Paradoxical hypotension following increased hematocrit and blood viscosity

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Paradoxical hypotension following increased hematocrit and blood viscosity. Am J Physiol Heart Circ Physiol 289: H2136–H2143, 2005. First published July 8, 2005; doi:10.1152/ajpheart.00490.2005.—Hematocrit (Hct) of awake hamsters and CD-1 mice was acutely increased by isovolemic exchange transfusion of packed red blood cells (RBCs) to assess the relation between Hct and blood pressure. Increasing Hct 7–13% of baseline decreased mean arterial blood pressure (MAP) by 13 mmHg. Increasing Hct above 19% reversed this trend and caused MAP to rise above baseline. This relationship is described by a parabolic function ($R^2 = 0.57$ and $P < 0.05$). Hamsters pretreated with the nitric oxide (NO) synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) and endothelial NOS-deficient mice showed no change in MAP when Hct was increased by <19%. Nitrate/nitrite plasma levels of Hct-augmented hamsters increased relative to control and L-NAME treated animals. The blood pressure effect was stable 2 h after transfusion. These findings suggest that increasing Hct increases blood viscosity, shear stress, and NO production, leading to vasodilation and mild hypotension. This was corroborated by measuring A1 arteriolar diameters ($55.0 \pm 21.5 \mu m$) and blood flow in the hamster window chamber preparation, which showed statistically significant increased vessel diameter ($1.04 \pm 0.1$ relative to baseline) and microcirculatory blood flow ($1.39 \pm 0.68$ relative to baseline) after exchange transfusion with packed RBCs. Larger increases of Hct (>19% of baseline) led blood viscosity to increase >50%, overwhelming the NO effect through a significant viscosity-dependent increase in vascular resistance, causing MAP to rise above baseline values.

nitric oxide; shear stress; vascular resistance; hypertension

It is a general medical and clinical perception that an increase in blood viscosity may lead to short- and long-term negative physiological conditions, and there appears to be universal agreement that increased blood viscosity is a factor in hypertension. Lowering blood viscosity, however, is not advocated as a means for controlling hypertension with the exception of erythrocytosis, substantial Hct increases consequent to adaptation to high altitudes, and cardiovascular impairment in premature infants. These conditions represent extremes of an increase in blood viscosity and clearly must be corrected by lowering Hct, because the extreme excess of red blood cells (RBCs) is superfluous in providing adequate oxygen-carrying capacity and is in fact a hindrance to blood flow and therefore oxygen delivery.

Clinical studies (14, 37) report a significant relationship between hypertension and high Hct levels. Hypertensive patients have higher Hct values than normotensive control individuals (23). Patients suffering from polycythemia vera or other erythrocytoses present with pathologically high Hcts leading to hypertension, thromboembolism, and other severe clinical complications (2, 15). There is evidence, however, that individuals, such as Peruvian miners, survive with Hct levels of 75–91% (18), suggesting the existence of an adaptive mechanism.

Endothelial cells play a key role in the regulation of blood pressure and blood flow because of their ability to detect changes in the environment to which they are exposed (25, 29, 35), responding to humoral factors in the circulation and mechanical conditions created by blood flow, particularly shear stress. The latter stimulates the release of vasoactive materials and modulates gene expression, cell metabolism, and cell morphology (4). Shear stress is determined by blood flow and blood viscosity. Smiesko et al. (32) show an increase in arterial lumen by increasing flow. Melkumyants et al. (26) report that increased shear stress, induced by elevated blood viscosity, causes vasodilation in the femoral artery of the cat, which returns shear stress to baseline values. Tsai et al. (34) report that in extreme hemodilution in the hamster skinfold model, normal levels of tissue perfusion can only be maintained when plasma viscosity is increased by the addition of a high-molecular weight Dextran 500. The underlying mechanism is believed to be shear stress-mediated production of nitric oxide (NO), a potent vasodilator generated from the conversion of L-arginine to L-citrulline by the enzyme NO synthase (NOS) (8).

