Microvascular correlates of blood pressure, plasma glucose, and insulin resistance in health

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Abstract

Objectives: The associations between hypertension, insulin resistance and glucose intolerance are poorly understood. Altered microvascular structure and function could contribute by increasing peripheral vascular resistance and decreasing tissue delivery of glucose. We addressed this hypothesis in a sample of healthy men. Methods: We studied 105 healthy young men aged 23–33 years. Insulin resistance was calculated using the Homeostasis Model Assessment (HOMA). Video capillaroscopy was used on the dorsum of the finger to measure skin capillary density, and in nailfold capillaries to measure capillary blood velocity. Skin vasodilatation was measured with laser Doppler fluximetry on the forearm following heating and iontophoresis of acetylcholine. Results: Higher systolic blood pressure was associated with insulin resistance ($r = 0.31$, $P < 0.005$), lower dermal capillary density ($r = -0.25$, $P < 0.05$), and impaired maximum dermal blood flow after heating ($r = -0.26$, $P < 0.01$), but not with capillary blood velocity ($r = 0.07$) or dilator responses to acetylcholine ($r = 0.09$). Insulin resistance did not correlate with indices of microvascular structure or function (all $r < 0.15$). However, higher fasting plasma glucose was associated with lower capillary density ($r = -0.27$, $P < 0.01$), and increased capillary blood velocity ($r = -0.30$, $P < 0.05$). Conclusions: The association between hypertension and insulin resistance is unlikely to be explained by altered microvascular structure and function. However, changes in the microvasculature are found in subjects with early and subtle elevations in blood pressure or fasting plasma glucose in advance of their crossing conventional thresholds for the diagnosis of hypertension or diabetes mellitus. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Small vessels (<100 $\mu$m luminal diameter) contribute most to vascular resistance [1] and thus are the likely site of any hemodynamically important disturbance of vascular structure and function. Hypertension is characterised by increased peripheral vascular resistance, and microvascular abnormalities [2–4] which may precede the development of hypertension: healthy young men at increased risk of developing hypertension show dermal capillary rarefaction and impaired skin hyperaemic response to local heating [5], and impaired vascular responses to the endothelium-dependent vasodilator acetylcholine [6]. Such changes in microvascular structure and function may also contribute to the association of hypertension with insulin resistance and glucose intolerance [7,8].

It has been postulated, although not widely accepted, that altered microvascular structure could contribute to insulin resistance, by limiting the rate of glucose delivery and therefore insulin-mediated glucose uptake [9]. Moreover, differences in microvascular function may also be important; glucose uptake may be enhanced by insulin-mediated vasodilatation, which is largely dependent upon nitric oxide synthesis [10]. Endothelial dysfunction may therefore contribute both to insulin resistance and hypertension. Furthermore, in normotensive subjects with
fasting hyperglycemia (in the non-diabetic range), raised fasting insulin levels are associated with impairment of the skin maximum hyperaemic response [11].

Against this background, relationships between insulin sensitivity or blood pressure and dermal microvascular structure or endothelial function have recently been observed in healthy volunteers [12]. To eliminate the possible confounding effects of age and sex in these findings, and to assess in addition their contribution to variance in plasma glucose concentrations, the present study examined the hypothesis that alterations in microvascular structure and function contribute to the range of blood pressure, insulin resistance and fasting plasma glucose concentrations in a large group of healthy young men.

2. Methods

2.1. Participants

These studies were approved by our local Research Ethics Committee and written informed consent was obtained. The investigation conformed with the principles outlined in the Declaration of Helsinki. Subjects were selected from a cohort which has been described elsewhere [13,14]. In brief, blood pressure was measured in 603 married couples in 1979 and in 864 of their offspring, then aged 16–24 years, in 1986. Age-adjusted Z-scores were used to define tertiles (i.e. cut-offs which divide the population distribution into equal thirds) for both offspring and mean parental blood pressures. Offspring for whom both their own blood pressure and the mean blood pressure of their parents were outwith the middle tertile were identified as belonging to one of ‘four corners’. Subgroups of offspring randomly selected from these corners have participated in further investigations [5,14] which have identified correlates of the inherited predisposition to high blood pressure. In 1992–94, we studied 105 Caucasian male offspring drawn at random from the four corners, according to the following protocol.

