Chapter 2
Effects of Insulin on the Vascular System

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Introduction

The function of the vascular system is to allow the delivery of blood (oxygen and nutrients) to the tissues according to their unique metabolic needs, and the function of insulin is to enhance the storage of nutrients and to support tissue growth. To accomplish this task for the ever-changing tissue requirements for oxygen and nutrients and without compromising the blood supply of vital organs, the vascular system responds in a variety of ways. It responds at the local tissue level via the release of short-acting vasoactive hormones, which redirect blood flow from less active to more active tissue units. The vascular system reroutes blood flow from organs with (relatively) lesser needs to organ systems, which require higher rates of blood flow, for example, by activation of the sympathetic nervous system (SNS). Finally, if tissue requirements cannot be met by the above mechanisms, cardiac output will increase to meet all requirements and to avoid dangerous reductions in blood pressure.

When this chapter was first published in 2005, more than 20 years after insulin's action on the vasculature had been demonstrated in the dog [1], most groups in the field had come to agree that insulin, in the human, in addition to its actions on glucose, protein, and fatty acid metabolism also exhibited distinct effects on the vascular system. Equally important, elevated circulating insulin levels had been found to be an independent risk factor for cardiovascular disease (CVD). These observations raised the question whether elevated insulin levels per se might cause macrovascular disease or whether the insulin levels were elevated to compensate for the insulin resistance seen in obesity, hypertension, and type 2 diabetes. Thus, the question to be answered was whether insulin itself possessed direct vascular effects, which might accelerate atherosclerosis or cause hypertension. Overall, in the last 15 years,

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most of what we knew in 2005 has been confirmed; insulin elicits a coordinated response at the level of the skeletal muscle vasculature, the heart, and the SNS, and possibly even in the larger conduit vessels. Equally importantly, no studies have shown that insulin's actions contribute to CVD.

Insulin, in lean insulin-sensitive subjects, increases skeletal muscle and adipose tissue blood flow at physiological concentrations. Simultaneously, cardiac output and SNS activity increase. The majority of the increment in cardiac output is directed toward skeletal muscle, suggesting that the blood-flow elevation as a result of insulin's vascular action may be instrumental in augmenting skeletal muscle glucose uptake. Over the last 15 years, due to the increased use of contrast-enhanced ultrasonography (CEU) in skeletal muscle, it has been shown that insulin increases skeletal muscle and adipose tissue perfusion and is also highly likely to increase capillary recruitment and perfusion, even before cardiac output increases. However, the accuracy of these observations has been questioned by results from animal studies that applied newer technologies [2, 3].

The role of the rise in sympathetic nervous system activity (SNSA) in response to insulin is less well understood. It has been proposed that the increase in SNSA may counteract insulin's vasodilator effect to avoid a decrease in blood-pressure levels; there are more recent studies that support this notion. The insulin-induced change in SNSA may also be important for blood-flow regulation in adipose tissue. Furthermore, insulin may also exert part of its cardiovascular effects indirectly via modulation of renal sodium and volume handling.

It has been demonstrated that insulin's effect on skeletal muscle blood flow is mediated through the release of endothelium-derived nitric oxide (NO), the most potent endogenous vasodilator. Importantly, NO is not only a vasodilator but also exhibits a host of anti-atherosclerotic properties. In addition to its effect on NO release, insulin also modulates the response to other vasoactive hormones such as angiotensin II or norepinephrine (NE) at the level of the vascular endothelium and the vascular smooth muscle cell. Therefore, insulin's effect on the vasculature of normal subjects appears to be beneficial in that it may counteract blood-pressure elevation and inhibit the atherosclerotic process.

Assessment of insulin's effect on human microcirculation has flourished over the last 15 years mostly due to increased availability of CEU technology; progress of this technology and other technologies combined with enhanced computational capabilities are likely to lead to interesting findings in the study of single arterioles or capillaries in the future [4, 5]. Results obtained with CEU and with various techniques, such as tracer, positron emission tomography (PET) scanning and magnetic resonance demonstrate, with few exceptions, that insulin increased microvascular perfusion through capillary recruitment.

Insulin's vasodilator effect on skeletal muscle and adipose tissue vasculature is blunted in states of insulin resistance such as obesity, hypertension, and type 2 diabetes mellitus; and more evidence for impaired endothelial function and decreased NO production in obesity, hypertension, and type 2 diabetes has been developed over the last 15 years. Additional findings suggest that the size of adipocytes and adipose tissue depot may constrain blood supply and, therefore, affect perfusion. The mechanism(s) by which obesity and type 2 diabetes impair endothelial function

are not fully elucidated and are likely multifactorial; a combination of factors such as elevated free fatty acid (FFA) levels, increased endothelin-dependent vascular tone, increased levels of asymmetric dimethyl-arginine (ADMA), or endothelin as observed in these insulin-resistant subjects may account, at least in part, for the vascular dysfunction.

The following review will focus mainly on data obtained from human studies, but data from animal or in vitro studies will be used when providing mechanistic insight into insulin's effects on the vasculature.

Technical Considerations

Before exploring insulin's vascular actions, several technical considerations should be made. In vivo studies of insulin's effect on the vascular system require, in most cases, systemic administration of glucose (euglycemic hyperinsulinemic clamp technique) to maintain stable glucose concentrations. Using the euglycemic hyperinsulinemic clamp technique [6] avoids hypoglycemia and the release of hormones such as epinephrine, NE, or cortisol, which can blunt the metabolic and vascular action of insulin. However, glucose metabolism will be increased by insulin administration, and therefore, it may be difficult to dissociate insulin's vascular and metabolic effects. Furthermore, even small amounts of insulin may result in a decrease of systemic FFA levels or in an increase in SNSA, which may alter vascular responses to different stimuli.

The experimental conditions under which the data are obtained may influence the vascular response to insulin and other vasoactive substances. For example, the cardiovascular response in part may depend on whether the study is performed with the subject in the supine or upright-sitting position [7], whether the forearm or the leg is studied and so on. Finally, in regard to the assessment of skeletal muscle perfusion and blood pressure, all methods (strain gauge plethysmography vs thermodilution or PET scanning or CEU) have different sensitivities, which may explain part of the divergent observations in the literature. Similarly, results of vascular function studies may differ according to the methods. Interestingly, flow-mediated vasodilation (FMD), the change in brachial artery diameter in response to ischemia, did not correlate with insulin sensitivity in a larger Canadian study.

Physiology

Insulin's Effects on Skeletal Muscle Blood Flow

Insulin increases skeletal muscle blood flow in lean insulin-sensitive subjects. This insulin effect is observed in the leg [8, 9] and the forearm [10]. Insulin's vasodilator action occurs at physiological concentrations and in dose-dependent fashion (Fig. 2.1). Limb blood-flow rates nearly double at insulin levels in the high

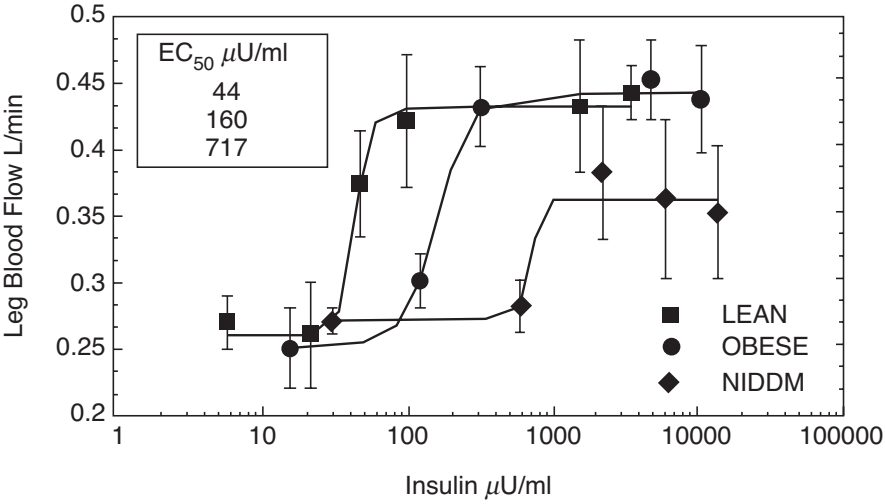

Fig. 2.1 Rates of leg blood flow in response to a wide range of steady-state insulin concentrations during euglycemic clamp studies in lean (filled square), obese (filled circle) and obese type 2 diabetic (filled diamond) subjects. The insert shows the insulin concentration required to achieve half-maximal increments in leg blood flow (EC50) in the different groups. (From ref. 11)

physiological range (~70–90 μU/mL). However, not all researchers have been able to observe the vasodilatory effects of insulin [12], except after a prolonged infusion, or at very high (~3000 μU/mL) systemic insulin levels [13]. The reasons for these divergent findings are not clear but are likely a result of differences in sensitivity and reproducibility of the methods used to determine blood flow.