In this study we investigated how acute changes in Hct affect blood pressure. Whole blood viscosity is an exponential function of Hct. Acute systemic changes in Hct should be a common occurrence in the treatment of hypertension by diuretics because they reduce plasma volume, presumably causing hemococoncentration and therefore increased whole blood viscosity, as shown in the study of Fazio et al. (11) who found that a significant decrease in blood pressure ($-6\%, P < 0.005$) corresponded to a significant increase in Hct in patients treated with the diuretic furosemide ($6\%, P < 0.005$). Increased blood viscosity could lead to increased shear stress and a release of NO (9, 11) and therefore vasodilation. This suggests that blood viscosity may be involved in the regulation of blood pressure in a counterintuitive direction.

In the following study we investigated how increased Hct and thus shear stress influence blood pressure in the hamster skinfold model. We used this model because we have experi-
ence in performing isovolemic blood exchanges, in the awake condition, while causing minimal cardiovascular perturbations. To validate the link between blood viscosity, shear stress, and NO and to corroborate our findings in a different species, we performed the same experiments in endothelial NOS (eNOS) knockout and wild-type mice.

MATERIALS AND METHODS

Animal preparation. Investigations were performed in golden Syrian hamsters (Charles River; Boston, MA), CD-1 mice (Charles River) and eNOS-knockout mice (B6.129P2-Nos3tm1Unc, Charles River). Animal handling and care were provided following the procedures outlined in the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). The study was approved by the local Animal Subjects Committee of the University of California, San Diego. The window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere (3, 10). This model was used to maintain the catheters in place in the awake animals and to prevent them from chewing or pulling them. Briefly, hamsters were prepared for chamber implantation with a 50 mg/kg ip injection of pentobarbital sodium anesthesia. Mice received a 0.1 mg/30 g ip injection of a mixture of 10 mg/kg xylazine, 200 mg/kg ketamin, and an equivalent volume of 0.9% saline. After hair removal, sutures were used to lift the dorsal skin away from the animal, and one frame of the chamber was positioned on the animal’s back. A chamber consisted of two identical titanium frames. The second frame was placed on the opposing side. Arterial and venous catheters (PE-50 for hamsters and PE-10 for mice) were implanted in the carotid artery and jugular vein. The catheters were filled with a heparinized saline solution (30 IU/ml) with a heparinized saline solution (30 IU/ml) to ensure their patency at the time of the experiment. Catheters were tunneled under the skin and exteriorized at the dorsal side of the neck where they were attached to the chamber frame with tape. The experiment was performed after at least 24 h but within 48 h after catheter implantation.

Inclusion criteria. Animals were suitable for the experiments if systemic parameters were within normal limits, namely, heart rate (HR) >540 beats/min, mean arterial blood pressure (MAP) >80 mmHg and PCO2 <125 mmHg. The latter criterion did not apply for eNOS knockout mice, which are spontaneously hypertensive (31).

Systemic parameters. MAP and HR were recorded continuously (MP 150; Biopac System; Santa Barbara, CA), except during the actual blood exchange. Hct was measured from centrifuged arterial blood samples taken in heparinized capillary tubes (Readacrit Centrifuge; Clay Adams, Division of Becton-Dickinson; Parsippany, NJ). Hemoglobin content was determined spectrophotometrically from a single drop of blood (B-Hemoglobin; Hemocue; Stockholm, Sweden).

Experimental groups. Experiments were carried out in five animal groups: hamster control group (n = 27, 37.6–88.8 g body wt), hamster N^O-nitro-arginine methyl ester (l-NNAME) group (n = 9, 42.5–64.4 g body wt), mouse control group (n = 10, 27.8–43.6 g body wt), eNOS knockout mouse group (n = 4, 19.4–22.9 g body wt), and hamster microcirculation group (n = 7, 48.6–69.5 g body wt).

