. Med.* 320: 1060–1068, 1989.
33. **Felber, J. P., M. U. Meyer, B. Curchod, H. U. Inselin, J. Rouselle, E. Maeder, P. Pahud, and E. Jequier.** Glucose storage and oxidation in different degrees of human obesity measured by continuous indirect calorimetry. *Diabetologia* 20: 39–44, 1981.
34. **Feldman, R. D., and G. S. Bierbrier.** Insulin mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 342: 707–709, 1993.
35. **Ferrannini, E., R. D. Buzzigoli, R. Bonadonna, M. A. Giorico, L. Oleggini, L. Graziadei, R. Pedrinelli, L. Brandi, and S. Bevilacqua.** Insulin resistance in essential hypertension. *N. Engl. J. Med.* 317: 350–357, 1987.
36. **Ferrannini, E., and R. A. DeFronzo.** The association of hypertension, diabetes and obesity: a review. *J. Nephrol.* 1: 3–15, 1989.
37. **Ferrannini, E., S. M. Haffner, B. D. Mitchell, and M. P. Stern.** Hyperinsulinaemia: the key feature of a cardiovascular and metabolic syndrome. *Diabetologia* 34: 416–422, 1991.
38. **Ferrannini, E., J. D. Smith, C. Cobelli, G. Toffolo, A. Pilo, and R. A. DeFronzo.** Effect of insulin on the distribution and disposition of glucose in man. *J. Clin. Invest.* 76: 357–364, 1985.
39. **Fisher, B. M., G. Gillen, H. J. Dargie, G. C. Inglis, and B. M. Frier.** The effects of insulin induced hypoglycemia on cardiovascular function in normal man: studies using radionuclide ventriculography. *Diabetologia* 30: 841–845, 1987.
40. **Fujita, N., T. Baba, T. Tomiyama, T. Kodama, and N. Kako.** Hyperinsulinemia and blood pressure in patients with insuloma. *Br. Med. J.* 304: 1157, 1992.
41. **Furchtgott, R. F., and J. V. Zawadski.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature Lond.* 288: 373–376, 1980.
42. **Ganrot, P. O.** Insulin resistance syndrome: possible key role of blood flow in resting muscle. *Diabetologia* 36: 876–879, 1993.
43. **Gans, R. O. B., L. V. D. Toorn, H. J. Bilo, J. J. P. Nauta, R. J. Heine, and A. J. M. Donker.** Renal and cardiovascular effects of exogenous insulin in healthy volunteers. *Clin. Sci. Lond.* 80: 219–225, 1991.
44. **Garg, U. C., and A. Hassid.** Nitric oxide-generating vasodilators and proliferation of cultured rat vascular smooth muscle cells. *J. Clin. Invest.* 83: 1774–1777, 1989.
45. **Gelfand, R. A., and E. J. Barrett.** Effect of physiologic hyperinsulinemia on skeletal muscle protein synthesis and breakdown in man. *J. Clin. Invest.* 80: 1–6, 1987.
46. **Greene, A. D., P. J. Tonellato, J. Lui, J. H. Lombard, and A. W. Cowley.** Microvascular rarefaction and tissue vascular resistance in hypertension. *Am. J. Physiol.* 256 (*Heart Circ. Physiol.* 25): H126–H131, 1989.
47. **Grubb, B., and J. F. Snarr.** Effect of flow rate and glucose concentration on glucose uptake by the rat limb. *Proc. Soc. Exp. Biol. Med.* 154: 33–36, 1977.
48. **Hall, J. E., M. W. Brands, S. D. Kivlighn, H. L. Mizelle, D. A. Hildebrandt, and C. A. Gaillard.** Chronic hyperinsulinemia and blood pressure. *Hypertension Dallas* 15: 519–527, 1990.
49. **Hiatt, W. R., S. Y. Huang, and J. G. Regensteiner.** Venous occlusion plethysmography reduces arterial diameter and flow velocity. *J. Appl. Physiol.* 66: 2239–2244, 1989.