In lean, insulin-sensitive subjects, the onset of insulin-mediated increments in skeletal muscle blood flow occurs early during a euglycemic/hyperinsulinemic clamp with a half-life of approximately 30 min, nearly identical to that of insulin's effect to increase glucose extraction [14]. A similar time course for insulin's vascular effect has also been described by Westerbacka and associates [15], who studied the effect of euglycemic hyperinsulinemia on pulse wave reflection in the aorta. In this study, the authors measured the pressure difference (central aortic augmentation) between the early and late systolic pressure peaks using applanation tonometry. They found that pressure augmentation and augmentation index decreased already after 30 min of hyperinsulinemia becoming statistically significant after 1 h. Because wave reflection is determined by compliance and vascular resistance, and because an early rise in skeletal muscle blood flow was not detected, which indicates a fall in peripheral vascular resistance, the authors concluded that insulin at physiological concentrations (~60 μU/mL) affects the caliber or distensibility (compliance) of large arteries. Studies in rats indicate [16] that insulin-mediated vasodilation may occur prior to an increase in cardiac output. Taken together, these studies provide evidence that insulin's effect on the vasculature occurs early in the course of hyperinsulinemia and parallels its effect on glucose metabolism.

Insulin does not only increase skeletal muscle blood flow at physiological concentrations but also augments the response to the endothelium-dependent vasodilator methacholine chloride (MCh). We have demonstrated [17] nearly a 50% augmentation of endothelium-dependent vasodilation at insulin levels of about 25 μU/mL. However, insulin did not augment the leg blood-flow response to the endothelium-independent vasodilator sodium nitroprusside (SNP). In support of our observation, insulin augmenter endothelium-dependent relaxation in response to the endothelium-dependent vasodilator acetylcholine in the isolated rat aorta but did not affect the response to SNP [18]. Taken together, these data indicate that insulin augments the production of but not the response to NO. In contrast to the above findings, euglycemic hyperinsulinemia was found to decrease FMD independent of insulin sensitivity or plasma lipid concentrations [19]; however, these results are difficult to interpret because this study used a less well-defined model to estimate endothelial function [20].

Insulin augmented the endothelial response to MCh, and therefore, we hypothesized that insulin causes skeletal muscle vasodilation via the release of NO. Using NG-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, we found that insulin's vasodilatory effects could be nearly completely annulled. In addition, the increment in leg blood flow was prevented by administration of L-NMMA into the femoral artery prior to initiating the systemic insulin infusion [14]. Moreover, leg blood flow, which nearly doubled in response to 4 h of euglycemic hyperinsulinemia returned to baseline (Fig. 2.2) levels within 5 min of an infusion of L-NMMA

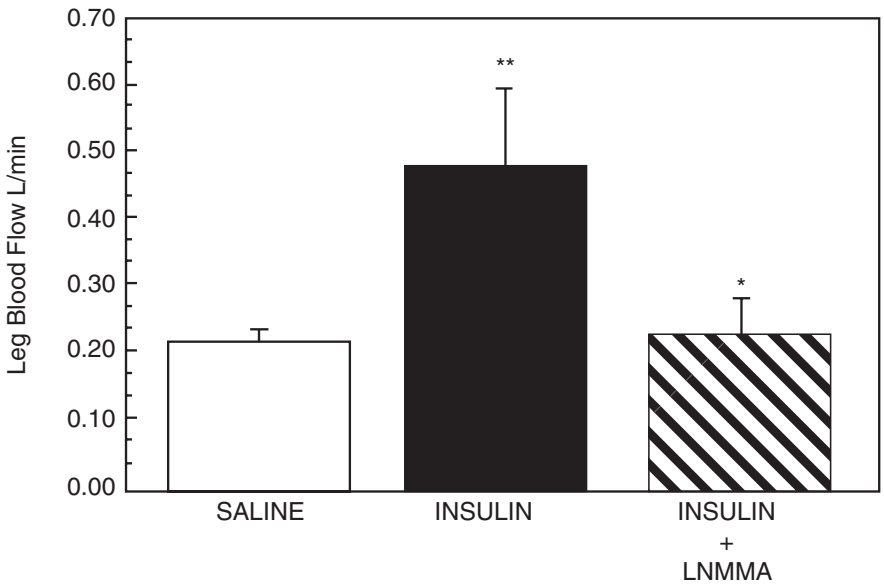

Fig. 2.2 Leg blood flow under basal conditions (saline), in response to 4 h of euglycemic hyperinsulinemia alone (insulin) and with superimposed intrafemoral artery infusion of L-NMMA (insulin + L-NMMA). (From ref. 17)

into the femoral artery [17]. Our findings have been confirmed by others in humans [21] and in animals [22]. The notion that insulin acts via release of NO from endothelial cell is supported by the observation that insulin directly releases NO from human umbilical vein endothelial cells [23]. This insulin-mediated NO release occurred in a dose-dependent fashion and could be completely abolished by N(omega)-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase. These results establish that insulin increases skeletal muscle blood flow, at least in part, via release of endothelial-derived NO.

Further investigation of the signaling pathway involved in insulin-mediated NO release revealed that genistein (an inhibitor of tyrosine kinase) nearly completely prevented the release of NO. Importantly, application of wortmannin, which inhibits phosphatidylinositol 3-kinase (PI3K), a signaling molecule required for insulin's effect to increase glucose uptake, caused about a 50% decrease in NO production. These in vitro results indicate that insulin-induced release of NO is mediated through signaling pathways involving tyrosine kinase, PI3K, and Akt downstream from the insulin receptor [24]. Importantly, Akt has recently been shown to phosphorylate endothelial NO synthase (eNOS), which results in increased activity of eNOS [25, 26]. Since insulin also increases the transport of amino acids into cells, the increased NO production may represent the complementary effects of eNOS phosphorylation and increased intracellular availability of arginine, the precursor of NO. Together, these findings suggest that insulin's metabolic and vascular actions share common signaling pathways which might explain the similar time course of skeletal muscle vasodilation and glucose uptake in response to insulin. Moreover, impairment of a common signaling pathway in obesity, hypertension, or diabetes could lead to both blunting of insulin-mediated blood-flow increments and decreased rates of skeletal muscle glucose uptake. In this regard, it is important to note that mice deficient of eNOS were insulin resistant and mildly hypertensive [27], but mice deficient of endothelial insulin receptors [28] exhibited normal glucose metabolism. Results from a recent study in primates [29] suggest that epoxyeicosatrienoic acids also mediate insulin-mediated augmentation in skeletal muscle perfusion.

Insulin's Effects on the Heart

Our lab [30] investigated the effect of different insulin infusion rates on stroke volume in groups of lean normotensive volunteers (Fig. 2.3a). Hyperinsulinemia in the low physiological range (35 ± 4 μU/mL) and in the high physiological range (78 ± 6 μ/mL) increased stroke volume by about 7%. A nearly 15% augmentation of stroke volume was observed with supraphysiological insulin concentrations (2145 ± 324 μU/mL). A similar effect of insulin on stroke volume was reported by Ter Maaten and associates [31], who observed a nearly 13% rise at insulin levels of about 30 μU/mL. The increase in stroke volume could be a result of either a decrease in peripheral resistance (see section "Insulin's Effect on Blood Pressure and Vascular Resistance") or as a result of an increase in inotropy of the heart muscle. Experiments in the isolated beating heart or with heart muscle preparation indicate that insulin

