Microvascular Function Relates to Insulin Sensitivity and Blood Pressure in Normal Subjects

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Background—A strong but presently unexplained inverse association between blood pressure and insulin sensitivity has been reported. Microvascular vasodilator capacity may be a common antecedent linking insulin sensitivity to blood pressure. To test this hypothesis, we studied 18 normotensive and glucose-tolerant subjects showing a wide range in insulin sensitivity as assessed with the hyperinsulinemic, euglycemic clamp technique.

Methods and Results—Blood pressure was measured by 24-hour ambulatory blood pressure monitoring. Videomicroscopy was used to measure skin capillary density and capillary recruitment after arterial occlusion. Skin blood flow responses after iontophoresis of acetylcholine and sodium nitroprusside were evaluated by laser Doppler flowmetry. Insulin sensitivity correlated with 24-hour systolic blood pressure (24-hour SBP; \( r = -0.50, P<0.05 \)). Capillary recruitment and acetylcholine-mediated vasodilatation were strongly and positively related to insulin sensitivity (\( r=0.84, P<0.001; r=0.78, P<0.001 \), respectively), and capillary recruitment was inversely related to 24-hour SBP (\( r = -0.53, P<0.05 \)). Waist-to-hip ratio showed strong associations with insulin sensitivity, blood pressure, and the measures of microvascular function but did not confound the associations between these variables. Subsequent regression analysis showed that the association between insulin sensitivity and blood pressure was not independent of the estimates of microvascular function, and part of the variation in both blood pressure (\( R^2=38\% \)) and insulin sensitivity (\( R^2=71\% \)) could be explained by microvascular function.

Conclusions—Insulin sensitivity and blood pressure are associated well within the physiological range. Microvascular function strongly relates to both, consistent with a central role in linking these variables. (Circulation. 1999;99:896-902.)

Key Words: hypertension ■ insulin ■ microcirculation ■ capillaries ■ endothelium

The association between insulin resistance and hypertension is well established but presently unexplained.\(^1,2\) One leading theory postulates that blood pressure elevation is caused by insulin resistance and compensatory hyperinsulinemia.\(^1\) An alternative hypothesis is that microvascular function, as a common antecedent, determines both blood pressure and insulin sensitivity. In several tissues, capillary density has been found to correlate inversely with blood pressure and peripheral resistance in hypertensive and normotensive subjects,\(^3,4\) and a decrease in capillary density may contribute to an increase in vascular resistance.\(^5,6\) Although previous investigations in skeletal muscle preparations from insulin-resistant subjects have convincingly shown the existence of insulin receptor and postreceptor defects,\(^7\) there is also evidence that both reduced capillary surface area and impaired microvascular endothelial function may contribute to insulin resistance.\(^8,9\) Muscle capillary density is positively correlated with insulin sensitivity, and diffusion distance of insulin and glucose from capillary to muscle cells (which increases with decreased capillary density) may play a role in determining insulin sensitivity.\(^10,11\) This decrease in capillary density may be a consequence of reduced endothelium-dependent vasodilatation at the precapillary level. Small precapillary vessels are considered the main regulators of capillary recruitment and in addition contribute to total peripheral resistance. Indeed, reduced endothelium-dependent vasodilatation of resistance vessels is associated with insulin resistance\(^12\) and hypertension.\(^13\)

Therefore, perturbed microvascular vasodilator capacity may link elevated blood pressure with insulin resistance. To test this hypothesis, we assessed capillary density and recruitment as well as endothelium-dependent vasodilatation of the skin microcirculation and analyzed the relation between these estimates of microvascular function and 24-hour ambulatory
blood pressure and insulin sensitivity in a group of 18 healthy normotensive and glucose-tolerant subjects. Because the relations between insulin sensitivity and blood pressure may be confounded by obesity and body fat distribution, body mass index and waist-to-hip ratio were also determined.

Methods

Subjects

Eighteen healthy, white volunteers participated in these studies with the approval of the local ethical committee. They were recruited from a larger group of 47 randomly selected volunteers who underwent assessment of their insulin sensitivity. These subjects were divided into tertiles on the basis of their insulin sensitivity. Next, we randomly selected 6 subjects from each tertile for more extensive investigations. All subjects were healthy as judged by medical history, had a normal 75-g oral glucose tolerance test, and were normotensive as determined by triplicate office blood pressure measurement. They did not use medication, and all were nonsmokers. Subject characteristics are summarized in Table 1.

