Clinical research

Haematocrit, type 2 diabetes, and endothelium-dependent vasodilatation of resistance vessels

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Aims In conditions such as type 2 diabetes, hypertension, and smoking, in which haematocrit (Hct) tends to be higher, endothelial function is impaired. In vitro, haemoglobin neutralizes nitric oxide very effectively. Whether red blood cells participate in the regulation of endothelial function in vivo has not been established.

Methods and results Clinical and haematological parameters and forearm blood flow responses to acetylcholine (ACh) and sodium nitroprusside (SNP) were measured in 84 type 2 diabetic patients and 19 control subjects. Diabetics showed blunted dose–response curves to both SNP and ACh. In diabetics, across quartiles of Hct, ACh blood flow responses were progressively lower (881±96, 652±81, 513±54, 307±46%, P<0.0001), and maximal SNP responses tended to be lower (706±72, 578±61, 607±69, 499±53%, P=0.06) despite similar age, body mass index, glycated haemoglobin (HbA1c), blood pressure, serum total and HDL-cholesterol levels, indices of insulin sensitivity, and markers of inflammation. After normalizing the ACh response for the SNP response (ACh/SNP ratio), a progressive reduction across Hct quartiles (1.54±0.23, 1.22±0.15, 0.93±0.09, 0.66±0.09, P<0.0001) was still observed, with patients in the III and IV quartile showing a blunted response compared with controls (1.44±0.08). Both in diabetics and controls, the ACh/SNP ratio was reciprocally related to Hct (r=−0.46 and r=−0.66, respectively, P<0.002 for both). This association was independent of comorbidities, gender, metabolic control, plasma lipids, or concomitant treatments, was stronger in the subjects with preserved endothelium-dependent dilatation, and was unchanged when haemoglobin replaced Hct.

Conclusion Both in diabetics and non-diabetics, haematocrit is inversely related to small vessel endothelium-dependent dilatation. Thus, in addition to blood rheology, a direct negative effect on nitric oxide availability might explain the link between high Hct and cardiovascular disease.

Keywords Endothelium; Nitric oxide; Haemoglobin; Haematocrit

Introduction

Haematocrit (Hct) is a risk factor for ischaemic heart disease. A recent meta-analysis calculated that the risk ratio of subjects in the top vs. bottom tertile of the distribution of haematocrit, independent of other cardiovascular risk factors, was 1.16 (95% CI: 1.05–1.29) in population-based studies, and 1.80 (95% CI: 1.19–2.76) in patients with pre-existing cardiovascular disease.¹ With regard to the mechanism(s) through which a high Hct increases cardiovascular risk, current opinion calls...
upon increased blood viscosity and altered blood rheology.

However, a recent analysis of the WOSCOPS study found that Hct was predictive of cardiovascular disease independently of blood viscosity. Other mechanisms, therefore, may be operative. Several in vitro studies have clearly shown that haemoglobin (Hb) is a major catabolic sink for the nitric oxide (NO) that is produced by the endothelium. NO reacts with Hb in three different ways: oxidation (withoxy-Hb to yield met-Hb and nitrate), addition [with Fe(II)-Hb to give NO-Fe(II)-

Hb], and S-nitrosylation (of a cysteine residue on a vacant haem moiety). Given the rate constant of these reactions and the physiological concentrations of substrates observed in vivo, it is possible to predict that NO has a significant spill-over into the arterial compartment and reaches Hb inside the erythrocyte where its bioactivity is either transiently or permanently buffered. Indeed, when NO is inhaled there is clear evidence that a significant amount of the gas is transported by red blood cells to the peripheral tissues where it is released in biologically active amounts.

