Haemoglobin and flow-mediated vasodilation

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ABSTRACT

A low [Hb] (Hb concentration) is out-balanced by peripheral vasodilation via mechanisms that are incompletely understood. Peripheral vasodilation is influenced by NO (nitric oxide) released from vascular endothelium in response to increased vessel wall shear stress, and absorption by Hb is the main mechanism by which the bioactivity of NO is disarmed. Thus we propose that graded NO absorption is the mechanism through which a low [Hb] is related to peripheral vasodilation. In the present study, we examined the relationship between [Hb] and FMD (flow-mediated vasodilation; 5 min of cuff ischaemia) of the radial and brachial arteries in 33 normal subjects and in 13 patients with Type II diabetes, known to have impaired NO-mediated vasodilation. The smaller radial artery provided the more sensitive test, as it had a 2-fold larger FMD than the brachial artery (22 ± 18% compared with 9 ± 18% respectively, in normal subjects; means ± S.D., P < 0.05). FMD of the radial artery had a negative correlation with [Hb] (r² = −0.66, P < 0.05; n = 27). In subjects with [Hb] below and above the median of 14.1 g/dl, the radial artery FMD was 30 ± 22% compared with 13 ± 12% respectively (P < 0.05). In diabetic patients, FMD was lower and a co-variation with [Hb] could not be established. Thus, in normal subjects, NO-mediated endothelium-related vasodilation at least partly out-balanced the ‘added burden’ of a low [Hb] during post-ischaemic reperfusion.

INTRODUCTION

A low [Hb] (Hb concentration) is out-balanced by peripheral vasodilation through mechanisms that are incompletely understood, but which may be related to NO (nitric oxide)-mediated vasodilation. The vasodilatory molecule NO is released by vascular endothelium in response to increased vessel wall shear stress, and absorption by Hb is the main mechanism by which the bioactivity of NO is disarmed [1]. Three reactions between Hb and NO have been considered important: (i) oxidation, in which NO reacts with HbO₂ to yield met-Hb and nitrate; (ii) the reaction in which NO reacts with Fe(II)-Hb to give NO-Fe(II)-Hb, and (iii) S-nitrosylation, yielding SNO-Hb [2]. The latter mechanism is influenced by the allosteric structural transitions that take place in Hb with changes in oxygen tension. A high oxygen tension favours S-nitrosylation of Hb and a low oxygen tension favours NO release, thereby suggesting a mechanism by which vasodilation may take place in ischaemic tissues [3,4]. NO scavenging is considered so complete that Hb in normal physiological concentrations is thought to take up nearly all NO thereby providing background for an ‘on-off’ action of NO. However, findings from chronically anaemic patients before and after transfusion of blood suggest that NO scavenging by Hb may be graded [5–7]. Thus [Hb] may influence vasodilation not only by providing an ‘on-off’ NO reaction and by ferrying NO from oxygenated to ischaemic tissues, but also indirectly through graded scavenging of NO.

It is not known if graded NO scavenging by Hb is a mechanism out-balancing variations in [Hb] within a
normal physiological range of concentrations, or if the mechanism is operational in normal subjects. A design in which [Hb] is changed, for example, by venesection, is appealing, but is associated with a number of neuro-hormonal changes; consequently, we initially chose to study the co-variation of [Hb] and endothelium-dependent vasodilation in healthy subjects and, as a means of internal validation of the technique, also in Type II diabetic patients with impaired endothelium-related vasodilation [8]. Reperfusion after cuff occlusion produces vasodilation in the conduit artery serving the hyperaemic tissue, the dilation mediated by NO produced by the vascular endothelial NOS (NO synthase) in response to the increased flow and vessel wall shear stress [1,9] and accounting for approx. 20% of the total flow increase during post-ischaemic reperfusion [10]. For facilitated ultrasound examination, the FMD (flow-mediated dilation) method has largely been validated on the larger conduit arteries, such as the brachial and femoral arteries [11,12]. However, model calculations suggest that the concentration of NO that reaches the vessel wall depends upon the diameter of the vessel [13], and studies on smaller arteries report larger relative changes in diameter than studies on larger arteries [11,14,15]. Hence, in addition to FMD of the brachial artery (luminal diameter of 3–5 mm), we studied FMD of the radial artery (1–3 mm). In normal subjects, we found the radial artery NO-mediated vasodilation to co-variate with [Hb].