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Methods

Sensitivity to insulin-mediated glucose uptake was assessed by the hyperinsulinaemic, euglycaemic clamp technique, as described previously. Briefly, insulin (Velsulin; Novo Nordisk) was infused in a primed continuous manner at a rate of 50 mU kg⁻¹ hr⁻¹ for 2 hours. Normoglycaemia was maintained by adjusting the rate of a 20% mannitol solution in water for injection and expressed per unit of plasma insulin concentration (M/I). Plasma insulin concentrations were measured by radioimmunoassay techniques (Immunoradiometric Assay, Medigenix Diagnostics). For convenience, the M/I ratio was multiplied by 100.

Microvascular measurements were conducted in a quiet, temperature-controlled room (T=23.4±0.4°C) after 30 minutes of acclimatization, with the subjects in the sitting position and the investigator, nondominant (in all cases, left) hand at heart level. Nailfold capillary studies and iontophoresis studies were performed on the same day. Subjects were asked to refrain from caffeine, alcohol-containing beverages, and meals for 4 hours preceding the test. During the tests, skin temperature was monitored and all subjects were studied at the same time of the day. Nailfold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope (Zeiss), linked to a television camera (Philips LDH 07/20), a video recorder (Blautopf RTV-915, S-VHS) and a monitor (Philips LDH 2135/10). Incident illumination was achieved by light from a 50-W vapor mercury lamp, which passes through a heat-absorption and heat-reflection filter, a polarizer, and a 50% mirror to illuminate the object. To visualize the capillaries, a 3.2× objective (Zeiss 3.2/0.07) was used with a total system magnification of 99×. Two separate visual fields were recorded before and after 4 minutes of arterial occlusion, each for 60 seconds, and the images were stored on videotape. Capillaries were counted using the naked eye from a freeze-framed reproduction of the videotape and from the running videotape, when it was uncertain whether a capillary was present or not. Capillary density was defined as the number of erythrocyte-perfused capillaries per square millimeter of nailfold skin. Percentage capillary recruitment was assessed by dividing the increase in capillary density after 4 minutes of arterial occlusion by the baseline capillary density. No knowledge of the subject’s insulin sensitivity or blood pressure was available to the person counting the capillaries. The intraobserver and interobserver coefficients of variation of the counting procedure were 4.5% and 10.1%, respectively.

Endothelium-(in)dependent vasodilatation of finger skin microcirculation was evaluated by iontophoresis in combination with laser Doppler flowmetry. Laser Doppler flowmetry is a noninvasive method to measure skin perfusion. A laser beam penetrates the skin and a fraction of the light is backscattered by moving blood objects and undergoes a frequency shift according to the Doppler principle, generating a signal proportional to tissue perfusion. Laser Doppler flux was measured on the middle phalanx of the finger with the Periflux 4000 system (Perimed) and expressed as arbitrary perfusion units. Iontophoresis is a noninvasive method of drug application that allows the local transfer of charged substances across the skin by use of a small electric current. A battery-powered iontophoresis controller (Phoresor II, Iomed) was used to provide a direct current for drug iontophoresis. Acetylcholine (1%, Bolaven (1%, Bolaven) was delivered with an anodal current; 9 doses (0.1 mA for 20 seconds) were delivered, with a 60-second interval between each dose. A 60-second interval between each iontophoresis period was required to achieve the plateau of the response following each delivery of acetylcholine. Day-to-day reproducibility was 15.9%±8.4%, as determined in 5 subjects on 2 occasions. Sodium nitroprusside (0.01%, Nipride, Roche) was delivered with a cathodal current; 7 doses (0.2 mA for 20 seconds) were delivered, with a 180-second interval between each dose. A 180-second interval between each iontophoresis period was required to achieve the plateau of the response after each delivery of sodium nitroprusside. Day-to-day reproducibility was 13.9%±9.0%, as determined in 5 subjects on 2 occasions. The response to acetylcholine and sodium nitroprusside were expressed uncorrected for their respective vehicle responses but were corrected for the biological zeros.

Ambulatory monitoring (Spacelabs 90207) was used to obtain 24-hour recordings of blood pressure and heart rate. The nondominant arm was used with an appropriately sized cuff. The monitors were programmed to take blood pressure and heart rate readings every 15 minutes from 7 AM to 10 PM and every 20 minutes from 10 PM to 7 AM. The subjects completed an activity diary. The readings were downloaded onto a computer spreadsheet and individually edited into daytime and nighttime periods from the subjects’ diaries. Body mass index (BMI) and waist-to-hip ratio (WHR), as a measure of body fat distribution, were determined. WHR was calculated by dividing waist circumference by hip circumference.