Experimental setup and isovolemic exchange transfusion with packed RBCs. The experimental procedure was the same for all five animal groups. The unanesthetized animals were placed in a restraining tube. They were given 30–60 min to adjust to the tube environment before baseline measurements were taken (MAP, HR, Hct, and hemoglobin). Fresh RBCs were obtained the same day from a donor hamster or a donor mouse. Donor blood was centrifuged, and RBCs were separated from plasma to obtain packed RBCs. After the determination of packed cells Hct, they were diluted with plasma to obtain Hct levels between 55% and 94%. The volume of exchange transfusion was calculated as a percentage of the animal’s blood volume, estimated as 7% of body weight for hamsters and 6% of body weight for mice. Different levels of Hct increase were obtained by varying the exchange-transfusion volume between 5% and 18% and by varying the Hct of the infused packed RBCs between 55% and 94%. Packed RBCs were infused with a dual syringe pump (model 33 syringe pump; Harvard Apparatus; Holliston, MA) into the jugular vein catheter at a rate of 100 µl/min (hamster) or 50 µl/min (mouse), and blood was simultaneously withdrawn from the carotid artery catheter at the same rate. Samples for Hct and hemoglobin measurements were taken after a 5-min resting period. Because of the small weight of the eNOS knockout mice (19.4–22.9 g) and their small blood volume, samples for Hct and hemoglobin measurements were taken only twice, at baseline and at the end of the 120-min observation period, and the increase of Hct after infusion of packed RBCs was estimated by means of Eq. 1:

\[
\text{Final Hct} = \left( BV \times BL \text{ Hct} + EV \times \text{Hct of PRBC} - EV \times BL \text{ Hct} \right) / BV
\]

where BV is blood volume, BL is baseline, EV is exchange volume, and PRBC is packed RBCs.

Animals were followed up for 120 min. Blood pressure and HR measurements were taken three times during a 30-min period (at 5, 15, and 25 min), each measurement representing an average blood pressure over 4 min. At the end of the observation period, Hct and hemoglobin were measured again and blood was withdrawn from the arterial catheter for measurements of plasma nitrate/nitrite concentrations.

Animals of the hamster microcirculation group were subject to microvascular measurements of vessel diameter, RBC velocity, and blood flow. The conscious animal in the tube was attached to the microscopic stage of an inverted microscope (IMT-2 Olympus; New Hyde Park, NY) equipped with a ×20 objective (numerical aperture = 0.32; series PHACO L1; Leitz, Germany). The tissue image was projected into a CCD camera (model 4815–2000; COHU; San Diego, CA) connected to a video cassette recorder (Panasonic AG-7355, Matsushita Electric; Osaka, Japan) and viewed on a Sony monitor (PMV-1271Q; Tokyo, Japan). Sites of investigation were chosen based on their visual acuity and location within the microvascular network. Because the large feeding arteries (A1) mainly contribute to total peripheral vascular resistance, these vessels were investigated in terms of diameter, RBC velocity, and blood flow. The same sites of measurements were followed throughout the experiments so that comparisons could be made directly with baseline levels.

Assessment of microcirculatory parameters. RBCs velocities in A1 arterioles were measured online by using the photodiode/cross-correlator system (16) (Photo Diode/Velocity Tracker Model 102 B; Vista Electronics; San Diego, CA). A video image-shearing method was used to measure vessel diameter. Blood flow was calculated from the measured values as \( Q = V \times \pi \left( D/2 \right)^2 \); measurements of vessel diameter (D), RBC velocity (V), and blood flow (Q) were made at baseline and 30, 60, 90, and 120 min after the exchange transfusion with packed RBCs.

Isovolemic exchange transfusion and pretreatment with l-NNAME. l-NNAME (Sigma) was dissolved in saline and infused through the jugular venous catheter within 1 min at a dosage of 30 µg/kg at a concentration of 20 µg/ml, previously shown to induce immediate hypertension after infusion (31). The volume of infusion was <5% of systemic blood volume. Animals were given 30-min resting time before the exchange with packed RBCs was started.