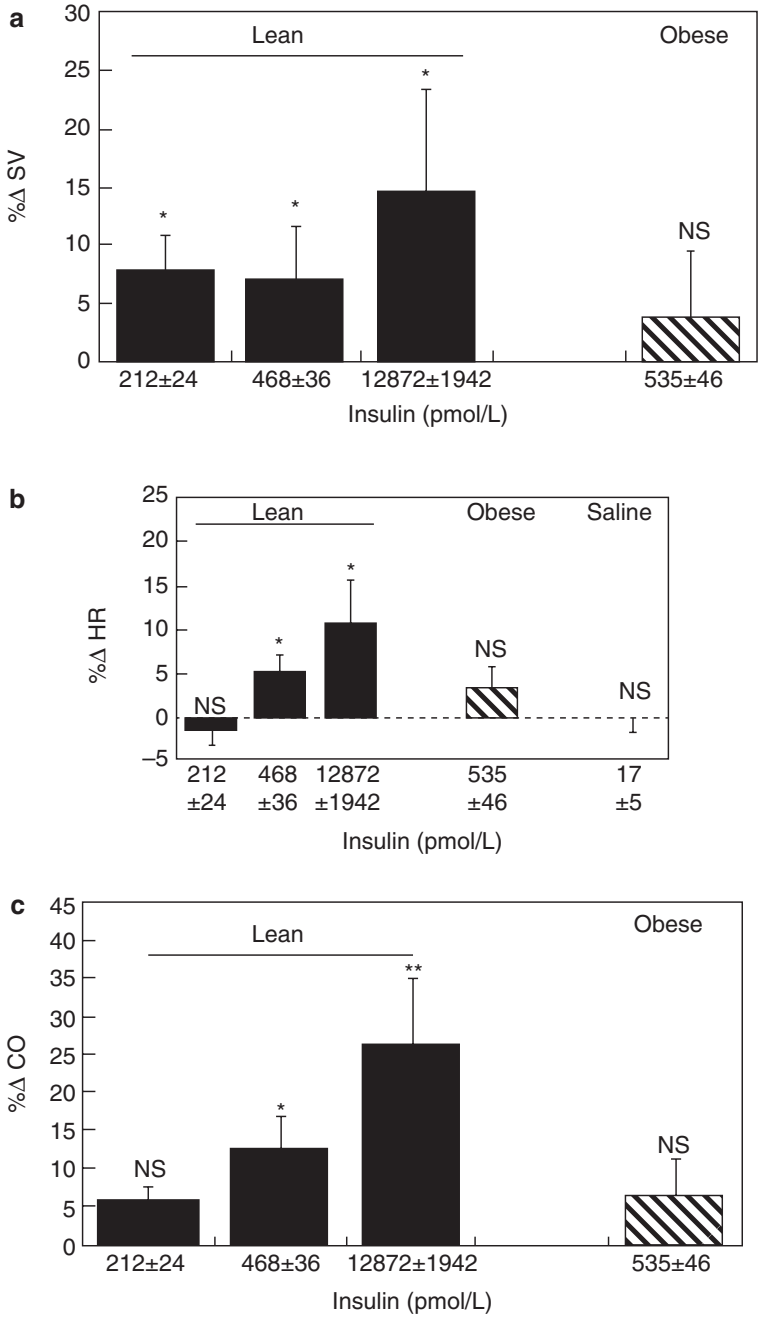

Fig. 2.3 Percent change (%Δ) from baseline (**a**) in stroke volume (SV), (**b**) in heart rate (HR), (**c**) in cardiac output (CO), (**d**) in mean arterial blood pressure (MAP), and (**e**) in total peripheral resistance (closed bar) and leg vascular resistance (hatched bar) during systemic hyperinsulinemic euglycemia and saline (control) infusion studies in lean and obese subjects. *$p < 0.05$, **$p < 0.01$ and not significant (NS) vs baseline. (From ref. 30)

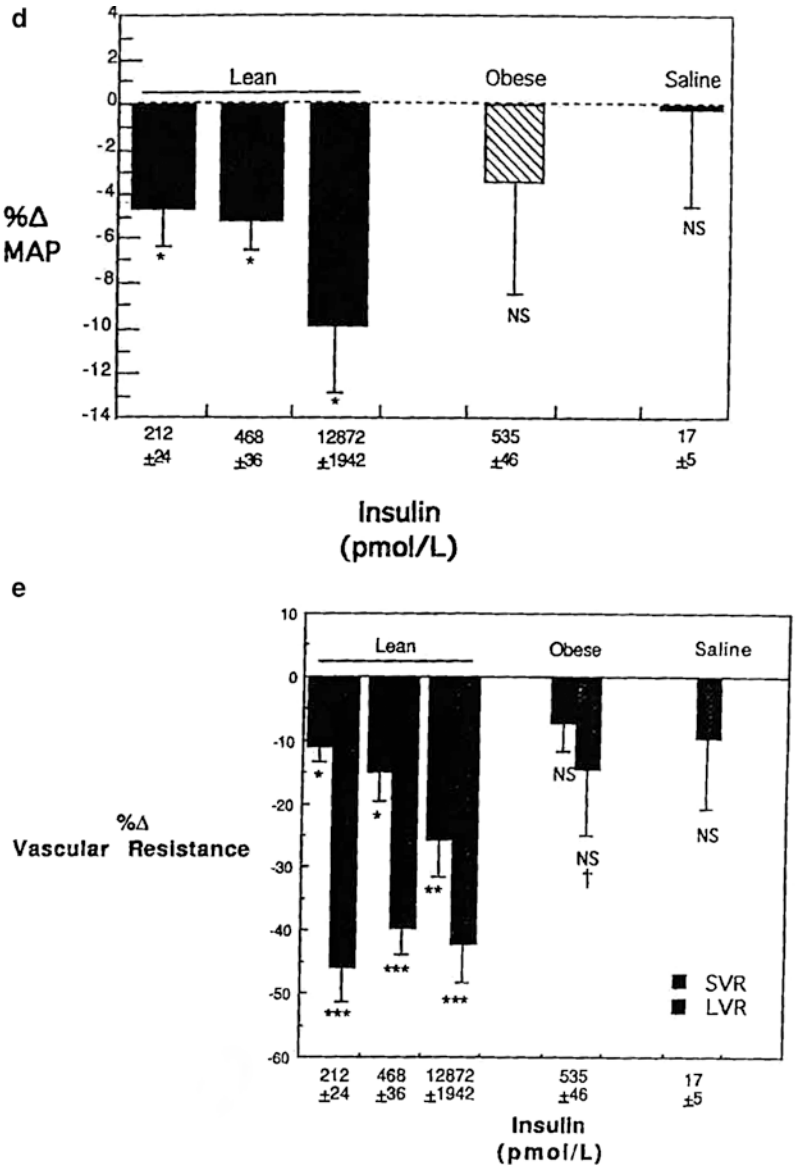

Fig. 2.3 (continued)

increases contractility of heart muscle. Taken together, these data indicate that insulin has a direct effect on the heart to increase cardiac stroke volume.

In addition to augmenting stroke volume, insulin increases heart rate. In our groups, heart rate did not change at low (~35 μU/mL) levels but increased by 5% and 10% at insulin concentrations of about 80 and about 2100 μU/mL,

respectively (Fig. 2.3b). Thus, our data indicate that insulin increases heart rate in a dose-dependent fashion. Increments in heart rate in response to hyperinsulinemia were also found by others [9, 10, 32] but not by all [31]. The reason for the discrepancy is not clear, but differences in volume status or position during the study may explain in part the different observations. Whether the increase in heart rate is a direct insulin effect or whether it is mediated by activation of the SNS is not known. Nevertheless, the increase in SNS activity likely represents normal physiology to maintain blood pressure [33, 34] and secure delivery of nutrients to the tissue.

As a result of the rise in heart rate and stroke volume in response to insulin, cardiac output upsurges. In our study groups, cardiac output increased by about 6%, 12%, and 26% in response to insulin concentrations of about 35, 80, and 2100 μU/mL (Fig. 2.3c). In support of our data, Ter Maaten and colleagues found about a 9% increase in cardiac output with insulin concentrations of about 50 μU/mL [31]. Moreover, Fugman and associates' study replicated most of the above findings in a more recent study [35], demonstrating increased cardiac output in response to high physiological levels of insulin. These insulin effects are not only of academic interest but also may have implications under conditions in which cardiac output needs to be augmented. For example, insulin's effect of increasing cardiac output has been used to improve severe heart failure in patients undergoing cardiac surgery who were unresponsive to catecholamines and vasodilators [36].

Over the last 15 years, there were no relevant new studies in the literature regarding insulin's hemodynamic effects on the heart, but there is current interest in insulin receptor signaling in the maintenance cardiomyocyte health and in models of cardiomyopathy.

Insulin's Effects on the Sympathetic/Parasympathetic Nervous System

Insulin had been shown to increase SNSA years before its vasodilator action was appreciated [37]. Systemic insulin infusion causes a dose-dependent rise in NE levels. In one study [6], NE levels in response to insulin increased from 199 ± 19 pg/mL under basal conditions to 258 ± 25 and 285 ± 95 pg/mL at insulin concentrations of 72 ± 8 μU/mL and 144 ± 13 μU/mL, respectively. In the same study, skeletal muscle SNSA measured by microneurography exhibited an even more impressive rise in response to insulin. Microneurography allows to measure frequency and amplitude of electric activity directly at the level of sympathetic nerve fibers. Determined by microneurography, SNSA increased from baseline of about 380 U to about 600 U and about 750 U in response to euglycemic hyperinsulinemia. Similar differences between the methods to assess changes in SNSA have been found by others [38], suggesting that plasma NE levels may underestimate the true effect of insulin to stimulate SNSA.

Interestingly, insulin modulates SNSA in a non-uniform manner. Van De Borne and colleagues [39] studied the effect of insulin on skeletal muscle SNSA with microneurography. The effect of hyperinsulinemia on cardiac SNSA and parasympathetic tone was assessed by power spectral analysis of the decrease in R–R interval. Power spectral analysis allows one to distinguish between low-frequency and high-frequency components of the changes in R–R intervals. The high-frequency component is thought to reflect parasympathetic nervous system activity (PNSA; vagal tone) whereas the low-frequency component reflects SNSA. Additionally, systemic infusion of the B-blocker propranolol allows to distinguish the contribution of the PNS and the SNS on the R–R interval variability.