**METHODS**

**Patients**

After informed consent was obtained, we studied 33 healthy subjects (12 female) and 13 Type II diabetic patients (three female) with diagnosed diabetes for 3.3 ± 2.3 years (mean ± S.D.). Patients were either diet-controlled or received biguanides or thiazolidinediones. The Oxford Ethics Committee approved all studies. The investigation conformed with the principles outlined in the Declaration of Helsinki. In diabetic patients, FMD is assessed for 30 min. Subjects were placed supine with their right lower arm in a custom-made support. The brachial artery was located with ultrasound at the medial side of the upper arm approx. 10 cm above the insertion of the distal brachial biceps muscle tendon. The radial artery was examined approx. 5 cm proximal to the head of the radial bone. The final measuring points were chosen as a > 1-cm-long non-tortuous segment of the respective arteries.

In random order and separated by a resting period of > 30 min, we studied both radial and brachial artery endothelium-related vasodilation in response to reperfusion after 5 min of ischaemia induced by supra-systolic cuff occlusion. For the radial artery study, the cuff was placed over the upper arm; and for the brachial artery study, the cuff was placed over the upper part of the lower arm. The cuff was inflated to 250 mmHg for 5 min and then released, resulting in an episode of reactive hyperaemia. Extending the ischaemic period beyond 5 min does not result in substantially higher increases in flow or artery diameter [18] and, in our experience, unduly increases the risk of the subject moving. Artery diameter changes were assessed for 120 s after deflation of the blood pressure cuff [12]. As seen in response to sublingual glyceryl trinitrate, both normal subjects and patients with diabetes mellitus have been shown repeatedly to have a normal non-endothelium-dependent vasodilation [8], so it was not measured.

Artery diameter was determined with duplex ultrasound with a 7.5 MHz linear phase-array transducer (Hewlett Packard SONOS 5500). The artery was studied in the ‘B-mode’ in a longitudinal scan corresponding to the largest diameter. An external fixation arm secured the position of the probe. Scans were stored on video tapes for off-line analysis. With correction for angle, Vmean (mean artery blood velocity) was determined by Doppler before cuff occlusion and after 60 s of reperfusion (in each case the average of three determinations was used for statistical analysis). As a reflection of lower arm muscle blood flow and oxygenation, during the radial artery study in the healthy subjects, Smo2 (muscle oxygen saturation) of the lower arm flexor muscle group was determined by near-infrared spectrophotometry (Somanetics 4100 [20]).

**Biochemical analysis**

In patients with diabetes and normal controls, fasting blood was taken to determine [Hb], HbC (haematocrit), glucose and HbA1c (glycosylated Hb) levels, lipid profiles and non-esterified fatty acid (‘free-fatty acid’ levels; NEFA C enzyme assay; Wako Chemicals). Systolic and diastolic blood pressures were measured by sphygmanometry after 10 min of supine rest.

**FMD**

Mental stress may blunt the FMD response [17] and, following blood sampling, subjects were allowed to rest

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between media and adventitia;[21]), corresponding to the last diastolic picture as determined by colour Doppler. Colour Doppler was chosen rather than the conventional use of ECG to better estimate the occurrence of diastole at the level of the vessel, since systolic and diastolic artery diameters may vary by approx. 0.3 mm (results not shown). Vessel walls were examined immediately outside the colour Doppler flow field to avoid visual interference. For the first 60 s, vessel diameter was determined for each heart beat. After 90 and 120 s respectively, additional scans were examined in order to ascertain that the dilatation was diminishing and that the maximal dilatation had thus been captured. To minimize the influence of transient variations in heart rate (e.g. extra-systoles), the resting value was taken as the mean and S.D. of the last ten heart beats before cuff inflation, and the value at maximal vasodilatation was taken as the mean of the five heart beats surrounding the largest measured artery diameter. To allow for comparisons, the flow-mediated increase in luminal diameter was reported as a percentage of the diameter at rest. Following normality testing, comparisons were made with Student’s t test for paired and unpaired samples respectively, but co-variation was quantified using Spearman’s ρ correlation, since FMD values and FMDs were not normally distributed. A two-sided P value < 0.05 was considered significant. Results are means ± S.D.

**RESULTS**

Six control subjects declined giving a blood sample, and off-line analysis of the artery diameter data revealed movement during the test in one subject during the radial study and in three subjects during the brachial study. Thus, for the radial artery, co-variation of [Hb] and FMD data were analysed in 27 normal subjects and 13 diabetic patients and, for the brachial artery, co-variation was analysed in 23 normal subjects and 11 diabetic patients. In comparison, the diabetic patients were older than the normal subjects and had higher BMI (body mass index), blood sugar and HbA1c (Table 1).

