INCREASED CARDIAC OUTPUT AND MICROVASCULAR BLOOD FLOW DURING MILD HEMOCONCENTRATION IN THE HAMSTER WINDOW MODEL

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Running head: Cardiac output during mild hemoconcentration

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ABSTRACT

The effect of small hematocrit (Hct) increases on cardiac index (cardiac output / body weight) and oxygen release to the microcirculation was investigated in the awake hamster window chamber model, by means of exchange transfusions of homologous packed red blood cells. Increasing Hct between 8-13% from baseline increased cardiac index by 5 - 31% from baseline (P < 0.05) and significantly lowered systemic blood pressure (P < 0.05). The relationship between Hct and cardiac index is described by a second order polynomial ($R^2 = 0.84; P < 0.05$) showing that Hct increases up to 20% from baseline increase cardiac index whereas increases over 20% from baseline decrease cardiac index. Combining this data with measurements of blood pressure allowed to determine total peripheral vascular resistance which was a minimum at 8 - 13% Hct increase and was described by a second order polynomial ($R^2 = 0.83; P < 0.05$). Oxygen measurements in arterioles, venules and the tissue at 8 - 13% Hct increase were identical to control, thus as a consequence of increased flow and oxygen carrying capacity, oxygen delivery and extraction increased but the change was not statistically significant. Previous results with the same model showed that the observed effects are related to shear stress mediated release of nitric oxide (NO), an effect that should be also present in the heart microcirculation, leading to increased blood flow, myocardial oxygen consumption and contractility. We conclude that a minimum viscosity level is necessary for generating the shear stress required for maintaining normal cardiovascular function.

KEY WORDS: Shear stress, blood viscosity, hematocrit, blood pressure, cardiovascular function;
The primary function of the circulatory system is the transport of oxygen and materials to metabolically active tissues and to remove waste products. The rate of oxygen transport is determined by the product of blood flow and arterial oxygen content. Under physiological conditions oxygen supply to tissues can be adjusted through changes in either blood flow or the oxygen content of blood. Blood flow is mainly affected by cardiac output and total peripheral vascular resistance whereas arterial oxygen content is a function of hematocrit (Hct).

It has been extensively shown that cardiac output and Hct are inversely related (31, 37, 38). It is well established that polycythemia reduces cardiac output while anemia has the opposite effect. In 1959 Richardson and Guyton performed landmark experiments on mongrel dogs (38) showing that increasing Hct by 47% of baseline decreases cardiac output by 51% of baseline. A similar result was recently reported by Lindenfeld et al. (33) who showed a non significant fall in resting cardiac output when hematocrit was increased from 35% to 50%. The decreased cardiac output in hemoconcentration was attributed to the increase of whole blood viscosity at higher Hcts and to the corresponding increase of total peripheral vascular resistance. The opposite condition of hemoconcentration, namely anemia, was found to cause an increased cardiac output (26% of baseline) even though the changes in cardiac output under anemic conditions were less pronounced than under hemoconcentration.

Richardson and Guyton also found that the maximum rate of erythrocytes oxygen delivery to the tissues occurred at an Hct of 40%, which was within 0.6% of the mean control Hct of the investigated dogs. From this observation arises the concept of “optimal hematocrit” in terms of tissue oxygen delivery. It has been reported that systemic oxygen
transport (arterial oxygen content times cardiac output) decreases following hemoconcentration (47). However Fan *et al.* (20) showed that oxygen supply to internal organs is maintained at a constant level until systemic Hct is increased above 60%.

A recent study of this laboratory (36) shows that increasing Hct between 7-13% of baseline decreases blood pressure by 10 mmHg and increases microvascular blood flow by 39% of baseline levels. It was hypothesized that the increased Hct leads to increased shear stress at the vascular endothelium causing vasodilatation via the release of nitric oxide (NO). It was proposed that this effect is eventually overwhelmed when Hct is increased more than 20% of control leading to a blood pressure increase. This study showed for the first time that elevating Hct above normal control levels is not necessarily associated with an increase in total peripheral vascular resistance and a decrease in blood flow. Whereas most of the previous investigations have explored the effect of greater Hct changes (20, 31, 33, 38) our study investigated the effect of small changes in Hct on blood pressure and blood flow.