Whether the vascular effects of endogenous NO are also modulated by red blood cells is unknown. The only two in vivo studies in man addressing this issue have evaluated the flow-mediated dilatation of conductance arteries and found opposite results. In one study, patients with polycythaemia vera were found to have a markedly impaired flow-dependent vasodilatation; in the other study, the removal of 500 mL of blood in patients with haemochromatosis acutely caused a 50% reduction of the brachial artery response. The latter experimental model, however, may be inadequate to test the hypothesis because flow-induced dilatation is largely dependent on shear stress, which, in turn, is positively influenced by blood viscosity. Hct will therefore have opposite effects on this specific vascular response of conductance arteries and the result of its manipulation will depend on which of the two prevails.

Type 2 diabetes is characterized by the presence of variable degrees of impaired endothelium-dependent dilatation, the pathogenetic basis of which is relatively poorly understood. Population-based studies have consistently shown that Hct is higher in diabetics, as it is in conditions such as smoking, hypercholesterolaemia, hypertension, and obesity, all characterized by the presence of a blunted response to endothelium-dependent vasodilators due to a reduced bio-availability of NO. We hypothesized that, given the well known negative interaction between haem and NO, a high normal Hct may modulate endothelial function in normal subjects and contribute to endothelial dysfunction in diabetic patients.

Methods

Study population

Patients with type 2 diabetes were recruited from two outpatient clinics: 31 in London (UK) and 53 in Pisa (Italy); 19 non-diabetic subjects, also recruited in Pisa, served as the control group. Exclusion criteria were: (i) female patients with childbearing potential, (ii) presence of severe anaemia (red blood cells < 3.5 x 10^12 cells/μL or Hb < 10 g/dL), polycythaemia (RBC > 6 x 10^12 cells/μL) or clinically significant renal, hepatic, or pulmonary disease, (iii) poor metabolic control (glycated haemoglobin [HbA1c] > 10%) or severe hyperglycaemia (> 15 mmol/L) after a 4-week antidiabetic therapy washout period, (iv) cardiac failure New York Heart Association (NYHA) grades III-IV or angina pectoris, (v) treatment with allopurinol, nitrates, calcium antagonists, hormones, or antioxidant vitamin supplementation.

Study design

After the screening visit, all patients began a 4-week washout period from antidiabetic drugs after which they underwent the vascular study. All subjects gave their written informed consent and the study was approved by the Ethics Review Committee of each participating institution. The majority of the diabetic subjects were enrolled in a double-blind, randomized intervention study of metformin and rosiglitazone. The data presented concern the baseline evaluations of this cohort. The non-diabetic subjects were selected from the outpatient clinic to match the diabetics by anthropometric and clinical characteristics. In more detail, a list with the above-mentioned exclusion criteria and the detailed description of the study population clinical characteristics (Table 1) was given to the physician in charge of the screening. The selected subjects were then evaluated by a study investigator (A.N.) who evaluated the adequacy of the matching relative to age and body mass index (BMI) (within ± 2 SEM from the diabetic patients’ mean), and equilibrated the group with regard to gender, hyper-tension, and smoking; when these criteria were satisfied, routine biochemistry was obtained to verify all exclusion criteria and also to exclude patients with either hypercholesterolaemia (total cholesterol > 6.3 mM) or abnormal (> 6.0%) HbA1c values. These subjects only underwent the vascular reaction study.

Test procedures

Vascular reactivity (by the perfused forearm technique) was measured with the patients in the fasting condition. In order to reduce inter-centre variability, all procedures were carefully standardized, the same strain-gauge plethysmograph (EC4, Hokanson, Bellevue, WA, USA), and the same test chemicals were used in the two centres, and a training session was attended by all investigators before the beginning of the study. The studies were performed with the subjects lying supine in a quiet, air-conditioned room kept at a constant temperature (21–24 °C). A teflon cannula (20G) was inserted percutaneously into the brachial artery of the non-dominant arm under local anaesthesia (2% lidocaine). This cannula was used for drug infusion and intra-arterial blood pressure/heart rate monitoring. Forearm blood flow (FBF) was measured at the end of each of five 5-min steps of intra-arterial acetylcholine (ACh) infusion (0.15, 0.45, 1.5, 4.5, and 15 μg/min/dL of forearm tissue). After a rest period of at least 30 min, to allow FBF to return to baseline levels, FBF was measured at the end of three 5-min steps of intra-arterial infusion of sodium nitroprusside (SNP, 1, 2, and 4 μg/min/dL). After the vascular tests, the diabetic subjects underwent a 2-h hyperinsulinaemic (40 μU/min/m, euglycaemic (6.0 mmol/L) clamp.
Table 1  Clinical characteristics of the study patients by quartile of haematocrit