Following 60 s of reperfusion, radial and brachial artery vmean had increased to a similar extent in normal controls and in diabetic subjects (Table 2), and neither resting vmean nor changes in vmean were related to [Hb]. In the radial artery study, 5 min of ischaemia induced SmO2 in the lower arm flexor group to decrease from 70 ± 6 to 28 ± 12 %. Following 60 s of reperfusion, SmO2 increased to 89 ± 2 %. Neither resting SmO2 nor changes in SmO2 during reperfusion were related to [Hb].

Examples of brachial and radial artery diameter data sets are shown in Figure 1. During reperfusion in normal subjects, the radial artery luminal diameter increased from 2.04 ± 0.57 to 2.44 ± 0.57 mm (22 ± 18 %; Figure 2) and decreased to 2.26 ± 0.61 mm after 90 s. The brachial artery luminal diameter increased from 3.78 ± 0.88 to 4.09 ± 0.83 mm (9 ± 7 %; Figure 2), and decreased to 3.85 ± 0.81 mm after 90 s. In both the radial (r² = 0.36) and the brachial (r² = 0.32) artery, FMD correlated with the resting artery luminal diameter (Figure 3), but the relationship between FMD and luminal diameter was steeper for the radial than for the brachial artery (α of linear regression lines, −19.0 for the radial artery compared with −4.5 for the brachial). Radial and brachial FMDs correlated with each other (r² = 0.30).

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**Table 1** Demographics, systolic and diastolic blood pressures and heart rate of 33 normal subjects and 13 patients with Type II diabetes mellitus.

<table>
<thead>
<tr>
<th></th>
<th>Normal subjects</th>
<th>Diabetic patients</th>
</tr>
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<tbody>
<tr>
<td>Age (years)</td>
<td>49 ± 11</td>
<td>60 ± 9*</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.75 ± 0.09</td>
<td>1.73 ± 0.07</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.8 ± 9.3</td>
<td>92.1 ± 14.7*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126 ± 19</td>
<td>133 ± 14</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76 ± 8</td>
<td>74 ± 9</td>
</tr>
<tr>
<td>Resting heart rate (beats/min)</td>
<td>70 ± 19</td>
<td>69 ± 9</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.06 ± 0.43</td>
<td>9.48 ± 2.89***</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.9 ± 1.37</td>
<td>13.9 ± 1.15</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>6.59 ± 0.52</td>
<td>8.68 ± 1.38**</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mEq/l)</td>
<td>0.40 ± 0.19</td>
<td>0.55 ± 0.18*</td>
</tr>
<tr>
<td>Lactate (mmol/l)</td>
<td>1.05 ± 0.66</td>
<td>1.51 ± 0.50*</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>4.93 ± 0.93</td>
<td>4.68 ± 1.28</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.35 ± 0.81</td>
<td>1.78 ± 0.69</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.33 ± 0.43</td>
<td>1.12 ± 0.27</td>
</tr>
</tbody>
</table>

**Table 2** Comparison of the vascular data before (rest) and after 5 min of cuff occlusion (reactive hyperaemia or ‘reperfusion’) in control subjects and diabetic patients.

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>Diabetic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radial artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vmean (cm/s)</td>
<td>14.6 ± 9.9</td>
<td>21.8 ± 10.3*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>2.04 ± 0.57</td>
<td>2.44 ± 0.57*</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>22 ± 18</td>
<td>12 ± 8†</td>
</tr>
<tr>
<td>Brachial artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>vmean (cm/s)</td>
<td>18.5 ± 8.5</td>
<td>28.7 ± 12.2*</td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>3.78 ± 0.88</td>
<td>4.09 ± 0.83*</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>9 ± 7</td>
<td>7 ± 6</td>
</tr>
</tbody>
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Figure 1  Radial (a and b) and brachial (c and d) artery diameter changes before and after 5 min of upper- and lower-arm suprasystolic cuff occlusion respectively, in a 30 year-old female control subject (a and c) and a 58-year-old female diabetic patient (b and d).

\[ \text{Hb} = 14.1 \text{ g/dl in the control subject; } \text{Hb} = 12.3 \text{ g/dl in the diabetic patient. Time (s) is relative to re-opening of the artery. The panels are constructed as if each heartbeat took up an equal time. The rest values shown are the last ten heart beats before inflation of the cuff. Any transient dilation seen immediately after re-opening of the artery relates to mechanical distension of the artery [22], whereas the dilation seen after approx. 45 s is flow mediated. The radial artery FMD of the diabetic patient was not established until after 90 s (outside plot range). Note, in both subjects, the relatively larger dilation seen in the radial artery.} \]

Figure 2  FMD of the radial and brachial arteries in 27 healthy subjects (open bars) and in 13 patients with Type II diabetes mellitus (hatched bars).