2.2. Protocol

After an overnight fast from 22:00 h, and abstention from proprietary drugs including aspirin for 10 days, subjects attended the Clinical Research Centre at 09:00 h. Height and weight were recorded before subjects rested supine and acclimatised to a controlled environmental temperature of 23–24°C. After 20 min, a 30-ml venous blood sample was obtained and recordings of blood pressure were made four times at 5-min intervals using a validated [15], semi-automated machine (Takeda UA 751 sphygmomanometer; Takeda Medical Inc., Tokyo, Japan). The means of the last three recordings were used in subsequent analyses. After 40 min, the following observations were made in sequence: (i) in the right arm, maximum vasodilatation of skin microvessels was measured by laser Doppler fluximetry in response to local heating to 42°C; (ii) in the left arm, nailfold capillary blood velocity and capillary numbers on the dorsum of the ring finger were measured by intravital videomicroscopy both before and during venous occlusion with a digital cuff (Peni-Cuff, Hokanson, Washington, USA) inflated to 40 mmHg for 10 min in order to expose non-perfused capillaries; (iii) in the right arm, forearm blood flow was measured before and after 12 min of ischemia applied using a sphygmomanometer at supra-systolic pressure, using strain gauge plethysmography; (iv) in the left arm, dermal vasodilatation was measured by laser Doppler fluximetry in response to transdermal delivery of acetylcholine by iontophoresis.

Further details of measurements in these subjects have been published previously [5], except for iontophoresis and assessment of insulin sensitivity. Iontophoresis was performed as previously validated [16]. Briefly, acetylcholine (Sigma, Poole, Dorset, UK) was prepared in 2% methylcellulose gel at a final concentration of 2 g/100 ml. Acetylcholine or vehicle (0.5 ml) were injected in random order into an iontophoresis chamber (Moor Instruments Ltd., UK) positioned on the volar aspect of the left forearm and flux was measured continuously by laser Doppler. Currents of increasing duration and intensity (100 μA for 10 s; 200 μA for 10 s; 200 μA for 20 s; and 200 μA for 40 s) were applied to deliver charges of 1, 2, 4, and 8 mC. Response periods were allowed after each charge (60 s for 1 and 2 mC, 90 s for 4 mC, and 120 s for 8 mC) which were sufficient for the response to plateau consistently. The iontophoresis chamber was then removed and cleaned before the procedure was repeated on a neighbouring site with the next solution (drug or vehicle). Mean flux, measured at the plateau of the response for each charge, was expressed in arbitrary flux units. We calculated maximum % increase in flux and area under the curve, and used both as summary statistics. However, there was no difference between these measures for any relationship with iontophoretic response, and maximum % changes in flux were used in regression analyses. In preliminary experiments, laser Doppler ‘biological zero’ values were recorded during ischemia, but flux values were negligible in relation to the vasodilatation to iontophoresed drugs so we did not adjust for biological zeros in this study.

2.3. Laboratory methods

Plasma was stored at −80°C until analysis. Insulin was measured by radioimmunoassay [17]. The assay (Eurogenetics Tasah, UK) has ≤3% cross-reactivity with proinsulin. Glucose was measured by autoanalyser. Insulin resistance was estimated using the Homeostasis Model Assessment (HOMA) [18].
2.4. Statistics

Comparison between subjects from the four corners was performed by ANOVA. Fasting plasma insulin, HOMA resistance index and maximal forearm blood flow were log-transformed to normalise their distribution. Other variables were normally distributed. Correlations in the whole sample were identified by linear regression. Multiple regression analysis was used to identify confounding factors between interrelated variables.

3. Results

3.1. Summary of measurements and comparison between four corners

Characteristics of subjects were: age (28.7±2.5, 23–33 years; mean±S.D., range); body mass index (BMI) (24.3±3.0, 18.6–36.8 kg/m²); systolic BP (119±10, 93–154 mmHg); diastolic BP (69±7, 51–98 mmHg); maximal forearm blood flow (39.3±12.7, 12.8–71.1 ml/100 ml per min); fasting plasma glucose (4.9±0.5, 3.0–6.5 mmol/l); and fasting plasma insulin (5.3±4.5, 1.1–29.6 mU/l). As expected, no participants had levels of blood pressure or glucose above cut-offs for the clinical diagnosis of hypertension or diabetes mellitus in this group, who were drawn from the upper and lower tertile of blood pressure distribution and therefore represent a random sample of 67% of the population.

Comparison of measurements of microvascular structure in these members of the four corners has been published previously [5]. Fasting plasma glucose was not different between corners (ANOVA P=0.24), but there were trends for higher HOMA insulin resistance index amongst subjects with higher blood pressure (ANOVA P=0.09), as previously described in a different sample of men and women from this cohort [14].

Iontophoresis of acetylcholine caused dose-dependent vasodilatation. There was no significant dilatation with vehicle (Fig. 1). There was no difference in response to acetylcholine between the four corners (ANOVA P=0.40).