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
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<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>20</td>
<td>21</td>
<td>21</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Female, n</td>
<td>6</td>
<td>0.512</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Age, years</td>
<td>55 ± 1</td>
<td>0.106</td>
<td>58 ± 2</td>
<td>57 ± 1</td>
<td>58 ± 2</td>
<td>56 ± 2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>167 ± 2</td>
<td>0.419</td>
<td>168 ± 2</td>
<td>168 ± 2</td>
<td>170 ± 2</td>
<td>171 ± 2</td>
</tr>
<tr>
<td>BMI, kg m⁻²</td>
<td>28.7 ± 1.1</td>
<td>0.805</td>
<td>28.4 ± 0.7</td>
<td>28.3 ± 0.7</td>
<td>28.3 ± 0.8</td>
<td>27.5 ± 0.7</td>
</tr>
<tr>
<td>WHR</td>
<td>0.90 ± 0.02</td>
<td>0.029</td>
<td>0.93 ± 0.01</td>
<td>0.95 ± 0.02</td>
<td>0.95 ± 0.01</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>-</td>
<td>-</td>
<td>7 ± 1</td>
<td>8 ± 1</td>
<td>5 ± 1</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>5.1 ± 0.3</td>
<td>&lt;0.0001</td>
<td>10.4 ± 0.6</td>
<td>9.7 ± 0.5</td>
<td>10.7 ± 0.5</td>
<td>10.1 ± 0.6</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>5.9 ± 0.1</td>
<td>&lt;0.0001</td>
<td>8.1 ± 0.2</td>
<td>7.6 ± 0.2</td>
<td>7.9 ± 0.2</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.0 ± 0.2</td>
<td>0.917</td>
<td>4.9 ± 0.2</td>
<td>5.2 ± 0.2</td>
<td>5.1 ± 0.3</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>HDL-cholesterol mmol/L</td>
<td>1.2 ± 0.1</td>
<td>0.683</td>
<td>1.1 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.1 ± 0.1</td>
<td>0.001</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.3</td>
<td>2.2 ± 0.7</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>5</td>
<td>0.215</td>
<td>8</td>
<td>6</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>126 ± 4</td>
<td>0.311</td>
<td>132 ± 4</td>
<td>132 ± 4</td>
<td>128 ± 3</td>
<td>129 ± 3</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>76 ± 4</td>
<td>0.286</td>
<td>75 ± 2</td>
<td>73 ± 2</td>
<td>74 ± 2</td>
<td>75 ± 2</td>
</tr>
<tr>
<td>Smokers, n</td>
<td>3</td>
<td>0.412</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Haemoglobin, g/dL</td>
<td>41 ± 1</td>
<td>0.589</td>
<td>37 ± 1</td>
<td>40 ± 1</td>
<td>43 ± 1</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>Haemoglobin, g/dL</td>
<td>13.8 ± 0.2</td>
<td>0.143</td>
<td>12.6 ± 0.2</td>
<td>13.8 ± 0.1</td>
<td>14.6 ± 0.1</td>
<td>15.5 ± 0.1</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*P* for the comparison controls vs. diabetics by Wilcoxon signed rank test for continuous variable and likelihood ratio χ² test for proportions.