In response to hyperinsulinemia (84 ± 5 U/mL), skeletal muscle SNSA increased more than twofold. In contrast, the SNSA effect on the reduction in R–R interval and variability in response to hyperinsulinemia was relatively small. This observation suggests that insulin's effect on the SNSA may be targeted specifically toward skeletal muscle, the place of insulin's metabolic action. Interestingly, the increase in skeletal muscle SNSA may delay insulin's vasodilator action [40].

The mechanism(s) for the increments in SNSA during hyperinsulinemia are not well understood. It may be mediated via the baroreceptor reflex to counteract insulin's vasodilator action or may represent a direct insulin effect on the central nervous system. Moreover, coupling of insulin's effects on the SNS and its effect to increase glucose uptake/metabolism cannot be excluded. Although activation of the baroreceptor reflex in response to a decrease in blood pressure causes activation of the SNS, it cannot explain all of the observed changes. First, time course of blood-pressure decline and SNSA were different [10] and second, the increments in SNSA in response to insulin were nearly two times those in response to blood pressure fall achieved by nitroglycerin infusion [41]. In support of a direct role of insulin on SNSA at the level of the brain, injection of insulin directly into the third ventricle has been shown to increase SNSA in rats [42]. This increase in SNSA activity could be abolished by generating a lesion in the surrounding the lateroventral portion of the third ventricle, a region implicated in sympathetic neural control. Overall, the results suggest a direct effect of insulin on the brain to increase SNSA, but other mechanisms cannot be excluded.

It has also been demonstrated that insulin modulates PNSA. Unfortunately, no biochemical markers of PNSA exist, which can be easily measured in vivo. As mentioned above, PNSA is studied by measuring the changes in R–R intervals using power spectral analysis. The PNSA (vagal component of heart rate control) is represented in the high-frequency part of the spectrum.

In 1996, Bellavere and associates [43] reported a decrease in high-frequency variability of R–R intervals in response to hyperinsulinemia indicating that PNSA decreased. Similar results were obtained by Van De Borne and associates [39] in which euglycemic hyperinsulinemia decreased both R–R interval and the high-frequency variability of the R–R intervals. Moreover, this insulin-induced reduction of both R–R interval and high-frequency variability could not be suppressed by the B-blocker propranolol. These data indicate that the reduction in PNSA and not increments in SNS were likely responsible for the changes in R–R interval and

variability. Furthermore, these data suggest that the effect of hyperinsulinemia on cardiac SNSA may be less than originally thought. Taken together, these data suggest that insulin's effect to stimulate SNSA may be mediated at least in part via a direct insulin effect on the brain. Furthermore, hyperinsulinemia appears to reduce parasympathetic tone at the level of the heart, which may contribute to the increments in heart rate.

Insulin's Effects on the Kidneys

The effect of euglycemic hyperinsulinemia on renal hemodynamics has not been studied by many groups. In one study [44], insulin at levels of about 100 U/mL has been reported to increase renal plasma flow by 10 ± 5%. A similar rise in renal plasma flow has been reported in response to L-arginine-induced insulin secretion.

Insulin's effect on electrolyte handling is well established. Insulin has been found to cause antinatriuresis [45, 46], antikaliuresis, and antiuricosuria in healthy volunteers. The antinatriuresis is achieved via a decrease in fractional sodium excretion. Fractional sodium excretion fell by 20–30% in response to euglycemic hyperinsulinemia with insulin levels of 50–60 μU/mL, well in the physiological range. Reductions in potassium and uric acid excretion in response to insulin were of similar magnitude [42]. Based on animal studies [47], it was thought that insulin exerts the antinatriuretic effect at the level of the distal tubule in which the highest density of insulin receptors is found, but it may be that the proximal tubule is the more likely site of insulin's antinatriuretic action in humans [48]. The mechanism of the antikaliuretic and antiuricoretic effects of insulin is less well elucidated.

Insulin's Effect on Blood Pressure and Vascular Resistance

Insulin's effect on skeletal muscle vasculature, stroke volume, heart rate, cardiac output, SNS, and renal sodium handling can affect blood pressure. Blood pressure is determined by cardiac output and total peripheral resistance (TPR). In other words, blood pressure in response to insulin may increase, stay unchanged, or decrease dependent on the changes in cardiac output and resistance. In lean, insulin-sensitive subjects, insulin causes a small but significant fall in blood pressure. In our study [30], hyperinsulinemia in the low (35 ± 4 μU/mL) and high (72 ± 6 μU/mL) physiological range caused about a 5% drop in mean arterial pressure (MAP), and supraphysiological insulin concentrations (2100 ± 325 μU/mL) were associated with about a 10% fall in MAP (Fig. 2.3d). However, although a drop in MAP has been reported by many groups, it has not been observed in all studies; MAP remained unchanged in a study reported by Scherrer [12] and even increased by nearly 7 mmHg in another study [31]. The reasons for the different effects of euglycemic hyperinsulinemia on blood pressure are not clear.

The decrease in MAP in light of increased cardiac output indicates [29] a fall in TPR. In fact, TPR decreased in a dose-dependent fashion by 11.1 ± 2.2, 15.0 ± 4.7, and 26.0 ± 6.0% at insulin concentrations of 35 ± 4, 72 ± 6, and 2100 ± 325 μU/mL, respectively (Fig. 2.3e). A similar decrease in TPR with comparable levels of hyperinsulinemia was also observed by Fugman and associates [35]. Even more impressive than the fall in TPR was the drop in leg vascular resistance (LVR) LVR decreased by nearly 45% at an insulin concentration of 35 ± 4 μU/mL (Fig. 2.3e). Higher prevailing insulin levels did not result in further decrements in LVR. Similar decrements in resistance have been observed by Anderson in the forearm [10, 49] and by Vollenweider in the calf [38]. However, in one study [31] in which both blood pressure and forearm blood flow increased, no changes in vascular resistance were detected.

Metabolic Implications of Insulin's Vascular Effects

Our lab has long championed the idea that insulin's vascular effects may contribute to the rate at which glucose is taken up by skeletal muscle, which represents the majority of insulin-sensitive tissues. In other words, insulin's vascular effects may determine, at least in part, insulin sensitivity and impairment of insulin's vascular effects may result in insulin resistance.

In support of this idea, we found that insulin's effect to increase skeletal muscle blood flow and cardiac output is positively and strongly associated with the rates of glucose uptake achieved in response to euglycemic hyperinsulinemia. In two studies [30, 50] performed nearly 5 years apart, the correlation coefficients between leg blood-flow increments and whole-body glucose uptake were 0.63 and 0.56, indicating that blood flow achieved during euglycemic hyperinsulinemia explains one-quarter to one-third of the variation in insulin sensitivity. Similarly, Ter Maaten and associates [31] found that the correlation coefficient between percent increments in leg blood flow and insulin sensitivity index was 0.88, again suggesting that insulin's effect to augment blood flow contributes to rates of glucose uptake. Furthermore, cardiac output or changes in cardiac output in response euglycemic hyperinsulinemia also correlated significantly albeit not as strongly as leg blood flow with rates of whole-body glucose uptake [30, 31]. Finally, the similar time courses [9] of insulin-mediated vasodilation and insulin-mediated glucose uptake suggest that metabolic and vascular actions of insulin might be coupled.

Taken together, these data suggest but do not prove that insulin's effects on metabolism and the vascular system are coupled. To test our hypothesis more rigidly, we assessed the effect of leg blood flow changes on leg glucose uptake. In one set of studies [50], we increased leg blood flow from 0.32 ± 0.12 L/min during euglycemic hyperinsulinemia to 0.60 ± 0.12 L/min ($p < 0.05$) by administering an intrafemoral artery infusion of the endothelium-dependent vasodilator MCh. As a result of the blood-flow increments, leg glucose uptake increased from 87.6 ± 13.4 to 129.4 ± 21.8 mg/min ($p < 0.05$). In a second set of studies [51], we decreased leg blood flow during euglycemic hyperinsulinemia by nearly 50% via an intrafemoral artery infusion of the NO synthase inhibitor L-NMMA. The fall in leg blood flow

