Effects of Hematocrit Changes on Flow-Mediated and Metabolic Vasodilation in Humans

Cristina Giannattasio, Alberto Piperno, Monica Failla, Anna Vergani, Giuseppe Mancia

Abstract—Endothelial function is noninvasively assessed by measuring nitric oxide–dependent increase in radial artery diameter accompanying the elevation in shear stress induced by increasing blood flow through a short-lasting ischemia of the hand. However, shear stress also depends on blood viscosity, whose changes might thus affect nitric oxide increase in a manner that is not properly reflected by blood flow changes. In 12 subjects with hemochromatosis, we measured ultrasonographically radial artery diameter and blood flow responses to a 4-minute ischemia of the hand. This was done also after removing 500 mL of blood (and concomitantly infusing 500 mL of saline), which significantly \( (P<0.01) \) reduced hemoglobin concentration and hematocrit. The increase in blood flow induced by the 4-minute ischemia was similar before and after blood removal \(+76\%\) and \(+80\%)\, which, in contrast, markedly attenuated the accompanying increase in radial artery diameter \(+25\%\ \text{versus} \ +13\%, \ (P<0.01)\). Thus, in humans, blood viscosity is involved in the endothelial response to an increase in shear stress. This implies that this response may not be accurately assessed and compared by quantifying the stimulus only through an increase in blood flow. \( \text{(Hypertension. 2002;40:74-77.)} \)

Key Words: blood flow • vasodilation • endothelium • hemoglobin • viscosity

Endothelial function plays an important role in the genesis of atherosclerosis, and for this reason it has been studied at various stages of cardiovascular diseases before and during treatment. This can be done by infusing in a brachial or coronary artery a substance (acetylcholine, bradykinin, and so forth) stimulating nitric oxide (NO) increase and measuring the resulting increase in blood flow. It can also be done in a noninvasive fashion by determining the increase in radial artery diameter that follows a short-lasting ischemia of the hand, because of the evidence that this vasodilation is due to NO increase triggered by an enhancement in blood flow shear stress.

However, shear stress also depends on blood viscosity, whose changes might thus affect NO in a manner that is not primarily reflected by changes in blood flow. The aim of our study has been to provide information on whether and how changes in blood viscosity modulate endothelial function in humans, as assessed and compared by quantifying the stimulus only through an increase in blood flow.

Methods

Subjects
We investigated 12 subjects (8 men; age, 44.2 ± 12.6 years) who had been diagnosed as having a genetic hemochromatosis based on (1) an increase in transferrin saturation and serum ferritin level; (2) hepatocellular hemosiderin deposits of grade III-IV; (3) hepatic iron index >2; (4) no iron-loading anemia and no history of blood transfusions; and (5) homozygosis for cysteine to tyrosine mutation of the hemochromatosis gene (HFE) at position 282 (C282Y). All patients were normotensive (average of 3 sphygmomanometric measurements, systolic and diastolic values identified by the 1st and 5th Korotkoff sounds, respectively) and had no history and physical or laboratory evidence of cardiovascular disease, including no atherosclerotic plaques in carotid and femoral arteries at an echocolor-Doppler examination. Liver histology was normal (except for iron overload), and no other hemochromatosis-related complication was present. No subject was a smoker. All subjects agreed to participate in the study after explanation of its nature and purpose. The study protocol was approved by the ethics committee of our hospital.

Endothelial Function
Radial artery diameter was measured by an A-mode ultrasonic echo-tracking device that recorded the displacement of the radial artery over the cardiac cycle (NIUS 02). The device used a transducer of 10 MHz, which was stereotaxically positioned over the radial artery 2 to 4 cm above the wrist, with a gel used as a medium. With the subject supine and the arm immobile at the heart level, the transducer was oriented perpendicularly to the longitudinal axis, based on the acoustic Doppler signal, so that its focal zone was located in the center of the artery and the backscattered echoes from both the anterior and the posterior walls could be visualized and acquired at 50 Hz. The device resolution allowed us to identify diameter changes of 0.0025 mm during blood pressure changes from diastole to systole. The device also made use of a photoplethys-
Blood pressure was recorded noninvasively from a finger ipsilateral to the radial artery examined with an accuracy similar to intra-arterial radial artery pressure and a resolution of 2 mm Hg. Heart rate was calculated as the reciprocal of two successive beats.