**P** for the Kruskal–Wallis rank sum test.

Assays

HbA₁c, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides, routine blood chemistry, and haematology (Technicon H-1 System, Bayer, Monaco) were assayed at the same central laboratory. Haematocrit was calculated as the product of red blood cell number and volume. Insulin (Human insulin-specific RIA kit, Linco, MO, USA), non-esterified fatty acids (NEFA, Wako Chemical, Germany), interleukin-6 (IL-6, ELISA kit, R&D Systems), tissue necrosis factor (TNF) α (ELISA kit, R&D Systems), and C-reactive protein (High Sensitive UL-CRP, Wako Chemical, Germany) were assayed in London.

Calculations

Plethysmographic trace recordings were analysed centrally by the same investigator (A.N.). Blood flow values were expressed both as mL/min/dL of forearm tissue and as percentage change from baseline values. Insulin sensitivity was estimated through the M value of the clamp as previously described.³

Statistics

Data are expressed as mean ± SEM unless otherwise stated. Differences in mean values between diabetics and non-diabetics and among quartiles of Hct were analysed by Wilcoxon and Kruskal–Wallis tests, respectively. Differences in the distribution of nominal variables were evaluated using the likelihood ratio χ² test. No correction for multiple tests on different variables was applied but the P-value for each comparison is always provided. Comparisons between each quartile of Hct vs. the non-diabetics were tested by using Dunnett’s method for the comparison with a control. Differences in the blood flow dose–response curves between diabetics and non-diabetics or among quartiles of Hct were tested by a multivariable model (MANOVA) with repeated measures.

The between-subjects effect (diabetes vs. non-diabetes or quartile of Hct) was modelled by fitting the sum of the repeated measures columns to the effect using a matrix that is a single vector of 1’s and the Wilks’ Lambda test. The within-subjects effects through the repeated measurements (ACh or SNP) and the interaction with the between-subjects effect (which, given the characteristics of the tests, is our main variable of interest) are modelled with a response function that fits differences in the repeated measures columns using the contrast response function. To further verify our hypothesis, we used a mixed model ANOVA with test drug dose (ACh or SNP) and Hct quartile (ordinal variables) as fixed effects and both subject ID and centre as random effects. By using Hct as a continuous variable, multivariable regression analysis was performed to test the following two hypotheses: (i) Hct and diabetes are independent predictors of endothelial dysfunction and there is a positive interaction between the two effects, (ii) The association between Hct and endothelial dysfunction is not explained by potentially confounding variables. For the former the following models were used:

\[
\text{ACh/SNP} = \text{constant} + \text{Hct} + \text{diabetes}
\]

and

\[
\text{ACh/SNP} = \text{constant} + \text{Hct} + \text{diabetes} + \text{Hct \times diabetes}.
\]

For the latter, two approaches were followed: first, the univariate associations were verified after stratifying the population according to relevant clinical parameters (gender, diabetes, hypertension, cardiovascular treatment, and HbA₁c); next, a correlation matrix was used to screen among the clinical variables known to exert a potential influence on vascular function (i.e. gender, age, lipids, hypertension, cardiovascular treatment), total serum protein—to account for haemodilution—and centre (as a dummy variable)—to account for inter-centre variability. All regression models were repeated on log-transformed dependent variables to test whether the association was affected by the non-normality of the variable distributions.
Results

The control subjects were generally similar to the diabetic patients with respect to most clinical characteristics and cardiovascular risk factors (Table 1), the diabetics showing only higher serum triglyceride levels and waist-to-hip ratio (WHR). In the patients, mean Hct was $41.4 \pm 3.5\%$ (mean $\pm$ SD, range 30.6--49.1) and Hb was $14.2 \pm 1.2$ g/dL (range 10.2--16.8); both were normally distributed and highly intercorrelated ($r = 0.96$). In the control group, Hct and Hb ranges were 35.0--47.0% and 11.5--15.3 g/dL, respectively. When patients were stratified into quartiles of Hct, the four groups showed similar clinical characteristics and differed only with respect to gender distribution (Table 1).