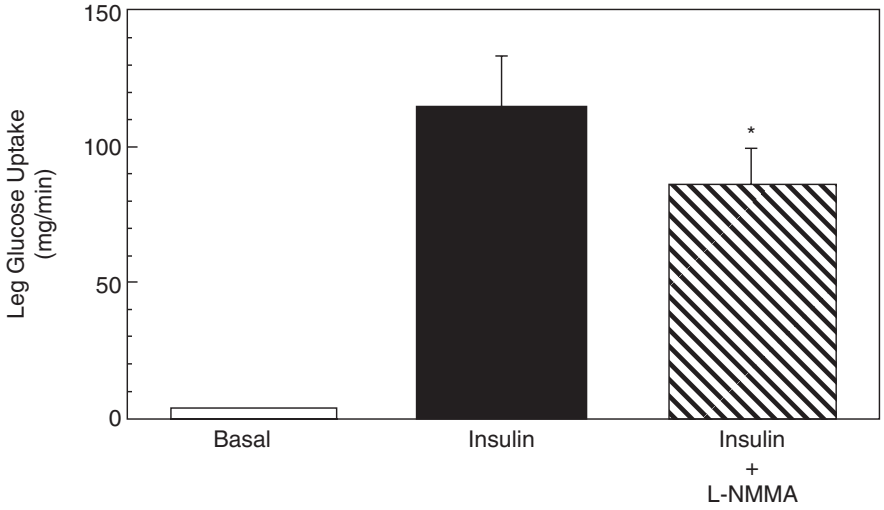

Fig. 2.4 Leg glucose uptake under basal conditions (basal), in response to 4 h of euglycemic hyperinsulinemia alone (insulin) and with superimposed intrafemoral artery infusion of L-NMMA (insulin + L-NMMA). (From ref. 51)

induced by L-NMMA caused leg glucose uptake to decrease from 114 ± 18 to 85 ± 13 mg/min ($p < 0.05$) representing about a 25% reduction of glucose uptake (Fig. 2.4), well in line with what had been predicted according to the experimentally defined correlation coefficients. In a third series of studies, we examined whether rates of skeletal muscle glucose uptake in response to changes in leg blood flow followed a noncapillary recruitment model as proposed by Renkin or whether changes in glucose uptake were dependent on capillary recruitment. The results of this study revealed that leg glucose uptake in response to pharmacological manipulation of blood flow was different than predicted by the Renkin model indicating that capillary recruitment is important for insulin's metabolic actions [52]. These findings are supported by studies of Bonadonna and associates [53] who looked at forearm glucose uptake using multiple tracer technique and Rattigan and associates [54] who measured glucose uptake in the isolated rat hindlimb. And Coggins and associates [55], using CEU, provided more direct evidence for insulin's effect to recruit skeletal muscle capillaries in men. Together, these data provide strong evidence that insulin's vascular effects relate to its metabolic effects and that this metabolic effect is mediated by capillary recruitment.

The above-discussed effects of insulin on the vascular system are also observed in response to meals [56]. Depending on the amount of carbohydrate or fat ingested and the circulating insulin levels achieved, heart rate, stroke volume, skeletal muscle blood flow, and SNSA increase substantially, indicating that this coordinated cardiovascular response occurs under physiological conditions and may be necessary to maintain both metabolic and hemodynamic homeostasis. Postprandial hypotension, which is frequently observed in the elderly, may be a result of insufficient increments in heart rate and/or stroke volume to compensate for insulin's vasodilator effect.

Interactions Between Insulin and Norepinephrine and Angiotensin II

Because elevated insulin levels were associated with higher rates of hypertension, it was hypothesized that insulin might augment the action of vasoconstrictor hormones such as NE or angiotensin II. Indeed, earlier studies [32, 37] reported that exogenous insulin enhanced the blood-pressure response to NE. About a 20% and 40% reduction of the NE concentrations required to rise diastolic blood pressure by 20 mmHg was reported after 1 and 6 h of euglycemic hyperinsulinemia. In contrast to this finding, we [57] observed that euglycemic hyperinsulinemia caused a right shift in the response to graded systemic infusions of NE. The reason(s) for the discrepant findings are not clear but are likely a result of differences in study protocol and the method by which blood pressure was determined (intra-arterial vs. cuff). Nevertheless, our data suggest that insulin attenuates vascular responsiveness to NE. In support of this notion, Sakai and associates [58] reported that an intra-arterial infusion of insulin attenuated the vasoconstrictor response to NE by nearly 50%. Moreover, Lembo and coworkers also demonstrated that that insulin augmented beta-adrenergic vasodilation in response to isoproterenol and attenuated a-adrenergic vasoconstriction [49]. Furthermore, this insulin action was blocked by L-NMMA and inhibitor of NO synthase. These results indicate that insulin's modulatory effect on adrenergic response is mediated via the release of NO.

The effect of hyperinsulinemia on blood-pressure response to angiotensin II has been studied by a number of groups [32, 59, 60]. Insulin does not augment nor attenuate the blood pressure response to systemic angiotensin II infusion. However, Sakai and associates [48] demonstrated that insulin, when directly infused into a vessel, may modulate the vasoconstrictor response to angiotensin II. In their study, the direct intrabrachial artery infusion of insulin caused a more than 50% attenuation of the forearm blood-flow response to angiotensin II.

Insulin modulates the response to vasopressor hormones such as NE, vasopressin, and angiotensin II not only at the level of the vascular endothelium but also directly at the level of the vascular smooth muscle cell independent of the endothelium. Insulin attenuates agonist-evoked calcium transients [61] resulting in decreased vascular smooth muscle contractions. Whether this insulin effect at the level of the vascular smooth muscle can be explained by its effect on shared signaling pathways as described with angiotensin-1 [62] or by a different mechanism remains to be clarified. It is clear, however that an imbalance between insulin's vasorelaxant effects and other vasoconstrictor hormones may result in the accelerated development of blood-pressure elevation and macrovascular disease. Interestingly, blood-pressure elevation by systemic administration of NE [57] or angiotensin II [59, 63, 64] failed to decrease rates of insulin-mediated glucose uptake and induce insulin resistance. To the contrary and somewhat unexpectedly, the blood-pressure elevation increased rates of insulin-mediated glucose uptake. The reason for this unexpected finding was most likely that limb blood flow increased which allowed for the higher delivery rates of substrate, glucose, and insulin and, thus, augmented skeletal muscle glucose uptake.

Interactions Between Insulin and Adipocytokines

Adipose tissue has been shown to release a number of hormones that may interact with the vasculature. Leptin, a hormone secreted from the adipocyte, causes not only the release of NO from endothelial cells and but also augments insulin's effect to release NO [65]. Furthermore, adiponectin, another adipocyte-derived hormone, has been shown to cause the release of NO from endothelial cells [66]. Finally, interleukin-6, released from intra-abdominal fat cells may decrease in endothelial NO production via increasing C-reactive protein [67] or via decreasing adiponectin secretion [68].

Pathophysiology: The Metabolic Syndrome

The metabolic syndrome which is also called "syndrome X" describes the clustering of a number of metabolic and hemodynamic abnormalities commonly seen in obesity and diabetes. More important, the metabolic syndrome is an independent risk factor for CVD. Syndrome X [69, 70] is associated with resistance to insulin-mediated glucose uptake, glucose intolerance, hyperinsulinemia, increased very low-density lipoprotein triglyceride, decreased high-density lipoprotein cholesterol, increased plasminogen activator inhibitor-1, and hypertension. Because classic risk factors account for only about 50% of the increased rates of cardiovascular morbidity and mortality associated with obesity and type 2 diabetes [71], other factors must play a role. One way to probe for potential candidates that might contribute to the higher rate of hypertension and the accelerated atherosclerotic process in insulin resistance is to evaluate the effect of obesity, hypertension, and type 2 diabetes on insulin's vascular effects.

The Metabolic Syndrome and Insulin's Effects on Skeletal Muscle Blood Flow

The effect of obesity, hypertension, and diabetes on insulin's vascular effects has been studied by a number of groups including our own. We [72] have demonstrated that obesity causes a left shift in the response to insulin's vasodilatory effect (Fig. 2.1); the dose that achieves half-maximal effect (ED) 50 for insulin's effect to increase skeletal muscle blood flow in the obese was nearly four times (~160 μU/mL) that of the lean (~45 μU/mL). Impaired insulin-mediated vasodilation in the obese was confirmed by Vollenweider and associates [73] who report about an 8% increment in calf blood flow in response to 2 h of euglycemic hyperinsulinemia in obese subjects, which is in stark contrast to the 30% increment achieved in the lean subjects.

Arterial stiffness is decreased in type 2 DM [74] and the effect of insulin to reduce arterial stiffness is impaired in obesity; Westerbacka [11] and colleagues demonstrated that in contrast to lean controls, arterial stiffness did not change in response to hyperinsulinemia with insulin levels of about 70 μU/mL and decreased only slightly in response to insulin levels of about 160 μU/mL.

Type 2 DM was associated with even more pronounced impairment of insulin-mediated vasodilation. In our study [72], only supraphysiological hyperinsulinemia (~2000 μU/mL) achieved about a 33% rise in blood flow and the limitation in flow increments could not be overcome by higher insulin concentrations (Fig. 2.1).