3.2. Relationships with blood pressure

Higher systolic blood pressure was associated with higher fasting plasma glucose (r=0.21, P<0.05), insulin resistance (log fasting plasma insulin r=0.32, P=0.001; log HOMA resistance index r=0.31, P<0.005), and higher body mass index (r=0.25, P<0.01). Higher systolic blood pressure was also associated with fewer dermal capillaries at baseline and during venous occlusion, and reduced maximal flow in dermal vessels (Table 1). However, systolic blood pressure did not correlate with maximal forearm blood flow following ischaemia, dermal vascular vasodilatation to acetylcholine or capillary blood velocity. Diastolic blood pressure correlated similarly with insulin resistance (log HOMA r=0.21, P<0.05; log plasma insulin r=0.21, P<0.05) and with skin hyperemia, but relationships with other measurements of microvascular structure or function were not as strong as for systolic blood pressure (Table 1).

3.3. Relationships with insulin sensitivity

In addition to the relationship with blood pressure described above, greater insulin resistance was associated with higher body mass index (r=0.53, P<0.001). However, there were no relationships between insulin resistance and indices of dermal microvascular structure (Table 1) or vasodilator response to acetylcholine (Fig. 2).

3.4. Relationships with fasting plasma glucose

Higher fasting plasma glucose was associated with fewer dermal capillaries at baseline and during venous occlusion, and with increased capillary blood velocity (Table 1). These effects were independent of systolic blood pressure in multiple regression analyses (after adjustment for effect of systolic blood pressure, r=−0.24, P<0.05 for plasma glucose vs. capillary density at baseline, r=−0.21, P<0.05 for plasma glucose vs. capillary density after venous occlusion, and r=0.29, P<0.01 for plasma glucose vs. capillary blood velocity).
Table 1

Correlations (r-values) of microvascular structure and function with blood pressure, fasting plasma glucose concentrations, and insulin sensitivity

<table>
<thead>
<tr>
<th></th>
<th>Mean (S.D.)</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
<th>BMI</th>
<th>Fasting plasma glucose</th>
<th>Log fasting plasma insulin</th>
<th>Log HOMA resistance index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log maximal forearm blood flow (ml/min)</td>
<td>5.28 (0.41)</td>
<td>-0.11</td>
<td>-0.07</td>
<td>-0.15</td>
<td>0.04</td>
<td>-0.13</td>
<td>-0.12</td>
</tr>
<tr>
<td>Maximal heated dermal blood flow (arbitrary flux units)</td>
<td>525 (211)</td>
<td>-0.27**</td>
<td>-0.31**</td>
<td>-0.09</td>
<td>0.03</td>
<td>-0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Capillary blood velocity (mm/s)</td>
<td>1.22 (0.65)</td>
<td>0.07</td>
<td>0.06</td>
<td>0.16</td>
<td>0.30*</td>
<td>0.11</td>
<td>0.15</td>
</tr>
<tr>
<td>Capillary density before venous occlusion (per 0.25 mm²)</td>
<td>23.6 (4.0)</td>
<td>-0.21*</td>
<td>-0.11</td>
<td>-0.14</td>
<td>-0.26**</td>
<td>-0.07</td>
<td>-0.11</td>
</tr>
<tr>
<td>Capillary density after venous occlusion (per 0.25 mm²)</td>
<td>25.3 (4.2)</td>
<td>-0.25*</td>
<td>-0.15</td>
<td>-0.15</td>
<td>-0.24*</td>
<td>-0.05</td>
<td>-0.07</td>
</tr>
<tr>
<td>Capillary recruitment (% change after venous occlusion)</td>
<td>7.7 (6.2)</td>
<td>-0.11</td>
<td>-0.13</td>
<td>-0.03</td>
<td>0.08</td>
<td>0.07</td>
<td>0.13</td>
</tr>
<tr>
<td>Dermal vasodilatation to acetylcholine (maximum % increase in flux)</td>
<td>596 (410)</td>
<td>0.09</td>
<td>0.11</td>
<td>-0.03</td>
<td>0.06</td>
<td>0.00</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01.

4. Discussion

It is intellectually attractive to link the common, associated conditions of high blood pressure, insulin resistance, and type 2 diabetes through shared abnormalities of microvascular structure and function causing, on the one hand, increased peripheral vascular resistance and high blood pressure, and on the other, impaired tissue delivery of glucose and insulin resistance. We have confirmed the associations between insulin resistance, higher blood pressure, and fasting hyperglycaemia in our cohort of 105 healthy young men. However, we have found that only higher blood pressure and fasting hyperglycaemia, and not insulin resistance, are associated with altered dermal microvascular structure. Moreover, we have not shown any association between dermal endothelial function and blood pressure, plasma glucose concentrations, or insulin resistance.