As shown in Figure 1, the FBF responses to ACh, both as absolute blood flow rates and as percentage increments above baseline, were blunted in the diabetic patients as a whole ($P < 0.0001$ for the effect of diabetes and $P < 0.002$ for the interaction with ACh dose by ANOVA for repeated measures). Among diabetics, the vascular response was progressively lower across quartiles of Hct ($P < 0.003$ for the effect of Hct quartile and $P < 0.002$ for the interaction with ACh dose). Also the vascular response to SNP showed a tendency to being reduced in the patients vs. controls ($P < 0.003$ and $P < 0.0003$ for the effect of diabetes alone and in interaction with SNP dose, respectively, when using absolute flow values, and $P = 0.726$ and $P = 0.023$ when percentage increments above baseline were analysed). Within the diabetic group, the effect of Hct quartile on SNP response was not statistically significant alone, while, in interaction with the SNP dose it showed a tendency to be statistically significant ($P = 0.160$ for the absolute flow values and $P = 0.061$ for their percentage increments). Similar results were obtained by using a mixed model ANOVA with patients IDs as random effect and test drug and Hct as fixed effects. The only exception was the SNP percentage response, which was found to be affected by Hct ($P < 0.001$), with post hoc analysis indicating that this was due to the first Hct quartile. Since SNP mainly evaluates smooth muscle cell relaxation and ACh tests both smooth muscle cell and endothelium reactivity, we used the ratio ACh to SNP (as the percentage increment above baseline during the final infusion step of each test drug) as a more refined index of endothelial function. As shown in Figure 2, the difference in the ACh to SNP ratio between non-diabetic and diabetic subjects and, among the latter, across quartiles of Hct, was partially attenuated but still present, with the patients in the top two Hct quartiles showing a marked impairment in endothelium-dependent reactivity. These patients had neither lower insulin sensitivity nor higher circulating NEFA, hs-CRP, IL-6, or TNF-α concentrations (Table 2). In the whole study group, the vascular responses to both ACh and SNP and their ratio were inversely related to Hct (Table 3). After stratifying for potential confounders, the strength of the associations with the response to ACh and the slope of the corresponding regression lines were similar in all the subgroups. Hct, in contrast, was not related to the SNP response in
neither Hct nor Hb was related to maximal SNP
for gender, HDL-cholesterol, and total serum
P, found (ACh: all the vascular indices and total serum proteins was
(group, among the measured clinical characteristics and
to an absolute 4% increment in Hct. In the whole study
negative predictors of endothelial dysfunction (ACh/SNP
dent and additive—with no significant interaction—
In multivariable analysis, diabetes and Hct were indepen-
diabetes or cardiovascular treatment, or in those
non-diabetics, in males, in normotensives, in those not
HbA1c ≤ 7%. With only the exception of female
subjects, the ACh/SNP ratio was significantly associated
Hct in all subgroups; a tendency towards a stronger
negative association was observed in male patients or in
those with HbA1c ≤ 7% regardless of their diabetic status.
In multivariable analysis, diabetes and Hct were independent
and additive—with no significant interaction—negative predictors of endothelial dysfunction (ACh/SNP
ratio), with these presence of diabetes being equivalent
to an absolute 4% increment in Hct. In the whole study
group, among the measured clinical characteristics and
vascular risk factors, the only variable influencing the
response to both ACh and SNP was male gender
(−147 ± 86 and −95 ± 34% for ACh and SNP, re-
spectively, P < 0.01 for both), whereas the ACh/SNP ratio
was negatively associated with HDL-cholesterol
(r = −0.23, P < 0.05). An inverse association between
all the vascular indices and total serum proteins was
found (ACh: r = −0.29, P < 0.005; SNP: r = −0.27,
P < 0.01; ACh/SNP: r = −0.23, P < 0.05). After adjusting
for gender, HDL-cholesterol, and total serum protein, neither Hct nor Hb was related to maximal SNP
response, while both were still associated with the
response to ACh (partial r of −0.49 and −0.48, re-
spectively) and with the ACh/SNP ratio (partial r of −0.39,
and −0.39, respectively). The pattern and strength of
these associations were unaffected by the introduction
into the model of dummy variables to account for inter-
centre variability or by log-transforming the dependent
variables.