Because insulin-mediated vasodilation depends on NO and is impaired in obesity and type 2 DM, we studied whether this impairment results from defective endothelial function or whether or defective NO activity. To this end, we generated dose–response curves for the leg blood-flow response to the endothelium-dependent vasodilator MCh and to the endothelium-independent vasodilator SNP. Leg blood flow in response to methacholine increased threefold in the lean but only twofold in both obese and type 2 diabetics (Fig. 2.5). In contrast, the leg blood-flow response to SNP did not differ between lean, obese and type 2 diabetics. Resistance to leg blood-flow increments in response to the endothelium-dependent vasodilator bradykinin has also been reported in obesity [76], thus, supporting our data that NO production is impaired.

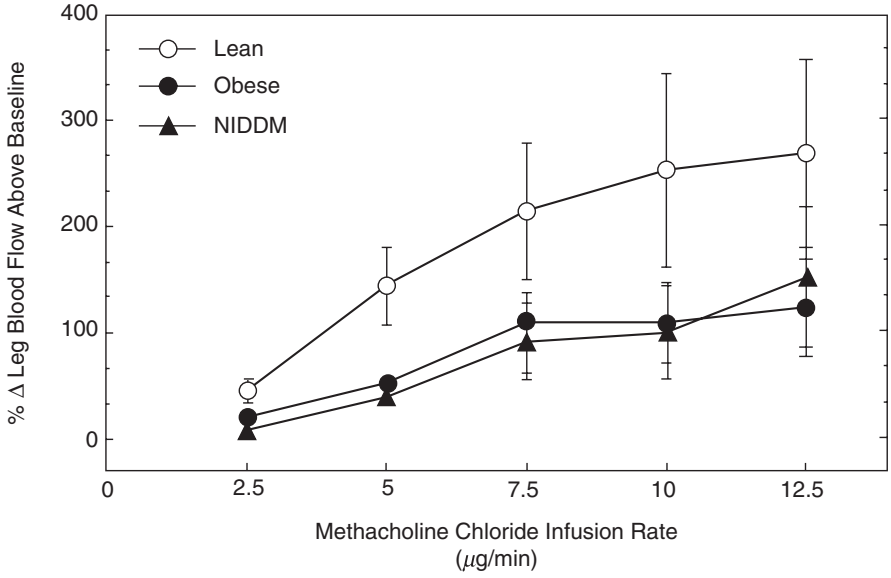

Fig. 2.5 Percent change (%Δ) from baseline in leg blood flow (LBF) in response to graded intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride in groups of lean (open circle), obese (filled circle), and obese type 2 diabetic (filled triangle) subjects. (From ref. 75)

In addition to obesity and type 2 diabetes, elevated blood-pressure levels are associated with impaired insulin-mediated vasodilation [77]. Laine and associates [76] demonstrated that insulin-stimulated leg blood flow increased by 91% in the control subjects but only by 33% in the hypertensive subjects. This is important because hypertension has been shown by Forte and associates [78] to be associated with significantly decreased rates of NO production. Therefore, it is likely that in hypertension, impaired NO production is responsible for the blunted vasodilation in response to hyperinsulinemia.

Direct measurements of NO production in the skeletal muscle vasculature of obese and type 2 DM subjects, however, have yielded conflicting data. In one preliminary study [75], we measured insulin-induced changes in NO flux rates in subjects exhibiting a wide range of insulin sensitivity. NO flux was calculated by multiplying the concentration of nitrite and nitrate times leg blood-flow rates before and after 4 h of euglycemic hyperinsulinemia. In this study, NO flux rates more than doubled in athletes who exhibited high insulin sensitivity but did not change in diabetics who were insulin resistant. However, Avogarro and associates [79], who measured NO flux rates in the forearm in obese and type 2 diabetic subjects, were unable to detect a difference in NO flux between the two groups. The reason for the discrepant observations is not clear, but further research will help to clarify this issue. Measurements of whole-body NO production using labeled L-arginine, the precursor of NO, revealed lower NO production rates in type 2 diabetics as compared to normal subjects [80] provides strong evidence for impaired NO production in type 2 diabetes.

Taking the data together, basal whole-body NO production is decreased in hypertensive and in type 2 diabetic patients, and it is likely that obesity, hypertension, and type 2 diabetes exhibit impaired NO production in response to insulin. Because NO is not only a potent vasodilator but also possesses a number of antiatherogenic properties, this defect in NO production could theoretically contribute to the increased rate of CVD in insulin-resistant states such as obesity, hypertension, or type 2 diabetes.

The mechanism(s) of impaired insulin-mediated vasodilation in obesity or type 2 DM are not known. One of the metabolic abnormalities consistently observed in insulin resistance is elevated FFA levels. Elevation of FFA levels also induces insulin resistance, which may be mediated, in part, via impairment of insulin-mediated vasodilation. Therefore, we studied the effect of FFA elevation on endothelial function in lean, insulin-sensitive subjects. The results of this study indicated that moderate two- or threefold elevation of FFA levels sustained for 2 h, achieved by systemic infusion of Intralipid plus heparin, blunted the response to the endothelium-dependent vasodilator MCh (Fig. 2.6) but not to the endothelium-independent vasodilator SNP [81]. Similar results were reported by de Kreutzenberg and colleagues, who measured forearm vascular responses to before and after elevation of FFA [82]. Interestingly, the postischemic flow response was also impaired by FFA elevation [74]. Importantly, elevation of triglyceride levels without inducing insulin resistance may not impair vascular function which is suggested by studies of patients with low lipoprotein lipase activity who exhibit normal endothelial function [83] despite markedly elevated triglyceride levels.

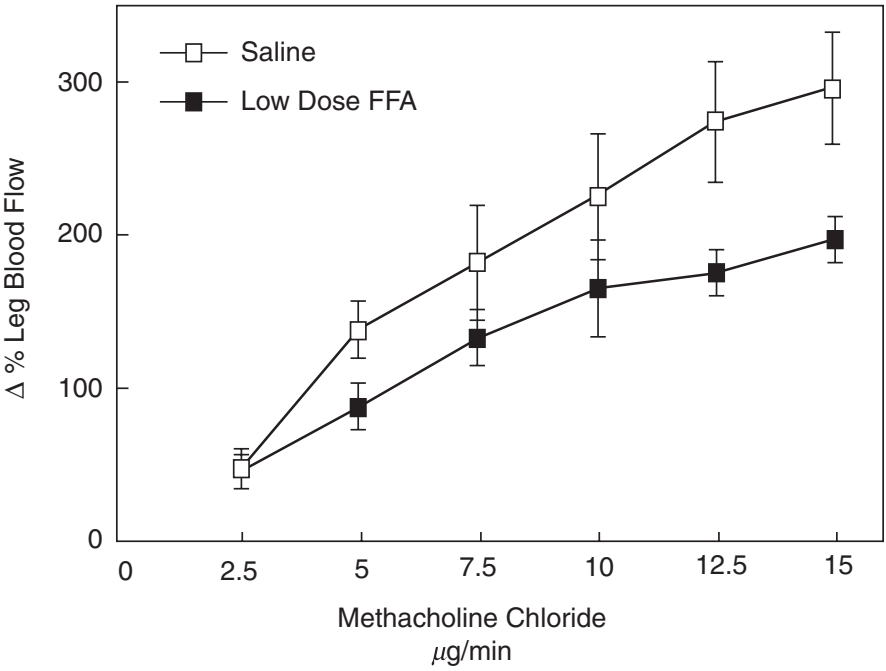

Fig. 2.6 Leg blood flow increments from baseline (%Δ) in response to graded intrafemoral artery infusion of methacholine chloride during infusion of saline (open squares) or during 20% fat intralipd emulsion (closed squares) combined with heparin designed to increase systemic circulating free fatty acid levels two- or threefold. (From ref. 81)

To further investigate the relation among elevated FFA levels, insulin sensitivity, and insulin-induced vasodilation, we investigated the time-course effect of FFA elevation on insulin-mediated increments in blood flow. Between 4 and 8 h, but not as few as 2 h of FFA elevation reduced insulin-mediated vasodilation [84]. Furthermore, increments in NO flux in response to euglycemic hyperinsulinemia were nearly completely abrogated by superimposed FFA elevation. This effect on insulin-induced vasodilation was only observed when FFA elevation also caused insulin resistance. These data indicate that insulin-mediated vasodilation is coupled with insulin's effect on glucose uptake. In contrast, muscarinergic-agonist-induced endothelium-dependent vasodilation appears to be regulated by other mechanisms as this signaling pathway can be disrupted by FFA elevations as short as 2 h [81]. Indirect evidence for this proposed effect of FFA elevation on insulin-mediated vasodilation comes from muscle biopsy studies in response to hyperinsulinemic euglycemia with and without superimposed FFA elevation [85]. Dresner and colleagues [85] demonstrated that insulin resistance induced by FFA elevation was associated with decreased PI3K activity in skeletal muscle. Therefore, if insulin-signaling pathways are shared in endothelial cells and skeletal muscle, one may expect impaired insulin signaling in the endothelial cells in response to euglycemic

hyperinsulinemia with superimposed FFA elevation. Other support of the negative effect of elevated FFA levels on endothelial NO production comes from in vitro studies that demonstrated a dose-dependent effect of oleic acid to impair NO release from cultured endothelial cells [86] and an attenuated the aortic strip relaxation in response to acetylcholine [87].