Blood flow velocity was measured at the same site of the diameter measurement by an 8-MHz probe positioned with an angle of 40° from the principal axis of the artery. Blood flow was calculated as the product of flow velocity and arterial diameter. Measurements were made before and immediately after a 4-minute inflation of a pediatric cuff placed around the wrist below the site of radial artery measurements. This maneuver leads to an increase in radial artery diameter that which is abolished by LMA administration, thus indicating its dependence on a flow-mediated local increase in NO.7

Radial artery diameter and blood flow were additionally measured after a 12 minutes inflation of an arm cuff at suprasystolic pressure to estimate the increase in radial artery diameter caused by maximal increase in blood flow.15

### Protocol and Data Analysis
Each patient came to our laboratory in the afternoon after a 24-hour abstinence from alcohol and caffeine and after a light morning meal. The study was performed at a constant temperature (21°C). The protocol of the study was as follows: (1) each subject was placed in the supine position and fitted with the radial artery echo-tracking device, the blood pressure measuring devices, and the wrist and arm cuff, (2) after a 10-minute interval, radial artery diameter, blood pressure, and blood flow velocity were continuously measured during 15 minutes (baseline) and venous samples were withdrawn to estimate the hematocrit and hemoglobin concentration, (3) in half of the patients the pediatric cuff placed on the wrist was inflated at suprasystolic pressure for 4 minutes and the above parameters were recorded for 4 minutes after the release of the inflation; after a 10-minute interval, the cuff placed around the arm was inflated at suprasystolic pressure for 12 minutes and measurements were made for 4 minutes after the release of the inflation, (4) in the other half of the patients, the 12-minute arm ischemia preceded the shorter 4-minute hand ischemia. (5) In each patient, 500 mL of venous blood was removed followed by 500 mL of saline infusion to maintain blood volume constant, and (6) a venous blood sample was withdrawn to estimate hematocrit and hemoglobin concentration, and the procedures described at points 2, 3, and 4 were performed again.

In each subject, baseline value for arterial diameter and blood flow was obtained by averaging five 30-second measurements obtained during the 15-minute period. The blood flow response to either the 4-minute or the 12-minute ischemia was assessed as the peak value during the 50 seconds after cuff pressure release. The radial artery diameter response to the short and long ischemia was assessed as the peak value during the 180 seconds after cuff pressure release. All measurements were made by a single investigator. The intraobserver coefficient of variation of radial artery diameter and blood flow (calculated for 2 sets of values obtained in standardized conditions) is, respectively, 2.5% and 8.0% in baseline conditions, 3.5% and 9.0% after 4-minute ischemia, and 1.9% and 7% after 12-minute ischemia. The statistical significance of the differences in mean values was assessed by 2-way ANOVA. The 2-tailed t test for paired observations was used to locate differences. A probability value of <0.05 was taken as the level of statistical significance. Throughout the text, values are given as ±SEM.