Discussion
In the present study, we found a close relationship
between Hct and endothelium-dependent vasodilatation
of resistance arteries within the physiological range of
Hct in a large group of relatively unselected type 2 dia-etic patients and in non-diabetic subjects. This relation-
ship, which was similar to that between Hb and
endothelium-dependent dilatation, was fairly linear
(Figure 2) across the range of Hct explored (30–50%),
and was independent of obvious confounders such as
diabetes, metabolic control, gender, HDL-cholesterol,
total serum protein concentration, and the presence of
hypertension or cardiovascular treatment. When compared

| Table 2 | Indices of insulin sensitivity and inflammation in the diabetic patients by quartile of haematocrit |
|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
|                        | D1                      | D2                      | D3                      | D4                      | P-value*               |
| NEFA, μmol/L           | 496 ± 43                | 527 ± 63                | 467 ± 46                | 422 ± 40                | 0.923                  |
| Plasma insulin, pmol/L | 71 ± 9                  | 89 ± 10                 | 86 ± 12                 | 64 ± 13                 | 0.329                  |
| M value, μmol/min/kg of LBM | 34 ± 3               | 31 ± 2                  | 28 ± 2                  | 27 ± 3                  | 0.329                  |
| HS-CRP, mg/L           | 1.3 ± 0.3               | 1.4 ± 0.3               | 1.0 ± 0.3               | 1.4 ± 0.3               | 0.577                  |
| IL-6, pg/mL            | 1.7 ± 0.3               | 2.1 ± 0.3               | 2.0 ± 0.3               | 2.2 ± 0.3               | 0.480                  |
| TNF-α, pg/mL           | 1.5 ± 0.3               | 1.8 ± 0.4               | 1.9 ± 0.3               | 2.2 ± 0.3               | 0.376                  |

Data are mean ± SEM.
*P-value for the Kruskal-Wallis rank sum test on haematocrit quartiles.

| Table 3 | Simple regression analysis: haematocrit and vascular responses in all study subjects and subgroups |
|------------------------|------------------------|------------------------|------------------------|------------------------|
|                        | ACh max (%)            | SNP max (%)            | ACh/SNP (ratio)        |
|                        | Slope ± SE             | P                      | r                      | Slope ± SE             | P                      | r                      |
| All (n = 103)          | −61 ± 9                | 0.0001                 | 0.54                   | −25 ± 8                | 0.002                   | 0.30                   | 0.076 ± 0.017           | 0.0001                 | 0.44                   |
| Diabetics (n = 84)     | −60 ± 9                | 0.0001                 | 0.59                   | −29 ± 9                | 0.02                   | 0.33                   | 0.072 ± 0.017           | 0.0001                 | 0.46                   |
| Non-diabetics (n = 19) | −50 ± 21               | 0.0034                 | 0.49                   | −38 ± 13               | 0.005                  | 0.46                   | 0.084 ± 0.023           | 0.002                   | 0.66                   |
| Male (n = 83)          | −55 ± 12               | 0.0001                 | 0.46                   | −44 ± 19               | 0.03                   | 0.49                   | 0.097 ± 0.018           | 0.0001                 | 0.45                   |
| Female (n = 20)        | −60 ± 24               | 0.0003                 | 0.51                   | −76 ± 7                | 0.076                  | 0.49                   | 0.077 ± 0.019           | 0.0001                 | 0.46                   |
| Normotensive (n = 67)  | −56 ± 11               | 0.0001                 | 0.54                   | −38 ± 13               | 0.005                  | 0.46                   | 0.071 ± 0.029           | 0.02                    | 0.39                   |
| Hypertensive (n = 36)  | −74 ± 20               | 0.0006                 | 0.54                   | −44 ± 19               | 0.03                   | 0.49                   | 0.071 ± 0.024           | 0.007                   | 0.46                   |
| No CVD treatmenta (n = 57) | −59 ± 12               | 0.0001                 | 0.54                   | −34 ± 11               | 0.004                  | 0.42                   | 0.076 ± 0.020           | 0.0004                  | 0.42                   |
| On CVD treatmenta (n = 46) | −63 ± 14               | 0.0001                 | 0.55                   | −34 ± 11               | 0.004                  | 0.42                   | 0.076 ± 0.020           | 0.0004                  | 0.42                   |
| HbA1c ≤ 7% (n = 44)    | −69 ± 17               | 0.0003                 | 0.52                   | −32 ± 11               | 0.005                  | 0.36                   | 0.059 ± 0.020           | 0.0005                  | 0.37                   |
| HbA1c > 7% (n = 59)    | −56 ± 11               | 0.0008                 | 0.56                   | −32 ± 11               | 0.005                  | 0.36                   | 0.059 ± 0.020           | 0.0005                  | 0.37                   |