Measurements of nitrate/nitrite concentrations in plasma. NO production was estimated by measuring the sum of nitrite and nitrate in plasma from blood samples obtained at the end of the experiment collected with a 5-ml syringe containing 0.14 ml of sodium citrate (26.35 mg/ml) per milliliter of withdrawn blood. Blood samples were centrifuged, separated from RBCs, and plasma was stored at −70°C. After ultrafiltration of the plasma through a 10-kDa mol mass cut-off filter, samples were incubated with nitrate reductase and enzyme

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cations for 3 h for conversion of nitrate to nitrite. Absorbance was read at 540 nm using a plate reader after addition of the Griess reagents that convert nitrite into a deep purple azo compound. Measurements were performed in the hamster control group \( n = 14 \), the hamster L-NAME group \( n = 8 \), and the untreated animals \( n = 8 \).

**Acquisition of viscosity data.** Blood viscosity was measured in samples from donor animals at different Hcts. Because the relationship between viscosity and Hct is approximately linear in the physiological Hct range, we performed a linear regression analysis of our data in the Hct range of 45–55% and obtained the relation:

\[
\eta = 0.1678 \times \text{Hct} - 4.348
\]

where \( \eta \) is whole blood viscosity, which was used to estimate whole blood viscosity for each animal after exchange transfusion. Viscosity measurements were performed with a cone/plate viscometer (model DV-II; Brookfield; Middleboro, MA) with the CPE-40 cone spindle at a shear rate 200/s.

**Measurements of blood volume.** Blood volume measurements were taken in three hamsters of the control group. At the end of the experiments, 0.1 ml (\( V_\text{b} = \) volume of the infused dye) of Evans blue dye (EBD) was infused intravenously at a concentration of 5 mg/ml (\( C_0 = \) dye concentration). Blood samples were taken at 5, 10, 15, and 20 min after the EBD injection and centrifuged for 5 min. Forty microliters of plasma of each sample were diluted in 1.960 \( \mu \)l of 0.1 M Tris- HCl buffer (pH 9.0). The optical density (OD) was measured at 620 nm using a Sequoia Turner model 690 spectrophotometer. Samples at 5, 10, 15, and 20 min after EBD injection were analyzed by linear regression to determine the extrapolated OD at time \( t = 0 \). Because of dilution, the acquired OD was multiplied by 50. The concentration of EBD (\( C \)) at \( t = 0 \) (\( C_{t=0} \) in mg/ml) was obtained by using the relation:

\[
C_{t=0} = (\text{OD} \times 50 - 0.0208)/74.877
\]

that describes the linear relationship between known concentrations of EBD dye and OD in this spectrophotometer.

Plasma volume (PLV, in ml) was determined from the relation:

\[
\text{PLV} = C_0 \times V_\text{b}/C_{t=0}
\]

Total blood volume (TBV, in ml) was calculated by using the formula:

\[
\text{TBV} = \text{PLV} / (1 - \text{Hct} \times \text{trapping factor} \times F_{\text{cell}})
\]

where Hct is the Hct right before the blood volume measurement; the trapping factor is 0.96; and \( F_{\text{cell}} \), the total body-to-venous Hct ratio, is 0.74/27.

**Statistical analysis.** Results are presented as means \( \pm \) SD unless otherwise noted. In the Microhemodynamics section data are presented relative to levels at baseline. Data within each group were analyzed using ANOVA for repeated measurements followed by the Bonferroni correction or if appropriate unpaired two-tailed Student’s \( t \)-test. All statistics were calculated by using GraphPad Prism 4.01 (GraphPad Software; San Diego, CA). Changes were considered statistically significant if \( P < 0.05 \).

**RESULTS**

**Hamster control group.** Experiments were performed in 27 animals. Blood pressure decreased in proportion to the increase in Hct after exchange transfusion with packed RBCs. Normal hamsters present a natural variability of both blood pressure and Hct, which in the present study was in the range of 39–48% in Hct and 84–122 mmHg in blood pressure. To account for this variability and reduce the number of animals that would be required to detect the effects found in this study, we present our results as percent changes from baseline.

Admittedly, this may be somewhat confusing for Hct changes because this itself is a percentage (percent volume of RBCs in blood). An alternative would be to express Hct in terms of hemoglobin concentration; however, blood viscosity has been traditionally presented as a function of Hct and not hemoglobin concentration. Thus we selected to present the data in terms of normalized blood pressure changes as a function of normalized Hct changes, considering that Hct is also a measure of concentration.