Additional mechanisms by which FFA may impair endothelial function include increased plasma levels of asymmetric dimethyl-L-arginine (ADMA) and/or increased endothelin action [88]. Lundman and associates [89] demonstrated that acute elevation of triglyceride (and likely elevated FFA) levels achieved by systemic infusion of a triglyceride emulsion was associated with elevation of ADMA levels and decreased flow-mediated vasodilation. Similarly, Fard and associates [90] showed that a high fat meal given to diabetic subjects resulted in increased plasma ADMA levels and impaired flow-mediated vasodilation.

Endothelin levels have been shown to increase in response to FFA elevation. Because elevated FFA levels are a hallmark of obesity and type 2 diabetes mellitus, Cardillo and associates [91] and Mather and associates [92] infused an inhibitor of endothelin, BQ 123 (a specific inhibitor of the endothelin-1-A receptor) directly into the brachial and femoral artery, respectively. Both studies revealed more pronounced vasodilation in response to BQ123 in the obese and diabetic subjects, indicating a higher endothelin-dependent tone in the insulin-resistant subjects. In addition, one study looking at vastus lateralis muscle biopsies showed reduced eNOS content and activity in type 2 diabetic subjects while endothelin-1 peptide and mRNA were higher [93]. Additionally, endothelin secretion may increase in response hyperinsulinemia and contribute to the impaired vasodilation observed in insulin-resistant states [94]. Results from studies in rats [95] have demonstrated that myeloperoxidase may also impaired vascular function in insulin resistance.

Taken together, these findings from in vivo and in vitro studies strongly suggest role of elevated FFA levels to impair endothelial function and decrease the rates of NO release, increase endothelin action, and increase vascular response to adrenergic stimulation.

The Metabolic Syndrome and Insulin's Effects on the Heart

Before discussing the effect of insulin on heart rate in insulin-resistant obese and diabetic subjects, two points should be made: first, basal heart rate and cardiac output [96] in obese and diabetic subjects is almost always increased as compared to lean subjects; second, heart function in diabetes may be abnormal as a result of autonomic neuropathy and third, since the heart is an on-demand pump, lesser increments in insulin's metabolic actions may be associated with a reduced need to supply tissues with additional oxygen and nutrients. Thus, the data have to be interpreted with caution especially when comparing relative changes between insulin-sensitive and insulin-resistant groups.

The effect of insulin resistance on insulin-induced change in stroke volume has received little attention. Stroke volume did not change in our group of obese subjects (Fig. 2.3a) exposed to insulin concentrations of about 90 μU/mL. However, we may have failed to detect a less than 5% increase in stroke volume because of small group size. Muscelli and associates [97], however, report a near 10% rise in stroke volume at insulin concentrations of about 120 μU/mL. The reason for the different results is not clear. Groups were comparable regarding body mass index or blood pressure. However, Muscelli and associates [97] used two-dimensional echocardiography, whereas we used dye dilution technique to determine stroke volume. Thus, the discrepant results may be explained, at least in part, by different sensitivities of the methods by which cardiac output was determined.

We did not observe a change in heart rate in response to hyperinsulinemia about 90 μU/mL in our obese subjects (Fig. 2.3b). In contrast to our findings, Vollenweider and associates detected about a 10% increase in heart rate in obese subjects with insulin levels comparable to our study (~100 μU/mL). Heart rate was also found to rise in a dose-dependent fashion in response to hyperinsulinemia [98] in type 2 diabetics.

Because stroke volume and heart rate did not change in our obese group (Fig. 2.3c), cardiac output did not change either. However, other studies report a significant 15% increment in cardiac output in obese subjects [97]. In type 2 diabetes, data on changes in cardiac output in response to hyperinsulinemia are not available. Nevertheless, because heart rate has been reported to increase in diabetics in response to hyperinsulinemia, it is reasonable to assume that cardiac output may increase as well. Taken together, the observations suggest that insulin's stimulatory effect on stroke volume, heart rate, and cardiac output may be intact in obese and type 2 diabetic subjects.

Insulin's action on the heart may extend well beyond modulation of hemodynamics. Cardiomyocytes possess insulin receptors which are important in postnatal development of the heart [99]. It is not known whether impaired insulin receptor signaling in the cardiomyocyte plays a role in the increased incidence of left ventricular hypertrophy and congestive heart failure which is often observed in obesity and diabetes. It may be of interest, however, that obesity and insulin resistance appear to be associated with a higher incidence of heart failure with preserved ejection fraction as compared to lean and more insulin-sensitive subjects in population studies [100].

The Metabolic Syndrome and Insulin's Effects on the Sympathetic/Parasympathetic Nervous System

When assessing the SNSA by measuring NE, no differences were detected between lean and obese subjects [38, 101, 102]. Tack and colleagues used tritiated NE combined with forearm blood-flow measurements to assess the effect of hyperinsulinemia on SNSA in the forearm of lean type 2 diabetic and controls; in response to

insulin, arterial and venous NE concentrations increased in both groups. For example, 45 min of hyperinsulinemia caused arterial NE levels to increase by 63.8 ± 14.1% and 41.3 ± 9.1% in diabetic and control subjects, respectively. In both groups, the rise in NE concentration was as a result of higher rates of total body and forearm NE spillover which were comparable between the diabetic and controls. Unfortunately, no obese subjects were studied, which would have allowed to distinguish the effects of diabetes (hyperglycemia) from those of obesity.

When measured by microneurography, basal skeletal muscle SNSA was found to be elevated more than twofold in obesity [101–103]. In response to euglycemic hyperinsulinemia, SNSA increased significantly [38]. Although the relative rise in SNSA was blunted in the obese subjects, the absolute levels of SNSA achieved during hyperinsulinemia were comparable between lean and obese subjects. These data suggest that SNSA is nearly maximally stimulated in obese insulin-resistant subjects and that added hyperinsulinemia is unable to increase SNSA above levels achieved in lean controls. SNSA appears to be abnormal in the states of metabolic syndrome, the prediabetic state [104, 105], and diabetes [106]. For example, Dell'Oro and colleagues [104] report 30–40% greater MSNA values in middle-aged prediabetic subjects when compared to matched control irrespective of being expressed as burst incidence over time or when corrected for heart rate. In addition, this neurogenic abnormality was associated with a 30–40% reduced spontaneous baroreflex MSNA sensitivity. Furthermore, in a multivariate analysis, MSNA values were directly and significantly related to HOMA index and inversely and significantly to baroreflex–MSNA sensitivity in the prediabetic group (Fig. 2.7).

Only two groups have thus far studied the effect of the metabolic syndrome on PNSA. Unfortunately, the results are somewhat contradictory. Muscelli and associates [107] report an increase in the low-frequency/high-frequency (LF/HF) ratio in

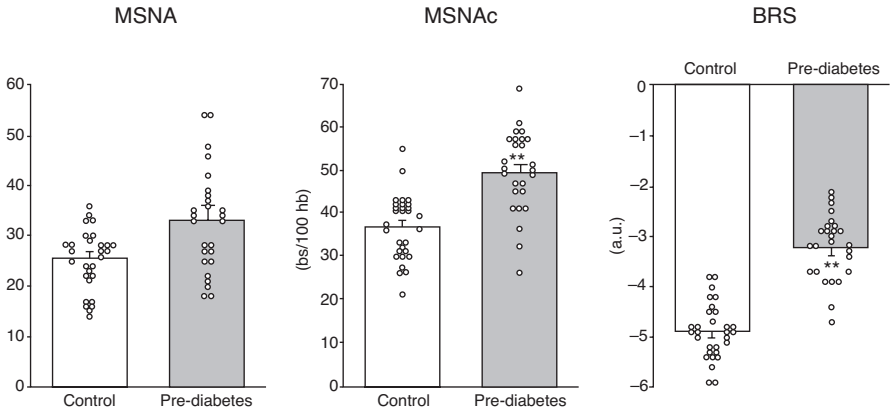

Fig. 2.7 Bar graphs refer to individual and mean (standard errors) values of muscle sympathetic nerve traffic, expressed as bursts incidence over time (MSNA, left panel) and bursts incidence corrected for heart rate values (MSNAc, central panel), and spontaneous baroreflex–MSNA (BRS, right panel) in 30 healthy controls (open bars) and in 26 patients with prediabetes (gray bars). Asterisks ($p < 0.01$) refer to the statistical significance between groups. (From ref. 104)

response to euglycemic hyperinsulinemia in lean normal subjects but not in obese insulin-resistant subjects. The authors conclude that insulin alters cardiac control by enhancing sympathetic outflow and withdrawal of parasympathetic tone. On the other hand, Laitinen and associates [108] demonstrate the opposite, an increase in the LF/HF in obese insulin-resistant subjects but not in the normal controls.