The reduction in capillary number that we have observed in men with higher blood pressure is consistent with the theory first proposed by Folkow in 1958 [19] that structural distortions in vascular networks maintain increased peripheral vascular resistance. These relationships are discussed in detail in our previous paper [5]. Here, we report a novel observation that higher fasting plasma glucose in healthy men is also associated with capillary rarefaction. This is unlikely to explain the impaired maximal dermal vasodilatation either in this or our previous study of a group with fasting hyperglycaemia [11] since capillary number and maximal dermal vasodilatation were not correlated. The differences in maximal flow may reflect altered structure or function elsewhere in the vascular bed. However, we have found evidence suggesting that rarefaction is functionally important in capillaries, since higher fasting plasma glucose was associated with increased capillary blood velocity.

The lack of relationship between indices of microvascular structure and insulin resistance contrasts with a previous study of normotensive older adults with elevated fasting glucose in the non-diabetic range in whom maximum skin blood flow to local heating was related to insulin resistance [11]. Perhaps in the presence of hyperglycaemia there may be a significant interaction between insulin resistance and vascular structure; whereas in our group, who were not selected for fasting hyperglycaemia, interactions between blood pressure and plasma glucose may be more important than insulin resistance as predictors of structural alterations in microvessels. Clearly, these negative results in the dermal circulation do not exclude the possibility that there are differences in capillary density in other beds, such as skeletal muscle, which are important in insulin resistance.

We used transdermal iontophoresis of acetylcholine to
assess endothelial function, but did not find relationships with blood pressure, insulin sensitivity or fasting plasma glucose. Previous descriptions of endothelial dysfunction in hypertension have examined either large arteries or the forearm muscle bed where nitric oxide is the major mediator of acetylcholine-induced vasodilatation [20], and impaired vasodilatation has been attributed to impaired nitric oxide synthesis. One explanation for the lack of relationship between blood pressure and iontophoresic response in this study is that iontophoresis of acetylcholine to dermal vessels does not assess purely nitric oxide-dependent vasodilatation, since it is not inhibited by the nitric oxide synthase inhibitor L-NMMA [16]. Other endothelium-dependent dilator mechanisms, which may not be deranged in hypertension, may be more important in dermal microvessels [11,16]. Alternatively, the relationship between blood pressure and endothelial function may not be continuous: our group did not include subjects with a clinical definition of hypertension; and endothelial dysfunction has not been a consistent finding in hypertensive patients [21]. Similarly, endothelial dysfunction has been observed in patients with fasting hyperglycaemia or diabetes mellitus [22–24], but our findings suggest that it is not related to plasma glucose in young healthy men at lower levels of plasma glucose. Our findings are consistent with those of Petrie et al., who failed to show a relationship between the forearm blood flow response to acetylcholine and insulin resistance in healthy men [25].

The lack of relationship between markers of insulin resistance and dermal microvascular function in our cohort contrasts with a previous study which showed striking associations between blood pressure or insulin resistance and both impaired dermal acetylcholine-mediated vasodilatation and impaired recruitment of nailfold capillaries following arterial occlusion [12]. Although the previous report had the potential advantage of measuring insulin sensitivity by euglycaemic hyperinsulinaemic clamp, this is closely correlated with HOMA resistance index [18]. Relationships with capillary density, and the possible confounding influence of fasting plasma glucose, were not reported in the previous paper, which was limited to just 18 participants with a wide age range (20–64 years) including both men and women. In the present study, there was a wide range of insulin sensitivity and obesity but potential confounding effects of age and sex were eliminated and plasma glucose was measured and corrected for. Whilst in the previous study, a 100% decrease in insulin sensitivity was associated with a ~250% decrease in response to iontophoresis of acetylcholine, in our study a 100% decrease in insulin sensitivity would be associated with only a 4–12% (95% confidence limits of slope) decrease in response to iontophoresis of acetylcholine. Given the greater statistical power of our study, and the absence of potential confounding effects of gender and age, it seems likely that the estimate of the relationship in the current study is more accurate. Whether functional differences in the microcirculation are well represented by recruitment of capillaries following arterial occlusion is also uncertain. Antonios et al. [4] found that capillary density decreased during reactive hyperaemia following 5 min of arterial occlusion (111–104/mm²) and that venous occlusion was the most effective way of demonstrating maximal numbers of capillaries [26]. We used the latter index.

In summary, this large study of healthy young men suggests that the association between hypertension and insulin resistance is unlikely to be explained by altered microvascular structure and function. However, changes in the microvasculature are found in subjects with early and subtle elevations in blood pressure or fasting plasma glucose in advance of their crossing conventional thresholds for the diagnosis of hypertension or diabetes mellitus. The early appearance of these abnormalities suggests that they could be important in the pathophysiology.

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References