Statistical Analysis

Data are expressed as mean±SD, unless stated otherwise. The paired Student’s t test was used to compare capillary densities before and after arterial occlusion. Wilcoxon rank-sum tests were used to compare vasodilatory responses before and after drug and vehicle administration. Subsequently, data were examined by use of Pearson’s correlation. A stepwise multiple regression analysis was used to analyze whether the observed associations between blood pressure and insulin sensitivity remained when allowing for microvascular function, WHR, and 24-hour heart rate. A 2-tailed P value of <0.05 was considered significant. All analyses were performed on a personal computer with the statistical software package SPSS version 6.0. Data of all 18 subjects were available for analysis.
**Results**

**Blood Pressure and Heart Rate**

Twenty-four-hour systolic blood pressure (SBP), mean arterial pressure (MAP), and diastolic blood pressure (DBP) averaged 118±7.4, 87±6.9, and 71±6.7 mm Hg. During daytime, SBP was 123±8.3, MAP 91±7.7, and DBP 76±7.3 mm Hg. During nighttime SBP, MAP, and DBP decreased to 107±7.7, 76±7.4, and 60±7.5 mm Hg, respectively. Twenty-four-hour heart rate averaged 71.7±8.4 bpm. During the daytime, heart rate was 74.7±9.0 bpm. During the nighttime, heart rate decreased to 58.5±15.9 bpm.

**Insulin Sensitivity**

Normoglycemia (4.5±0.4 mmol/L) was maintained during the insulin infusion. Attained serum-free insulin concentrations averaged 409±128 pmol/L. The rate of glucose uptake, expressed per kilogram of body weight, was 8.2±4.1 mg·kg⁻¹·min⁻¹.

**Microvascular Function**

After 4 minutes of arterial occlusion, capillary density increased from 38.3±5.9 to 53.8±8.4 capillaries/mm² (P<0.0001). Blood flow increased significantly from 19.1 (median, range 9.1 to 59.6) to 133.3 (median, range 54.3 to 263.9) arbitrary perfusion units (PU) after iontophoresis of acetylcholine (P<0.001). The mean percentage of acetylcholine-mediated vasodilatation was 683.4%±274%. Acetylcholine-vehicle (mannitol 3%) elicited a small but significant increase in blood flow from 14.7 (median, range 6.2 to 33.7) to 16.2 (median, range 9.4 to 44.6) PU (P<0.01); the percentage increase was 12.7% (median, range 0% to 131.4%). After iontophoresis of sodium nitroprusside (SNP), blood flow increased significantly from 19.8 (median, range 8.5 to 36.9) to 148.2 (median, range 56.2 to 211.6) PU (P<0.001). The mean percentage SNP-mediated vasodilatation was 768.8%±381%. SNP-vehicle (water for injection) also elicited a small but significant increase in blood flow from 15.4 (median, range 7.2 to 30.6) to 17.7 PU (median, range 9.7 to 72.8; P<0.01); the percentage increase was 26.6% (median, range 0% to 82.3%). Correction for vehicle response did not importantly affect any of the subsequent analyses (data not shown).

**Multiple Regression Analysis**

Table 2 shows the correlations among the main variables and demonstrates that insulin sensitivity, blood pressure, and estimates of microvascular function were significantly correlated. A reduction in microvascular vasodilator capacity was associated with an increase in insulin resistance and 24-hour SBP (Figure 1).

A leading theory postulates that blood pressure elevation is caused by insulin resistance and compensatory hyperinsulinemia.1 We wished to examine whether any such relation can instead be explained by microvascular function. Table 3 shows that statistically, this is indeed the case. Model 1 shows that insulin sensitivity (independent variable), as expected, was univariately related to blood pressure (dependent variable). Model 2 shows that the relation between insulin sensitivity and blood pressure was lost when microvascular function (ie, percentage capillary recruitment and percentage acetylcholine-mediated vasodilatation) was entered into the regression analysis. Next, we forced WHR into the analysis (model 3). The results were similar to those in model 2 in that insulin sensitivity was not significantly related to blood pressure. The relation between microvascular function and blood pressure was now borderline significant, but in fact the regression coefficients (β) were very similar to those in model 2, so that WHR did not confound the relation between microvascular dilator capacity and blood pressure. Despite significant univariate associations with insulin sensitivity (r=−0.56, P<0.05) and percentage capillary recruitment (r=−0.49, P<0.05), 24-hour heart rate, a measure of sympathetic tone, did not confound the relation between blood pressure, insulin sensitivity, and microvascular dilator capacity (model 4). To restrict the number of variables in models 3