50. **Holmang, A., P. Björntorp, and B. Rippe.** Tissue uptake of insulin and insulin in red and white skeletal muscle in vivo. *Am. J. Physiol.* 263 (*Heart Circ. Physiol.* 32): H1170–H1176, 1992.

51. Honig, C. R., Odoroff, C. L., and J. L. Frierson. Capillary recruitment in exercise: rate, extent, uniformity and relation to blood flow. *Am. J. Physiol.* 238 (Heart Circ. Physiol. 7): H31–H42, 1980.
52. Honig, C. R., C. Odoroff, and J. L. Frierson. Active and passive capillary control in red muscle at rest and in exercise. *Am. J. Physiol.* 243 (Heart Circ. Physiol. 12): H196–H206, 1982.
53. Huang, S. C., M. E. Phelps, E. J. Hoffman, K. Sideris, C. J. Selin, and D. E. Kuhl. Non-invasive determination of local cerebral metabolic rate of glucose in man. *Am. J. Physiol.* 238 (Endocrinol. Metab. 1): E69–E82, 1980.
54. Jackson, R. A., J. B. Hamling, P. M. Blix, B. M. Sim, M. I. Hawa, J. B. Jaspan, J. Belin, and J. D. N. Navarro. The influence of graded hyperglycemia with and without physiological hyperinsulinemia on forearm glucose uptake and other metabolic responses in man. *J. Clin. Endocrinol. Metab.* 63: 541–549, 1986.
55. James, D. E., K. M. Burleigh, L. H. Storlein, S. P. Bennett, and E. W. Kreagen. Heterogeneity of insulin action in muscle: influence of blood flow. *Am. J. Physiol.* 251 (Endocrinol. Metab. 14): E422–E430, 1986.
56. Jarrett, R. J. In defence of insulin: a critique of syndrome X. *Lancet* 340: 469–471, 1992.
57. Jarrett, R. J., H. Keen, M. McCartney, J. H. Fuller, P. J. S. Hamilton, D. D. Reid, and G. Rose. Glucose tolerance and blood pressure in two population samples: their relation to diabetes mellitus and hypertension. *Int. J. Epidemiol.* 7: 15–24, 1978.
58. Jorfeldt, S., and H. Rutberg. Comparison of dye dilution and plethysmographic blood flow measurements: an evaluation of the influence of invasive techniques on blood flow and on arterial and femoral venous substrate variables in man. *Clin. Sci. Lond.* 79: 81–87, 1990.
59. Juhlin-Dannfelt, A., F. Frisk-Holmberg, J. Karlsson, and P. Tesch. Central and peripheral circulation in relation to muscle-fiber composition in normo- and hypertensive man. *Clin. Sci. Lond.* 56: 335–340, 1979.
60. Julius, S., T. Gudbrandsson, K. Jamerson, S. T. Shahab, and O. Andersson. The hemodynamic link between insulin resistance and hypertension. *J. Hypertens.* 9: 983–996, 1991.
61. Kahn, A. M., J. S. Flier, R. S. Bar, J. A. Archer, P. Gorden, M. D. Martin, and J. Roth. The syndromes of insulin resistance and acanthosis nigricans: insulin receptor disorders in man. *N. Engl. J. Med.* 294: 739–745, 1976.
62. Kahn, A. M., C. L. Seidel, J. C. Allen, R. G. O'Neil, H. Shelat, and T. Song. Insulin reduces contraction and intracellular calcium concentration in vascular smooth muscle. *Hypertension Dallas* 22: 735–742, 1993.
63. Kelley, D. E., J. P. Reilly, T. Veneman, and L. J. Mandarino. Effects of insulin on skeletal muscle glucose storage, oxidation and glycolysis. *Am. J. Physiol.* 258 (Endocrinol. Metab. 21): E923–E929, 1990.