FMD was expressed as a percentage of the pre-cuff value. Results are means ± S.D. *P < 0.05 compared with control; #P < 0.05 compared with the radial artery values in diabetic patients.

In the diabetic patients, the radial artery luminal diameter increased from 2.28 ± 0.39 to 2.55 ± 0.48 mm (12 ± 8%; Figure 2), and decreased to 2.47 ± 0.43 mm after 90 s. With the applied analytical technique of choosing the maximal diameter during reperfusion, the brachial artery luminal diameter could be said to have increased from 4.10 ± 0.58 to 4.39 ± 0.62 mm (7 ± 6%; Figure 2), and to have decreased to 4.20 ± 0.49 mm after 90 s. As shown in Figure 1, however, visual inspection of the curves often did not reveal any significant changes.

Resting artery luminal diameters did not co-variate with [Hb]. In the radial artery of the normal subjects, the maximal dilation correlated with [Hb] \((r^2 = -0.64; \text{Figure 4})\), but not with Hbc. One subject with an [Hb] of 10.0 g/dl had a very high FMD of 91%; even if this subject was taken out, however, the correlation was only slightly smaller \((r^2 = -0.61)\). FMD was 30 ± 22 compared with 13 ± 12% in subjects with [Hb] below and above the median value of 14.1 g/dl respectively, although blood pressures, heart rate, \(\text{Vmean}, \text{S\text{\text{2}}O}_2\) and resting luminal
Figure 3  FMD of the radial and brachial arteries in healthy subjects

\[\text{Radial artery (n = 32)}; \quad \text{Brachial artery (n = 30). In both arteries, FMD is related to the vessel luminal diameter, as the relationship is steeper for the radial than for the brachial artery (ex of linear regression lines, -19.0 for the radial artery compared with -4.5 for the brachial artery; } P < 0.05). Radial and brachial FMDs were correlated with each other (r^2 = 0.30, P < 0.05; Spearman’s ρ).\]

Diameters were comparable. In the brachial artery of the normal subjects, the maximal dilation did not correlate with either [Hb] or Hbc. In the diabetic patients, [Hb], Hbc and HbA1c did not correlate with radial or brachial artery dilation.

DISCUSSION

In normal subjects, flow-mediated vasodilation correlated with [Hb] independent of basal blood flow and resting luminal diameter. Flow-mediated vasodilation was more than 2-fold higher in subjects with an [Hb] lower than the median (14.1 g/dl) in comparison with those with an [Hb] above the median. In patients with Type II diabetes, this relationship between [Hb] and flow-mediated arterial vasodilation could not be demonstrated.

Although a number of vasodilatory compounds, other than NO, have been identified to explain the vasodilation of ischaemic hyperaemia (potassium, oxygen, carbon dioxide, hyperosmolality, pH, \[\text{PO}_4^{3-}\], adenosine, kinins, prostaglandins and total osmolarity of the tissue interstitium [22]), dilation of the conduit arteries supplying post-ischaemic tissue depends almost solely on endothelial-NOS-produced NO [1,9,15]. The main physical factor involved is vessel wall shear stress that depends on blood flow and blood viscosity [9,23]. The viscosity of blood is 3–5 times higher than the viscosity of water and closely related to Hbc. In our present study, blood flow velocities and hence shear rates were comparable, and increased vasodilation by a low [Hb] cannot have been due to altered blood viscosity, as this would have tended to go in the opposite direction [24]. If the mechanism is not one of altered shear stress, we suggest the mechanism is by graded Hb scavenging of NO; a mechanism apparently so finely tuned as to be able to out-balance changes in [Hb], even within the normal range. Although other endothelium-derived vasodilating substances (including prostaglandins and endothelium-derived hyperpolarizing factor) exist and have been speculated to be implicated in flow-mediated vasodilation, NOS blockade almost completely abolishes FMD in both radial and brachial arteries; in vitro NO is a factor known to depend heavily on scavenging by Hb [2,13].