Increasing Hct between 2% and 19% (45 ± 3% at baseline vs. 49 ± 3% after exchange transfusion) decreased blood pressure between 1 and 13 mmHg of baseline with the maximum pressure drop of 13 mmHg occurring when Hct was increased 7–13% of baseline. Increasing Hct between 19% and 23% (42 ± 3% at baseline vs. 52 ± 4% after exchange transfusion) increased blood pressure between 1 and 15 mmHg above baseline. Blood pressure changes occurred 30–60 min after exchange transfusion and remained stable for the whole observation period (120 min). Changes in blood pressure versus percentage of Hct increase were fitted by a second-order polynomial (\( R^2 = 0.57 \) and \( P < 0.05 \)) (Fig. 1).

Following the trend line obtained by curve fitting with a second-order polynomial, we divided the animals into four groups: animals with no increase in Hct (group A) versus baseline, animals with Hct increases between 1% and 7% (group B) versus baseline, animals with Hct increases between 8% and 14% (group C) versus baseline, and animals with Hct increases between 15% and 20% (group D) versus baseline.

We found that the blood pressure measured in group A (no Hct increase and no blood pressure change from baseline) was statistically significantly different from the blood pressure of group B (Hct increases between 1% and 7% from baseline; blood pressure, \(-7 \pm 2 \) mmHg from baseline) \((P < 0.05)\) and from group C (Hct increases between 8% and 14% from baseline; blood pressure, \(-9 \pm 3 \) mmHg from baseline) \((P < 0.05)\).
The blood pressure of group C (Hct increases between 8% and 14% from baseline, blood pressure $-9 \pm 3$ mmHg from baseline) was statistically significantly different from blood pressure values of group D (Hct increases between 15% and 20% from baseline, blood pressure $-1 \pm 6$ mmHg from baseline) ($P < 0.05$). At the end of the experiment, Hct was $\sim 50\%$ lower than after the exchange transfusion.

Increasing whole blood viscosity by $4\text{–}45\%$ of baseline due to the increase in Hct by $2\text{–}19\%$ of baseline decreased blood pressure by $1\text{–}13$ mmHg of baseline. Increasing Hct beyond $19\%$ of baseline increased blood viscosity $>50\%$ of baseline, which corresponded to an increase in blood pressure above baseline. Changes in blood pressure vs. percentage of viscosity increase are best fitted by a second-order polynomial ($R^2 = 0.66$ and $P < 0.05$) (Fig. 2).

Nitrate/nitrite concentrations in plasma of Hct-increased animals were significantly elevated, compared with untreated animals ($7.54 \pm 2.76$ μmol vs. $4.98 \pm 1.48$ μmol; $P < 0.05$).

**Microhemodynamics.** Seven hamsters were used to assess microrcirculatory parameters in 45 A1 arterioles (baseline diameter, $55 \pm 21$ μm). Arterioles dilated significantly ($1.04 \pm 0.1$ vs. baseline; $P < 0.05$) after exchange transfusion with packed RBCs. RBC velocity increased significantly compared with baseline ($1.2 \pm 0.6$ vs. baseline; $P < 0.05$), and microvascular blood flow increased $1.39 \pm 0.68$ compared with baseline ($P < 0.05$) (microvascular data are normalized relative to baseline). All animals were studied for 120 min, and microrcirculatory changes were stable during this time (Fig. 3).