64. Kisseebah, A. H., and A. N. Peiris. Biology of regional fat distribution: relationship to non-insulin dependent diabetes mellitus. *Diabetes Metab. Rev.* 4: 622–632, 1988.
65. Kolterman, O. G., J. Insel, M. Saekow, and J. M. Olefsky. Mechanisms of insulin resistance in human obesity. Evidence for receptor and postreceptor defects. *J. Clin. Invest.* 65: 1272–1284, 1980.
66. Krogh, A. *The Anatomy and Physiology of Capillaries*. New Haven, CT: Yale Univ. Press, 1929.
67. Laakso, M., S. V. Edelman, G. Brechtel, and A. D. Baron. 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.
68. Laakso, M., S. Edelman, G. Brechtel, and A. D. Baron. Impaired insulin mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41: 1076–1083, 1992.
69. Laakso, M., S. V. Edelman, G. Brechtel, and A. D. Baron. Effects of epinephrine on insulin-mediated glucose uptake in whole body and leg muscle in humans: role of blood flow. *Am. J. Physiol.* 263 (Endocrinol. Metab. 26): E199–E204, 1992.
70. Landsberg, L. Diet, obesity and hypertension: a hypothesis involving insulin, the sympathetic nervous system and adaptive thermogenesis. *Q. J. Med.* 236: 1081–1090, 1986.
71. Lembo, G., R. Virgilio, G. Iaccarino, F. Lamenza, M. Volpe, and B. Trimarco. Insulin reduces reflex forearm sympathetic vasoconstriction in healthy humans. *Hypertension Dallas* 21: 1015–1019, 1993.
72. Levenson, J. A. Simon, and I. Pitthois-Merli. Brachial arterial changes in response to wrist occlusion in normotensive and hypertensive man. *Am. J. Physiol.* 253 (Heart Circ. Physiol. 22): H217–H224, 1987.
73. Liang, C.-S., J. U. Doherty, R. Faillance, K. Maekawa, S. Arnold, H. Gavras, and W. B. Hood, Jr. Insulin infusion in conscious dogs. Effects on systemic and coronary hemodynamics, regional blood flows and plasma catecholamines. *J. Clin. Invest.* 69: 1321–1336, 1982.
74. Lillioja, S., A. A. Young, C. L. Cutler, J. L. Ivy, W. G. H. Abbott, J. K. Zawadski, H. Yki-Järvinen, L. Christin, T. W. Secomb, and C. Bogardus. Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J. Clin. Invest.* 80: 415–425, 1987.
75. Miles, D. W., and C. J. Hayter. The effects of intravenous insulin on the circulatory responses to tilting normal and diabetic subjects with special reference to baroreceptor reflex block and atypical hypoglycemic reactions. *Clin. Sci. Lond.* 34: 413–430, 1968.
76. Milley, J. R. Effect of insulin on the distribution of cardiac output in the fetal lamb. *Pediatr. Res.* 22: 169–172, 1987.
77. Milley, J. R., and J. S. Papacostas. Effect of insulin on metabolism of fetal sheep hindquarters. *Diabetes* 38: 597–603, 1989.
78. Modan, M., H. Halkin, S. Almog, A. Lusky, A. Eshkol, M. Shefi, A. Shitrit, and Z. Fuchs. Hyperinsulinemia. A link between hypertension, obesity and glucose intolerance. *J. Clin. Invest.* 75: 809–817, 1985.
79. Moncada, S., and A. Higgs. Mechanisms of disease: the L-arginine-nitric oxide pathway. *N. Engl. J. Med.* 329: 2002–2012, 1993.
80. Moore, R. D. Effects of insulin upon ion transport. *Biochim. Biophys. Acta* 737: 1–49, 1983.
81. Muntzel, M. S., A. L. Mark, and A. K. Johnson. Anteroventral third ventricle lesions abolish sympathetic neural responses to hyperinsulinemia (Abstract). *Hypertension Dallas* 22: 420, 1993.