Slope ± SE indicates effect (± standard error) on the vascular response of unit increase in Hct estimated through simple regression analysis.

*aCVD treatment consisted of: low-dose aspirin (n = 15), statins (n = 10), fibrates (n = 8), ACE-inhibitors (n = 15), ATII antagonists (n = 2), α- or β-blockers (n = 15), diuretics (n = 3); alone (n = 31) or in combination (n = 15).
with non-diabetic subjects, type 2 diabetic patients as a group showed a blunted endothelium-dependent vasodilatation, but among them those with high normal (>41%) values of Hct showed a more severe defect. In agreement with some previous reports, we have also observed that type 2 diabetic patients tend to have a reduced vascular response to SNP and that this defect tends to be accentuated in the patients with higher Hct. This implies blunted NO-mediated smooth muscle cell relaxation and makes the interpretation of data more complex. In fact, under these conditions, the response to ACh (assessing both endothelium and smooth muscle cell function) does not provide a pure estimate of endothelial function, which will be rather more closely reflected by the ACh/SNP ratio. When we compared the ACh and the ACh/SNP responses (Figure 2), the former, resulting from the combination of the endothelial and the non-endothelial defects, appears more compromised than the latter in diabetics vs. controls and across quartiles of Hct. Thus, we conclude that non-endothelium-mediated vasodilatation is also negatively affected by Hct, although less severely. This observation is congruent with the known effect of blood viscosity on tissue perfusion at high rates; the persistence of significant associations between Hct or Hb and the ACh/SNP ratio strongly suggests that a high Hct negatively influences endothelium-dependent dilatation through mechanisms other than blood viscosity.

Given the cross-sectional nature of the study, we cannot establish a cause–effect relationship between Hct and agonist-induced vasodilatation. However, the associations between the haematological and the vascular reactivity variables were surprisingly similar in all subgroups of patients stratified according to potentially confounding clinical variables (diabetes, hypertension, metabolic control, gender, treatment). With regard to treatment, the strongest potential confounder consisted of ACE-inhibitor treatment, alone (n = 15) or in combination with diuretics (n = 3), but also within this small group of diabetics the association held true (r = 0.55, P < 0.05, slope: –0.090 ± 0.037). In addition, among the diabetic patients with higher Hct, the presence of endothelial dysfunction was not related to the coexistence of more severe insulin resistance, mild degree of inflammation, or elevated TNF-α levels (Table 3). We can also exclude that the association was influenced by some factor related to the study centre as it was present in the patients of with centres [although a slightly steeper relationship was observed in the Italian (slope: –0.082 ± 0.026, P < 0.003) than British (slope: 0.039 ± 0.011, P < 0.05) patients]. This was probably determined by the fact that the latter showed a slightly higher mean Hct (43.0 ± 6 vs. 40.5 ± 0.5%, P < 0.005) and a narrower Hct range (38–40 vs. 31–48%) coupled with a slightly more depressed endothelial function (ACh/SNP ratio: 0.8 ± 0.1 vs. 1.1 ± 0.1, P < 0.01) with a narrower range (0.1–1.4 vs. 0.1–2.7).