**Hamster i-NAME group.** Blood pressure increased immediately from $95 \pm 7$ to $123 \pm 6$ mmHg in nine hamsters pretreated with i-NAME. Exchange transfusion with packed RBCs, increasing Hct between 8% and 19% ($43 \pm 3\%$ at baseline vs. $49 \pm 2\%$ after exchange transfusion), did not produce the reduction in blood pressure found in the group with no i-NAME pretreatment. After 1 h, blood pressure tended to decrease according to the degradation of i-NAME (31). Blood pressure remained elevated from baseline ($120 \pm 5$ mmHg vs. $95 \pm 7$ mmHg) 120 min after exchange transfusion and was significantly elevated compared with blood pressure levels of the hamster control group at 30, 60, 90, and 120 min after the exchange transfusion with packed RBCs ($P < 0.05$) (Fig. 4). This result parallels previous observations by Sakai et al. (31), who also found significant A1 arteriolar constriction after the administration of i-NAME. Nitrate/nitrite concentrations in the plasma were significantly lower compared with Hct augmented hamsters ($4.88 \pm 1.64$ μmol vs. $7.54 \pm 2.76$ μmol; $P < 0.05$).

**Mouse control group.** To assess whether increasing Hct produces the same trend in blood pressure behavior in a different animal species, experiments were made in 10 CD-1 mice. Exchange transfusion with packed RBCs increased Hct 6–18% ($47 \pm 2\%$ at baseline vs. $51 \pm 3\%$ after exchange transfusion). Blood pressure began to change between 30 and 60 min after transfusion. Increasing Hct by 6–15% of baseline was followed by a decrease in blood pressure ($2\text{–}10$ mmHg) compared with baseline. A maximum decrease in blood pressure ($10$ mmHg) corresponded to a 9–11% increase in Hct versus baseline. Increasing Hct more than 17% of baseline increased blood pressure by 10 mmHg of baseline. A comparison of this response to that of the hamster control group showed that the lowering blood pressure effect in CD-1 mice started at a higher percentage of Hct increase, being first observed when Hct was increased by 5% of baseline. These results are also described by a second-order polynomial ($R^2 = 0.70$ and $P < 0.05$) (Fig. 5).

**eNOS knockout mouse group.** Our results in hamsters showed a maximum blood pressure drop when Hct was increased 8–13% of baseline; therefore, we analyzed the change in blood pressure in wild-type and eNOS knockout mice subjected to a similar increase in Hct. Wild-type mice with a Hct increase of 7–15% of baseline showed significantly lower
blood pressure (−5 ± 3 mmHg) compared with animals with normal Hct.

The same experiments were made in four eNOS knockout animals, which were spontaneously hypertensive at baseline (130 ± 7 mmHg). After infusion of packed RBCs, Hct increased 4–11% (45 ± 4% at baseline vs. 48 ± 3% after exchange transfusion). Unlike control wild-type mice, these Hct increases were not followed by a blood pressure decrease during the 120 min of observation (Fig. 6) (P > 0.01).

Similar to Hct in hamsters, Hct in mice decreased after 120 min to about half of the previous increase in Hct. Measurements of nitrate/nitrite concentrations were not made in mice because of the small size of these animals and the resulting small sample volume.

**DISCUSSION**

The present study shows that an acute increase in Hct is not necessarily associated with an increase in blood pressure. We found that after isovolemic exchange transfusion with packed RBCs in hamsters, blood pressure decreased in proportion to the percentage rise in Hct. Increasing Hct between 7% and 13% of baseline produced the maximal blood pressure drop (7–13 mmHg of baseline). When Hct was further increased, blood pressure returned to baseline values, and increasing Hct by more than 19% of baseline (19–23%) increased blood pressure above baseline (1–15 mmHg). Even though our number of animals (n = 4) with Hct increases >19% is relatively small, the blood pressure behavior of this group parallels the finding of Kuo et al. (21) who showed that acute Hct increases of 30% from baseline increased blood pressure by 10 mmHg of baseline, a result that was statistically significant. From our data, it is likely that acute increases in Hct of >20% from baseline values represent the limit of an acute viscosity change that can be compensated for by the mechanisms explored in the present study, with the result that greater viscosity increases lead to the increase of blood pressure shown by Kuo et al. (21).

Our results show that NO release counteracts the increase in vascular resistance due to increased blood viscosity; however, as shown in Figs. 5 and 6, arterial pressure did not increase in the L-NAME-treated hamsters or the eNOS knockout mice when blood viscosity increased. This result may be explained by considering that shear stress also mediates the release of additional vasodilatory mediators such as prostacyclin (12). Therefore, the lack of increase in blood pressure should be due to the remaining effect of increased release of other vasodilatory mediators of whose its effects appear to be of lesser magnitude than those attributable to NO.