82. Natali, A., G. Buzzigoli, S. Taddei, D. Santoro, M. Cerri, R. Pedrinelli, and E. Ferrannini. Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes* 39: 490–500, 1990.
83. Natali, A., D. Santoro, C. Polombo, M. Cerri, S. Ghione, and E. Ferrannini. Impaired insulin action on skeletal muscle metabolism in essential hypertension. *Hypertension Dallas* 17: 170–178, 1991.
84. Neahring, J. M., K. Stepniakowski, and B. M. Egan. Forearm vascular alpha-receptor sensitivity is normal and not altered by insulin in obese men with mild hypertension. *Hypertension Dallas* 22: 147–151, 1993.
85. O'Brien, R. M., and D. K. Granner. Regulation of gene expression by insulin. *Biochem. J.* 278: 609–619, 1991.
86. Olefsky, J. M. Lilly lecture 1980. Insulin resistance and insulin action. An in vitro and in vivo perspective. *Diabetes* 30: 148–162, 1981.
87. Page, M. M., R. B. W. Smith, and P. J. Watkins. Cardiovascular effects of insulin. *Br. Med. J.* 1: 430–432, 1976.
88. Page, M. M., and P. J. Watkins. Provocation of postural hypotension by insulin. *Diabetes* 25: 90–95, 1976.
89. Pollare, T., H. Lithell, and C. Berne. Insulin resistance is a characteristic feature of primary hypertension independent of obesity. *Metab. Clin. Exp.* 39: 167–174, 1990.
90. Porcellati, F., C. Fanelli, P. Bottini, L. Epifano, A. M. Rambotti, C. Lalli, S. Pampanelli, L. Scionti, F. Santeusanio, P. Brunetti, J. Hilsted, and G. B. Bolli. Mechanisms of arterial hypotension after therapeutic dose of subcutaneous insulin in diabetic autonomic neuropathy. *Diabetes* 42: 1055–1064, 1993.
91. Prager, R., P. Wallace, and J. M. Olefsky. In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects. *J. Clin. Invest.* 78: 472–481, 1986.

92. **Prakash, T. R., S. H. MacKenzie, J. L. Ram, and J. R. Sowers.** Insulin (INS) stimulates gene transcription and activity of Na^+/K^+ -ATPase in vascular smooth muscle cells (VSMC) (Abstract). *Hypertension Dallas* 20: 443, 1992.
93. **Rabinowitz, D., and K. L. Zierler.** Forearm metabolism and its response to intra-arterial insulin. *J. Clin. Invest.* 41: 2173–2181, 1962.
94. **Randin, D., P. Vollenweider, L. Tappy, E. Jequier, P. Nicod, and U. Scherrer.** Effects of adrenergic and cholinergic blockade on insulin induced stimulation of calf blood flow in humans. *Circulation* 86: 1–369, 1992.
95. **Reaven, G. M.** Role of insulin resistance in human disease. *Diabetes* 37: 1595–1607, 1988.
96. **Renkin, E. M.** Control of microcirculation and blood-tissue exchange. In: *Handbook of Physiology. The Cardiovascular System. Microcirculation*. Bethesda, MD: Am. Physiol. Soc., 1983, vol. IV, pt. 2, chapt. 14, p. 627–687.
97. **Richter, E. A., K. G. Mikines, H. Galbo, and B. Kiens.** Effect of exercise on insulin action in human skeletal muscle. *J. Appl. Physiol.* 66: 876–885, 1989.
98. **Rosen, O. M.** After insulin binds. *Science Wash. DC* 237: 1452–1458, 1987.
99. **Rowe, J. W., J. B. Young, K. L. Minaker, A. L. Stevens, J. Pallotta, and L. Landsberg.** Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man. *Diabetes* 30: 219–225, 1981.