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**Table 2. Pearson’s Correlation Analysis of Blood Pressure, Insulin Sensitivity, Microvascular Function, and Waist-to-Hip Ratio in 18 Healthy Subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin Sensitivity</th>
<th>Acetylcholine–Mediated Vasodilatation</th>
<th>% Capillary Recruitment</th>
<th>WHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-Hour SBP</td>
<td>−0.50*</td>
<td>−0.34, NS</td>
<td>−0.53*</td>
<td>+0.66†</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td></td>
<td>+0.78†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Acetylcholine-mediated vasodilatation</td>
<td>+0.76†</td>
<td>−0.58*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Capillary recruitment</td>
<td></td>
<td>−0.64†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05, †P<0.01, ‡P<0.001, NS (P=0.165).
and 4, age, sex, and BMI were not entered. However, univariate analyses showed that these variables were not significantly associated with blood pressure, insulin sensitivity, or microvascular function, and entering them in the multivariate model did not affect the conclusions (analysis not shown). In sum, the regression analyses shown in Table 3 were consistent with the idea that the association of insulin sensitivity with blood pressure can be explained by altered microvascular function.

Our hypothesis in addition stipulated that microvascular function might to some extent determine insulin sensitivity. Therefore, we next examined whether microvascular function (independent variable) was associated with insulin sensitivity (dependent variable). Table 4 shows that capillary recruitment and acetylcholine-mediated vasodilatation were strongly and positively related to insulin sensitivity, and capillary recruitment was inversely related to blood pressure. This is the first study to report on such relations within 1 group of humans, and therefore to assess the possible role of microvascular function in linking insulin sensitivity to blood pressure.

**Discussion**

The results of the present studies in healthy subjects demonstrate, as expected, an inverse relation between blood pressure and insulin sensitivity. An important new finding was that a physiological association exists between skin microvascular function on the one hand and insulin sensitivity and blood pressure on the other. Capillary recruitment and acetylcholine-mediated vasodilatation were strongly and positively related to insulin sensitivity, and capillary recruitment was inversely related to blood pressure. This is the first study to report on such relations within 1 group of humans, and therefore to assess the possible role of microvascular function in linking insulin sensitivity to blood pressure.
On the basis of these findings, we suggest that perturbed microvascular vasodilator capacity needs to be considered as an explanation for the relation between insulin resistance and elevated blood pressure. Figure 2 shows how reduced microvascular vasodilator capacity may link insulin resistance and elevated blood pressure, both of which are associated with increased cardiovascular risk. Recent experimental data support the potential contribution of a reduction of microvascular vasodilator capacity to an increase of tissue vascular resistance in hypertension. Perturbed capillary recruitment may decrease insulin sensitivity by several mechanisms: first, by increasing diffusion distance of insulin and glucose to glucose-metabolizing tissues; second, by impairing trans-endothelial insulin transport, a major determinant of insulin action during hyperinsulinemic clamps, if this should be surface-area dependent; third, by impairing recruitment of previously underperfused muscle tissue. In support, studies with the use of the perfused rat hind limb have shown that skeletal muscle metabolism (ie, oxygen uptake and insulin-mediated glucose uptake) can be increased by increased capillary perfusion, even if total flow to the muscle remains constant.

Our findings give considerable support to the role of microvascular function in linking insulin resistance to elevated blood pressure. The data suggest that there is no direct, independent association between insulin sensitivity and blood pressure, and that this association could in large part be explained by microvascular vasodilator capacity, in particular percentage capillary recruitment. It is important to emphasize that these observations were not confounded by differences in sex or age and pertained in the absence of hypertension and impaired glucose tolerance. Consistent with our hypothesis is the recent finding of impaired microvascular vasodilatation and capillary rarefaction in young adult men with a familial predisposition to high blood pressure. In addition, it has previously been shown that normotensive offspring of hypertensive parents exhibit insulin resistance and hyperinsulinemia compared with an appropriate control group.