The change in blood pressure occurred 30–60 min after the end of the exchange transfusion and was stable during 120 min of observation. We hypothesize that the effect is related to the
increase in whole blood viscosity, which increases shear stress at the vascular wall and thus NO production, leading to vasodilation. This concept is supported by the finding that the Hct-treated hamster control group showed significantly elevated nitrate/nitrite concentrations compared with animals pretreated with the NOS inhibitor L-NAME and with untreated animals. We found that a 7–13% increase in Hct of baseline corresponds to an 18–30% increase in whole blood viscosity versus baseline. Higher viscosity levels resulting from further increased Hcts led to a progressively smaller decrease in blood pressure and an eventual increase in blood pressure.

According to the Hagen-Poiseuille law, the flow of blood is directly related to the fourth power of the radius and inversely related to fluid viscosity. Therefore, total peripheral resistance is determined by vessel diameter and blood viscosity, whereas blood pressure is determined by cardiac output and total peripheral vascular resistance. The most important factor affecting whole blood viscosity is Hct. Diseases with an elevation of blood viscosity, so called hyperviscosity syndromes, are associated with hypertension and vascular occlusive complications leading to transient episodes of neurological dysfunction, such as amaurosis fugax, hemiparesis, and epileptic attacks (17).

Increased whole blood viscosity is also reported to be associated with cardiovascular diseases (37). The classical risk factors for cardiovascular diseases like hypertension, diabetes, obesity, and hyperlipidemia have been shown to be accompanied by elevated whole blood viscosity (6, 22). Hyperviscosity in hypertensive patients is associated with a poor prognosis, because it is correlated with the blood pressure level and severe complications, including left ventricular hypertrophy (24).

The present study shows that blood pressure is lowered by the increase in blood viscosity through an increase in Hct, a finding that may, in part, be due to the small changes in Hct that were induced. The range of Hct increase of baseline in hamsters in which blood pressure was either decreased or remained constant was 2–19%, which led to an increase in blood viscosity by 50% of baseline. This finding suggests that there is a limit in the ability of the vascular system to compensate for increased viscosity. Because this increase in viscosity is accomplished via an increase in Hct, and therefore oxygen carrying capacity, it would seem that phenomena like adaptation to high altitude by erythrocytosis may become pathological only after a specific Hct threshold is surpassed. Because of the link between NO production and compensation for increased viscosity, it is likely that further elevations in viscosity increase blood pressure because the capability of endothelial cells to release NO reaches a plateau that maximizes NO-mediated vasodilation. Therefore, hyperviscosity syndromes become pathological when the elevation in viscosity exceeds the ability of endothelial cells to release NO, causing hypertension.

The finding that eNOS knockout mice, which do not produce NO via eNOS, showed no decrease in blood pressure, underlines the role of endothelial NO in the observed effect. Similarly, hamsters pretreated with L-NAME, the NOS inhibitor that induces immediate hypertension after infusion, did not show a decrease in blood pressure after exchange transfusion with packed RBCs. The small pressure decrease as a function of time observed in these animals was related to the degradation process of L-NAME that begins ~60 min after infusion.

Our study showed that animals with an increased Hct have increased plasma nitrate/nitrite concentrations relative to untreated animals (P < 0.05). Intact endothelial cells release NO in response to vasoactive substances and shear stress (13, 36). Endothelial NO has been shown to maintain normotension to prevent cardiovascular dysfunction and to influence survival in polyglobilic mice (30). The associated increase in Hct would increase shear stress and stimulate vasodilation via NO release (11).