100. **Schultz, T. A., S. B. Lewis, D. K. Westbie, J. E. Gerich, R. J. Rushakoff, and J. D. Wallin.** Glucose delivery—a clarification of its role in regulating glucose uptake in rat skeletal muscle. *Life Sci.* 20: 733–736, 1977.
101. **Schultz, T. A., S. B. Lewis, D. K. Westbie, J. D. Wallin, and J. E. Gerich.** Glucose delivery: a modulator of glucose uptake in contracting skeletal muscle. *Am. J. Physiol.* 233 (Endocrinol. Metab. Gastrointest. Physiol. 2): E514–E518, 1977.
102. **Shehin, S. E., J. R. Sowers, and M. B. Zemel.** Impaired vascular smooth muscle $^{45}\text{Ca}^{2+}$ efflux and hypertension in Zucker obese rats. *J. Vasc. Med. Biol.* 1: 278–282, 1989.
103. **Sowers, J. R., P. R. Standly, J. L. Ram, M. D. Zemel, and L. M. Resnick.** Insulin resistance, carbohydrate metabolism and hypertension. *Am. J. Hypertens.* 4: 466S–472S, 1991.
104. **Steinberg, H. O., G. Brechtel, A. Johnson, and A. D. Baron.** Insulin modulates endothelium derived relaxing factor/nitric oxide dependent vasodilation in skeletal muscle. *Hypertension Dallas* 22: 74–436, 1993.
105. **Tsutsu, N., V. Nurior, T. Kodama, R. Noniyama, M. Iwase, and M. Fujishimi.** Lack of association between blood pressure and insulin in patients with insulinoma. *J. Hypertens.* 8: 479–482, 1990.
106. **Tuck, M. L.** Obesity, the sympathetic nervous system and essential hypertension. *Hypertension Dallas* 19, Suppl. 1: I67–I77, 1992.
107. **Vane, J. R., E. E. Ånggård, and R. M. Botting.** Regulatory functions of the vascular endothelium. *N. Engl. J. Med.* 323: 27–36, 1990.
108. **Vicaud, E.** Hypertension and the microcirculation: a brief overview of experimental studies. *J. Hypertens.* 10, Suppl. 5: S59–S68, 1992.
109. **Vollenweider, P., D. Randin, L. Tappy, E. Jequier, P. Nicod, and U. Scherrer.** Low dose insulin infusion evokes sympathetic activation in lean healthy humans. *Hypertension Dallas* 22: 149–451, 1993.
110. **Vollenweider, P., L. Tappy, D. Randin, P. Schneiter, E. Jequier, P. Nicod, and U. Scherrer.** Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J. Clin. Invest.* 92: 147–154, 1992.
111. **Wright, C. E., D. D. Rees, and S. Moncada.** Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc. Res.* 26: 48–57, 1992.
112. **Yagi, S., S. Takata, H. Kiyokawa, M. Yamamoto, Y. Noto, T. Ikeda, and N. Hattori.** Effects of insulin on vasoconstrictive responses to norepinephrine and angiotensin II in rabbit femoral artery and vein. *Diabetes* 37: 1064–1067, 1988.
113. **Yang, Y. J., I. D. Hope, M. Ader, and R. N. Bergman.** Insulin transport across capillaries is rate limiting step for insulin action in dogs. *J. Clin. Invest.* 84: 1620–1628, 1989.
114. **Yki-Järvinen, H., A. A. Young, C. Lamkin, and J. E. Foley.** Kinetics of glucose disposal in whole body and across the forearm in man. *J. Clin. Invest.* 79: 1713–1719, 1987.
115. **Zemel, M. B., B. A. Johnson, and S. A. Ambrozy.** Insulin stimulated vascular relaxation: role of Ca^{2+} -ATPase. *Am. J. Hypertens.* 5: 637–641, 1992.
116. **Zierler, K. L.** Theory of the use of arteriovenous concentration differences for measuring metabolism in steady and non-steady state. *J. Clin. Invest.* 40: 2111–2125, 1961.