WHR, as a measure of body fat distribution, showed strong associations with insulin sensitivity, blood pressure, and the measures of microvascular function. However, WHR did not confound the association between microvascular function and insulin sensitivity or blood pressure. Abdominal fat may secrete substances, such as free fatty acids and cytokines, which influence microvascular function. Free fatty acids have been shown to increase vasoconstrictor responses in dorsal hand veins and to impair endothelium-dependent vasodilatation at the level of the resistance vessels. In addition, proinflammatory cytokines have been proposed to link obesity to endothelial dysfunction. Alternatively, WHR may be a marker of a certain type of body composition that is itself linked to microvascular dysfunction. Associations between body fat distribution and muscle fiber type have been reported, with obesity and high WHR linked to fast-twitch predominance. WHR was even more closely related to muscle capillary density. Interestingly, in the same study insulin sensitivity showed a positive relation with capillary density and an inverse relation with the percentage of fast-twitch muscle fibers. In another small study, the percentage slow-twitch muscle fibers and capillary density have been found to correlate inversely with intra-arterial blood pressure and peripheral resistance in untreated hypertensive and normotensive subjects. Taken together, these studies are consistent with our findings and the contention that muscle fiber type and muscle capillary density play a role in linking insulin sensitivity with blood pressure. Muscle fiber composition and, thereby, capillary density, as well as insulin sensitivity may be negatively influenced by sustained sympathetic activation. Furthermore, increased sympathetic nervous activation, as indexed by elevated heart rate, has been proposed to link hypertension with insulin resistance. In our study, however, 24-hour heart rate did not confound the associations between blood pressure, insulin sensitivity, and microvascular function. Nevertheless, it is important to emphasize that because of the cross-sectional nature of our study, we cannot exclude the possibility that the demonstrated associations can all be explained by an as yet unmeasured variable.

Because muscle is the main peripheral site of insulin action and of pressure dissipation, and therefore of vascular resistance, it might have been more straightforward to study muscle rather than skin microvascular function. However, the study of muscle capillary density, in contrast to skin capillary density, requires invasive techniques, and capillary recruitment cannot be studied. Furthermore, although skin is not a primary target organ of the insulin-mediated glucose uptake, the vascular effects of insulin can also be demonstrated at this site. Insulin has been demonstrated to induce vasodilatation in skin microcirculation independent of its hypoglycemic effect. Moreover, skin microvascular vasodilator capacity is associated with insulin sensitivity in subjects with fasting hyperglycemia. Likewise, although skin microvascular resistance does not make a major contribution to the total peripheral vascular resistance, an association between skin capillary density and blood pressure in normotensive and hypertensive subjects can nevertheless be demonstrated. Moreover, in subjects with hypertension, changes in skeletal muscle microvascular function are paralleled by changes in skin microvascular function. Therefore, skin microcirculation resembles muscle microcirculation in many ways, and the variation in skin microvascular function found in this...
study may merely be a manifestation of a generalized variation in microvascular function not confined to a single organ. Although direct comparisons between skin and muscle microvascular function have yet to be reported, it seems justified to use the skin microvascular model to assess the role of microvascular function in the relation between insulin sensitivity and blood pressure.

It may not be obvious that acetylcholine, applied topically through iontophoresis instead of intravasally, evokes an endothelium-dependent vasodilatation. In a recent study, however, the local vasodilatation caused by intradermally injected acetylcholine (into the extravascular space) was blocked by L-NMMA, a competitive inhibitor of nitric oxide synthesis. Also, this local vasodilatation is not dependent on prostaglandin production and functioning nociceptive C-fibers. Moreover, the vasodilator response to iontophotically applied acetylcholine is reduced in patients with non–insulin-dependent diabetes and hypertension, and in these patients impaired endothelial vasomotor function has been demonstrated with other techniques. Therefore we and others find it possible that the effect of acetylcholine is chiefly endothelium dependent.

Our study subjects were recruited to show substantial variability in their insulin sensitivity, which resulted in an approximately 8-fold variation. Insulin sensitivity was expressed as M/I ratio, which is a measure of the quantity of glucose metabolized per plasma insulin concentration. By correcting for differences in steady-state plasma levels, the M/I value is a better index than the M value for comparing changes or differences in tissue sensitivity to insulin. The larger variation in insulin sensitivity and the use of the M/I value in the present study may explain the differences with a recent study of Petrie et al, in which a dissociation between insulin sensitivity of glucose uptake and endothelial dysfunction in normal subjects was demonstrated. In summary, there is a continuous association between insulin sensitivity and blood pressure well within the physiological range. Microvascular function strongly relates to both, consistent with a possible central role in linking these variables. These findings may have important implications with respect to the high prevalence of hypertension and cardiovascular disease in insulin resistance states.

Acknowledgments

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