The Metabolic Syndrome and Insulin's Effect on the Kidney

The effect of euglycemic hyperinsulinemia on renal hemodynamics in obesity has not been studied. In one study assessing the effect of euglycemic hyperinsulinemia on renal function in type 2 diabetes, no differences in estimated renal plasma flow were observed. Thus, the scarce data suggest that insulin's effect on renal blood flow is intact in obesity and type 2 diabetes.

Insulin's effect on electrolyte handling has been well studied in type 2 diabetes but data on obesity are not available. The antinatriuretic effect of insulin is well preserved in type 2 diabetes. Gans and associates [98] report a fall in fractional sodium excretion fell by 43 ± 6% and 57 ± 9% in response to euglycemic hyperinsulinemia with insulin levels of 64 ± 12 μU/mL and 1113 ± 218 μU/mL, respectively. Because no control group was available in this study, it is not possible to determine whether the antinatriuretic response was normal or exaggerated in type 2 diabetes. Exaggerated antinatriuresis could lead to volume retention and contribute to the development of hypertension.

The Metabolic Syndrome and Insulin's Effect on Blood Pressure

Insulin's effect on the heart, the SNS, and the kidneys appear to be intact in subjects with the metabolic syndrome. This is in contrast to the impairment of insulin's effect to vasodilate skeletal muscle vasculature, which contributes to the decrease in peripheral vascular resistance during euglycemic hyperinsulinemia. Therefore, because the product of cardiac output and vascular resistance determine blood pressure, one might expect euglycemic hyperinsulinemia to result in blood-pressure elevation. In our study, acute euglycemic hyperinsulinemia did not alter blood pressure in the obese subjects (Fig. 2.3d). Other groups have reported that blood pressure in response to euglycemic hyperinsulinemia increased [38], decreased [109], or remained unchanged [110] in obese and diabetic subjects. Thus, the current data do not support the idea that hyperinsulinemia per se is causally related to the blood-pressure elevation associated with the metabolic syndrome. Since the publication of this chapter in 2005, no dedicated studies to study the effect of insulin on blood pressure have been published, but there were a few studies looking at the effect of a fatty meal and lipid infusion on blood pressure and endothelial function [111]

showing that orally or intravenously administered lipids increased blood pressure and impaired endothelium-medicated vasodilation, determined by FMD in the forearm.

The Metabolic Syndrome and Interactions Between Insulin and Norepinephrine

Although there is a great interest in the effect of the metabolic syndrome on the vascular responses to vasopressors such as NE or angiotensin II, few data are available in humans. We have demonstrated that the pressure response to systemic infusion of NE is augmented in obesity [57]. At similar NE concentrations, the obese subjects exhibited a nearly 50% more pronounced blood pressure rise than the lean controls. Furthermore, insulin's effect to attenuate the pressure response to NE was abolished by obesity. In another study (unpublished data), we found that elevation of FFA enhanced the blood-pressure response to intra-arterial as well as systemic infusion of a selective alpha-one adrenergic agonist while blunting baroreceptor-medicated vasodilation in the leg.

The effect of insulin resistance on the pressure response to angiotensin II was evaluated by Gaboury and associates [112] in normotensive and hypertensive subjects. In normotensive subjects, no relationship between insulin sensitivity and the blood-pressure response to angiotensin II was detected. However, insulin sensitivity correlated inversely with the blood-pressure response to angiotensin II in the hypertensive subjects.

Taken together, these data suggest that vascular responses to pressors may be increased in insulin resistance, which could contribute to the development of hypertension. The data also indicate that the relationship between insulin resistance and pressure responsiveness is not linear and may be modulated by additional factors that are poorly understood.

Interventions to Ameliorate the Effects of the Metabolic Syndrome on the Vascular System

If the increased rate of CVD associated with metabolic syndrome is partially mediated via the effects of insulin resistance on the vascular system, amelioration of insulin resistance should improve the abnormalities of the vascular system, which have been described above. In other words, maneuvers that improve insulin sensitivity should result in lower blood pressure, decreased heart rate, reduced SNSA, and improved endothelial function. Over the last 15 years, additional studies have been conducted to assess the effect of improved insulin sensitivity on insulin-mediated vasodilation and endothelial function.

It has been known for a long time that weight loss improves insulin sensitivity and lowers blood pressure [113]. Weight loss also decreases heart rate and reduces the heightened SNSA [114–116] and improves blunted SNS responsiveness to glucose ingestion [117]. Weight loss has been shown to improve blood flow in adipose tissue in some but not all studies [118, 119].

Troglitazone, a thiazolidinedione derivative, has been described to improve insulin sensitivity [120] and lower blood pressure in obese subjects. Furthermore, troglitazone decreased peripheral vascular resistance in diabetics [121], and pioglitazone decreased blood pressure in diabetic subjects [122]. Rosiglitazone which is also an insulin sensitizer was shown to improve insulin sensitivity, increase blood flow and glucose uptake in subjects with newly diagnosed type 2 diabetes [123]; in the same study, metformin improved neither insulin sensitivity nor blood flow. These data suggest that improvement of insulin sensitivity without changes in body fat content ameliorates cardiovascular abnormalities observed with the metabolic syndrome.

Our own findings [124] using 600 mg of troglitazone per day for 3 months in obese females suffering from polycystic ovary syndrome suggest a beneficial effect of troglitazone on both insulin-mediated vasodilation and the blood-flow responses to the endothelium-dependent vasodilator MCh. In contrast to our study, Tack and coworkers [125] found no effect of troglitazone (400 mg/day for 8 weeks) on insulin-induced blood-flow increments in obese insulin-resistant subjects despite a 20% improvement in insulin sensitivity. While the above studies represented longer-term interventions, an acute infusion of autonomous nervous system blockade with trimethaphan [126] improved insulin action in insulin-resistant but not in insulin-sensitive subjects. Thus, given the sparse and somewhat contradictory literature about the effect of increased insulin sensitivity on insulin-mediated increments in blood flow and endothelial function, further studies are required. Nevertheless, reduction of insulin resistance leading to improved endothelial and vascular system function may result in decreased cardiovascular morbidity and mortality in obese, hypertensive, and diabetic subjects.

Conclusion

Over the last 25 years, it has been established that insulin is a vascular hormone. Insulin's vascular actions extend beyond its effect to increase skeletal muscle blood flow and glucose uptake. Current data suggest that insulin modulates vascular tone and vascular smooth muscle cell proliferation and migration via the release of NO and other yet unidentified mechanisms (Fig. 2.8). Thus, insulin's effects on the vascular system may be important to prevent or delay the progression of CVD. The metabolic syndrome affects the vascular system at multiple levels. Resistance to the vascular actions of insulin may explain, at least in part, the abnormalities associated with the metabolic syndrome. The altered state of the vascular system in metabolic syndrome may contribute to higher rates of hypertension and macrovascular disease. States of insulin resistance that occur naturally or due to an intervention are

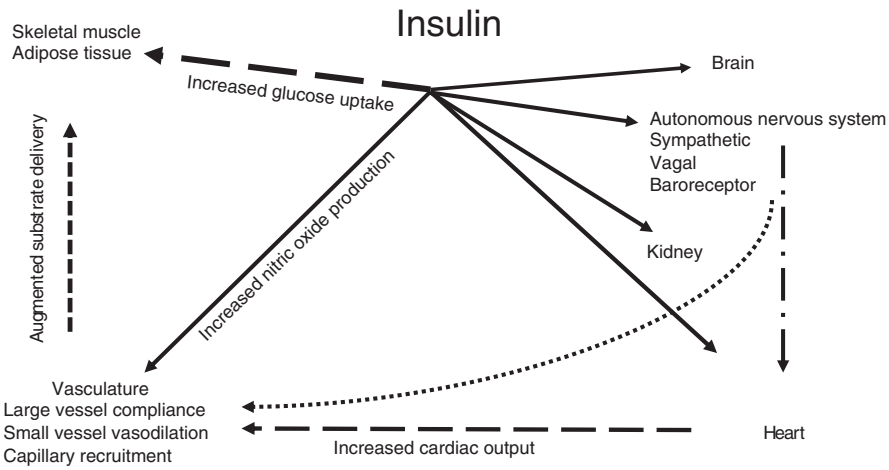

Fig. 2.8 Schema of classic and non-classic insulin action on different targets to enhance insulin delivery and glucose uptake

almost always associated with impaired endothelium-dependent vasodilation. However, the opposite that impaired endothelial function induces insulin resistance, is not the case [127]. Future research assessing the interaction between insulin's effect on the vasculature and newly discovered adipocytokines and other vasoactive hormones will better define the pathophysiological abnormalities underlying insulin-resistant states and help design therapies to improve endothelial function and reverse the accelerated atherosclerotic process.

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