In vitro, Hb effectively absorbs NO [1], but in vivo the interaction of NO and Hb is not well characterized under normal physiological conditions [2]. Despite estimated half-lives of 0.05–0.18 ms, an intra-arterially infused aqueous NO bolus can be detected far from the infusion.
site, and NO thus ‘escapes’ Hb scavenging longer than previously expected [25]. The influence of Hb on NO-mediated vasodilatation has mainly been studied in chronically ill patients with extreme [Hb] values. Transfusion of packed erythrocytes into patients with chronic anaemia results in a lower peripheral conductance and a corresponding decreased level of NO bioavailability [5]. Also, in patients with cirrhosis of the liver and a resultant hyperdynamic circulation, anaemia aggravates peripheral vasodilatation and transfusion reverses it [6, 7]. In patients with polycythaemia secondary to chronic obstructive pulmonary disease, acetylcholine infusions failed to lower pulmonary vascular resistance until after haemodilution [26]. Our present study bridges these investigations by demonstrating a near-linear relationship of [Hb] and NO-mediated vasodilatation within the normal physiological range of [Hb].

The way Hb is presented to the endothelium is important, as are changes in viscosity. Free Hb in plasma is a more potent absorber of NO than Hb packed inside erythrocytes [27], and patients with sickle cell disease and associated haemolysis have episodes of excessive vasoconstriction, even vasospasm, although they are often anaemic [28]. Using ultrasound of the radial artery, Giannattasio et al. [24] demonstrated a 50% lower increase in diameter following reperfusion of an ischaemic hand after isovolumetric haemodilatation in patients with haemochromatosis. Thus, with a high Hbc the increase in viscosity may override the NO-scavenging effect of Hb. As suggested by Figure 4a, the effect of [Hb] on FMD seems to be most pronounced in the lower range of [Hb].

In resting subjects, cardiac output does not increase until [Hb] has decreased below approx. 6.5 g/dl, and we could not demonstrate any influence of [Hb] on resting artery diameters. In non-ischaemic tissue, any endothelium-related tendency for vasodilatation may be outbalanced by vasoconstrictive mechanisms. Thus anaemic patients have increased sympathetic out-flow that may affect the diameter of conduit arteries as large as the radial artery [29]. In the post-ischaemic tissue, the vasodilatory mechanism was unmasked, since this vasodilatation is independent of sympathetic nervous innervation [22]. On the other hand, FMDs were not related to pretest indicators of autonomous nervous system activity (blood pressure and heart rate) or arterial blood velocity, as determined by Doppler ultrasound, or the tissue oxygen saturation, as determined by near-infrared spectrophotometry. Therefore the larger increase in radial artery diameter observed in subjects with low [Hb] cannot be explained by, for example, relaxation after prominent sympathetic vasoconstriction.

Upstream cuff occlusion, as applied during the present study of the radial artery, theoretically raises the possibility of an added component of ischaemia of the endothelium itself, as can be seen with a cuff situated close to the measuring point [30], but findings are mainly in line with the radial artery dilating by the same mechanism as the brachial artery. Thus the radial artery luminal diameter did not change during the 5 min period of cuff occlusion and radial and brachial FMD were correlated, demonstrating that the two techniques measured the same phenomenon in the normal subjects. Also, with upstream cuff occlusion, Joannides et al. [15] found the vasodilatation of the radial artery to be abolished by infusion of l-NAME (N\textsuperscript{G}-monomethyl-l-arginine), and our present study demonstrates a temporal profile of the radial artery dilation (Figure 1) comparable with the profile of NO-mediated vasodilatation in the brachial artery (after approx. 45 s). In contrast, reactive hyperaemia is characterized by an increase in flow within the first few seconds and a subsequent slow exponential decay.

In patients with Type II diabetes, our present study shows that even in the radial artery, where flow-mediated vasodilatation may be apparent even if examination of the conventionally studied brachial artery indicates an abolished flow-mediated response, FMD of the radial artery was almost halved compared with normal subjects. Blunting of an endothelial response in diabetics has been suggested to come from accumulation of superoxide anions generated by HbA1c [31], but in vitro studies have been conflicting [32], and we found no correlation between FMD and HbA1c. Also, we could not demonstrate any relationship between [Hb] and NO-mediated vasodilatation, possibly because the statistical power was too low to demonstrate such relationships. With respect to influence from medication, no improvement in FMD in patients with overt diabetes mellitus has been demonstrated with biguanides and thiazolidinediones [33].

In conclusion, [Hb] in the normal range is correlated with flow-mediated endothelium-dependent vasodilatation, a high [Hb] associated with diminished dilatation. We suggest that graded NO absorption by Hb underlies this interaction. The established interaction between Hb and vasodilatation offers a mechanism whereby normal tissue blood flow is upheld, despite changes in [Hb].

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REFERENCES


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