It should be noted that populations at high altitudes have a higher Hct and have been reported to present high levels of exhaled NO (1). Our findings suggest that a possible explanation for this phenomenon is the increased NO production in the pulmonary circulation due to increased shear stress. Thus maintaining the Hct level in the physiological range or increasing it within limits is crucial to guarantee the necessary shear stress for producing vasodilation via inhibition of smooth
muscle tone, as well as modulating platelet aggregation and leukocyte binding to the endothelium and lipoprotein metabolism (33). Therefore, our data suggest that maintaining Hct within normal limits may be a requirement for blood pressure homeostasis.

De Simone et al. (5) recently showed a negative relationship between systolic blood pressure and whole blood viscosity among American Indians participating in the Strong Heart Study, whereby lower whole blood viscosity and lower Hct were related \( (P < 0.01) \) to higher pulse pressure. This finding in a population of untreated persons could be explained by our hypothesis that maintaining whole blood viscosity in its physiological range is crucial in generating the necessary shear stress to stimulate NO release of endothelial cells. In this study, blood pressure ranged from normotensive \((120/71 \pm 11/8)\) to mild hypertension \((149/82 \pm 12/11)\), approximately corresponding to the initial portion of the bimodal relationship between blood pressure and whole blood viscosity found in our study. Although significantly increased blood viscosity is associated with elevated blood pressure, our data suggest that small increases in blood pressure might be related to lowered whole blood viscosity as found by de Simone et al.

In a clinical context, the acute changes in Hct reported in our study may occur during treatment of hypertension with diuretic drugs and during hemodialysis. Diuretics are widely used for treatment of hypertension and are presumed to reduce blood pressure mainly by reducing plasma volume. However, plasma volume contraction leads to hemoconcentration, which in turn increases blood viscosity and presumably shear stress at the vascular endothelium, stimulating the release of NO. The same mechanism of hemoconcentration after hemodialysis might contribute to the severity of hemodialysis-related hypotension. Hypotensive episodes are a major complication of hemodialysis. Nette et al. (28) recently showed that dialysis-related hypotension seems to result from a reduction in left ventricular function and the inability of the vasculature to adequately increase arteriolar tone after reduction of plasma volume. Thus acute hemoconcentration and the correspondingly increased shear stress-induced NO release could be an additional factor in preventing the vasculature to maintain arteriolar tone. The results of this study may be explained by experiments with blood flowing in capillary tubes, which show that the RBC column is surrounded by a plasma layer that shields the intima from extensive contact with RBCs (20). With the increase of Hct, the RBC column expands, which in turn decreases the size of the plasma layer, causing erythrocytes to more effectively interact with endothelial cells, leading to increased shear stress and thus stimulation of NO release. Shear stress is essential to maintain endothelial cell integrity and functioning, and decreased shear stress can lead to apoptosis (7, 19). Because cardiovascular diseases have been shown to be accompanied by endothelial dysfunction, it could be of clinical interest to focus on patients’ Hct levels, given the importance of Hct in generating shear stress.

In our study there was a statistically nonsignificant increase in blood volume during the course of the experiment, which was corroborated by the finding that the increase in Hct induced by the exchange transfusion of packed RBCs was halved by the end of the experiment. This latter effect was consistent and present in all ranges of Hct increase. It is probably because of the decrease in capillary blood pressure due to the lowering in blood pressure for the initial stages of Hct increase and because of the augmented viscous losses in the precapillary circulation as Hct is increased to higher values. This small increase in blood volume is notable, because, in general, it would be associated with an increase in blood pressure. In principle, diuretics are supposed to lower blood pressure through a mechanism that lowers blood volume. Thus the noted decreases in blood pressure cannot be associated to a decrease in blood volume; furthermore, although Hct tended to return to near normal values, blood pressure remained at the new steady state.

In conclusion, this study shows that increasing Hct has a biphasic effect on blood pressure, which is initially lowered due to stimulation of NO release by the endothelium that may be due to increased shear stress. This effect is eventually overwhelmed by the increase in peripheral vascular resistance due to the exponential increase of blood viscosity as Hct increases further. The finding that small increases in Hct lower blood pressure provides an additional explanation of the action of diuretics in the treatment of hypertension and suggests that in some conditions Hct and blood viscosity may be lower than the level needed for ensuring the normal function of the cardiovasculature.

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