### Results
After removal of 500 mL of blood and saline infusion, patients showed a significant reduction in hemoglobin concentration (from 13.3±0.3 to 12.2±0.4 g/dL, P<0.01) and in hematocrit (from 39.9±0.8 to 37.1±0.4%, P<0.01) and no significant change in baseline systolic (from 126.8±4.5 to 129.2±4.9 mm Hg) and diastolic blood pressure (from 67.4±4.0 to 69.6±4.2 mm Hg), heart rate (from 67.5±3.5 to 65.0±3.5 b/min), radial artery diameter (from 2.4±0.2 to 2.4±0.2 mm), and flow (from 17±0.3 to 26±1.1 mL/min). As shown in the Figure, systolic blood pressure, diastolic blood pressure, and heart rate were not changed significantly by the 4-minute ischemia either before or after blood removal, this being the case for the 12-minute ischemia as well. After blood removal, the increase in radial artery blood flow induced by the 4-minute ischemia was modestly enhanced, the change in hematocrit showing a significant inverse relation with the change in flow in a close fashion (r=0.64, P=0.03). The concomitant increase in radial artery diameter was on the other hand strikingly reduced, the change showing no significant relation with the hematocrit (r=0.39, NS).

Blood removal also significantly though modestly enhanced the much greater increase in radial artery blood flow induced by the 12-minute ischemia, but in contrast with the 4-minute ischemia it attenuated to a much lesser degree the concomitant greater increase in radial artery diameter, which remained statistically significant.

### Discussion
In our patients, removal of blood followed by infusion of a similar volume of saline caused a reduction in hemoglobin concentration and hematocrit, thereby leading to a reduction in blood viscosity. This reduction did not impair the increase in radial artery blood flow induced by a 4-minute ischemia of the hand, which was actually increased after the hematocrit reduction. It caused, however, a marked impairment of the increase in radial artery diameter, which is triggered by this maneuver through an increase in NO.10 This allows us to confirm in humans what has been documented in animal studies.8–11 That is, that (1) also in humans, blood viscosity importantly participates in the modulation of endothelial function through changes in shear stress; (2) the influence of shear stress on NO increase cannot be properly quantified by only measuring blood flow alterations; and (3) greater increases in blood flow by ischemia can represent a lesser shear stimulus to the endothelium if hematocrit is reduced.

A few other aspects of our study deserve to be mentioned. In our patients, the NO-mediated radial artery diameter response to the 4-minute ischemia was markedly impaired by a reduction in blood viscosity despite (1) only a modest reduction of hemoglobin concentration and hematocrit and (2) a concomitant enhancement in the ischemic-dependent increase in blood flow. We can speculate that the important role of blood viscosity in the relation between shear stress and NO may be permissive in nature. Namely, that blood viscosity may maintain the ability of shear stress forces triggered by blood flow changes to act as a NO stimulus. We can also speculate that this is the case also when these forces are even more markedly recruited because the larger increase in radial artery diameter that accompanied the increase in blood flow induced by the 12-minute ischemia was also attenuated after the reduction in blood viscosity. The only difference with the data obtained by the short-term ischemia was that the residual increase in diameter remained large presumably because of its predominant dependence on nonendothelial factors.
Our results obviously pertain to the vascular district that we examined, and it is thus impossible to exclude that in other vessels endothelial cells are differently sensitive to the interaction between blood viscosity, blood flow, and shear stress stimuli. It is also impossible to exclude that this interaction is different for chronic rather than acute blood viscosity changes and in subjects other than those with hemochromatosis. We were unable, however, to extend our investigation to subjects other than those with hemochromatosis because of the ethical problems posed by removal of blood when this intervention is not therapeutic.

Finally, our findings have implications for current studies on endothelial function in humans that are more and more frequently based on the increase in radial artery diameter induced by a short-term ischemia of the hand. It is clear from our data that considering the “endothelial” stimuli only in terms of increase in blood flow is not enough. Assessment of blood viscosity directly or through hemoglobin concentration, hematocrit, or other techniques is also needed to ensure that differences or similarities in endothelium-dependent responses really reflect changes or lack of changes in endothelial function.

Perspectives

Our study has important methodologic implications for current studies on endothelial function in humans, more and more frequently based on the increase in radial artery diameter induced by a short-term ischemia of the hand. It is clear from our data that considering the “endothelial” stimuli only in terms of increase in blood flow is not enough. Assessment of blood viscosity directly or through hemoglobin concentration or hematocrit is also needed.

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References


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