In terms of which is the cause and which the effect, while the rheological consequences of a high Hct and the buffering effect of Hb on NO are well-known phenomena, the alternative hypothesis—that this vascular dysfunction stimulates erythropoiesis—appears not to be supported by plausible mechanisms—or by transgenic animal models null for endothelial NO-synthase. In addition, the association of vascular dysfunction with Hct values in the high physiological range reconciles a number of apparently unrelated observations. Thus, Hct levels are higher in patients with essential hypertension, correlate with diastolic blood pressure, predict the development of type 2 diabetes, cluster with features of the metabolic syndrome, and increase in women after menopause. In each and all of these circumstances, vascular (mainly endothelial) dysfunction has been shown and proposed as a relevant step in the pathogenesis of the excess cardiovascular disease associated with these conditions.

Intra-arterial ACh and SNP infusions clearly do not reproduce physiological conditions; therefore, strictly speaking, whether Hct takes part in the physiological modulation of vascular tone remains to be demonstrated. However, this approach is generally recognized as a powerful tool to evaluate the integrity of endothelium mediated vasodilatation of resistance arteries. It has been widely used both in case–control and intervention studies and, more importantly, also in longitudinal studies, in which it has been shown to predict subsequent cardiovascular events. Our results strongly suggest that Hct exerts an important role in chronically modulating this vascular function in vivo, the effect probably being mediated through the increased impedance of blood and the chemical reactions between NO and Hb that take place in the arterial compartment. In quantitative terms, the impact of enhanced blood impedance—as estimated through the association between Hct (as well as total serum proteins) and the response to SNP—appears to be rather modest but not negligible. NO is only one of the mediators released by the endothelium upon ACh stimulation and it is unknown whether it reaches the intravascular compartment and whether it diffuses through the cell plasma membrane. However, elegant in vitro studies have recently demonstrated that NO metabolism is affected by the presence of red blood cells and that it also depends on the degree of Hb oxygenation within the erythrocyte. Whether acute or subacute changes in Hct result in a measurable effect on ACh- and/or SNP-induced dilatation cannot be established by our study. On the basis of our data we would predict a 7% greater maximal blood flow increase over baseline for each point reduction in Hct. An interesting corollary of our finding is that endogenous NO, at least when its release is highly stimulated, appears not only to diffuse along an abluminal gradient to act on local smooth muscle cells but also to enter the bloodstream to act on distal smooth muscle cells. The recent observations showing the relevance of the NO–Hb interaction to the haemodynamic response to inhaled NO, to the pathogenesis of oedema in severe anaemia, of erythropoietin-induced hypertension, and of the thrombo-embolic complications of polycythæmia vera, coupled with accurate biochemical modelling of vascular NO metabolism, strongly support the notion that endothelial-derived NO, in addition to its paracrine...
action, might enter the bloodstream and exert an effect on downstream vascular tissues. In agreement with this possibility, we observed a greater effect of Hct and Hb in the subjects with preserved endothelial function with respect to the subjects with a blunted response (Figure 3): when the generation of NO is already compromised, it is expected that the additional negative contribution of Hct will be less pronounced. Alternatively, or in addition, since the enzyme acetylcholinesterase is not only present in the plasma but is also bound to the erythrocyte, it is possible that local ACh clearance is accelerated if Hct is high. As a corollary, and independently of the underlying mechanism(s), studies of endothelial function utilizing ACh as the test agonist should take Hct into account in the comparison of patient groups or in the evaluation of changes in endothelial function with time or treatment.

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References