The noted increase in microvascular blood flow in the presence of a lowering of systemic blood pressure at the optimal Hct suggests that these effects are centrally mediated, and not limited to the tissue of the hamster chamber window model studied. Therefore we carried out this study to test the hypothesis that small increases in hematocrit in an otherwise normal population significantly change cardiac output and total peripheral vascular resistance. To further explore our hypothesis we also investigated the effects of small Hct increases on oxygen transport in the hamster chamber window model.
MATERIALS AND METHODS

Animal preparation. Investigations were performed in 55 - 65 g male Golden Syrian Hamsters (Charles River Laboratories, Boston, MA). 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. The window chamber model is widely used for microvascular studies in the unanesthetized state, and the complete surgical technique is described in detail elsewhere (12, 19). Briefly, hamsters were prepared for chamber implantation with a 50 mg/kg ip injection of pentobarbital sodium anesthesia. 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 with a 15-mm circular window. With the aid of backlighting and a stereomicroscope, one side of the skinfold was removed following the outline of the window until only a thin layer of retractor muscle and the intact subcutaneous skin of the opposing side remained. Saline and then a cover glass were placed on the exposed skin held in place by the other part of the chamber. The intact skin of the other side was exposed to the ambient environment. The area of microscopic observation is originally located just behind the large front vessels that feed and drain the chamber network. A modified preparation technique was used in this study, where the investigated tissue is nearer to the animal’s head to allow microscopic observation of the large A1 arterioles right as they branch off the large feeding arteriole (A0) and the large collecting venules shortly before they merge into the large draining vein (V0) of the chamber network. The animal was allowed at least 2 days for recovery before the preparation was assessed under the microscope for any signs of edema, bleeding or unusual neovascularization. Barring these complications, the animal was anesthetized again with pentobarbital sodium. Arterial and venous catheters (PE-50) were implanted in the carotid artery and jugular vein.
The catheters were filled with a heparinized saline solution (30 IU/ml) to ensure their patency at the time of 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. Three to four days after the initial surgery the microvasculature was examined and only animals passing established systemic and microcirculatory inclusion criteria such as having tissue void of low perfusion, inflammation, and edema were entered into the study (46).

**Inclusion criteria.** Animals were suitable for the experiments if 1) systemic parameters were within normal limits, namely, heart rate (HR) > 340 beats/min, mean arterial blood pressure (MAP) > 80 mmHg and < 125 mmHg, systemic Hct > 45%, arterial PO2 (PaO2) > 50 mmHg; and 2) microscopic examination of the tissue in the chamber observed under X650 magnification did not reveal any signs of edema and bleeding.

**Systemic parameters and blood chemistry.** 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). Arterial blood was collected in heparinized glass capillaries (0.05ml) from the carotid catheter and immediately analyzed for PaO2, PaCO2, and pH (Blood Chemistry Analyzer 248, Bayer; Norwood, MA). The comparatively low PaO2 and high PaCO2 of these animals is a consequence of their adaptation to a fossorial environment.

**Experimental groups.** Experiments were carried out in three animal groups: Increased Hct group (n = 6), control group (n = 5) and cardiac output group (n = 13).
Isovolemic exchange transfusion with packed red blood cells (RBCs). 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. Donor blood was centrifuged and RBCs were separated from plasma in order to obtain packed RBCs. The volume of exchange-transfusion was calculated as a percentage of the animals’ blood volume. Different levels of Hct increases were obtained by varying the exchange transfusion volume between 8 and 15% of the animals’ blood volume (estimated as 7% of the animals’ body weight). Since animals weights ranged between 55 - 65 g, their blood volumes ranged between 3.9 - 4.6 ml and their actual exchange transfusion volumes ranged between 0.3 - 0.7 ml. The actual transfusion volume varied between the animals in order to achieve different levels of hematocrit increases. Packed RBCs were infused with a dual syringe pump (‘33’ syringe pump, Harvard Apparatus Inc., Holliston, MA) into the jugular vein catheter at a rate of 100µl/min. 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. Animals were followed up for 120 min. BP and HR measurements were taken 3 times during a 30 min period (at 5, 15 and 25 minutes) each measurement representing an average BP over 4 minutes. At the end of the observation period Hct and hemoglobin were measured again.

Assessment of microcirculatory parameters. Animals 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 a transillumination intravital microscope (BX51WI, Olympus, New Hyde Park, NY). The tissue image was projected onto a CCD camera (COHU 4815-2000, San Diego, CA) connected to a video cassette recorder.
(Panasonic AG-7355, Tokyo, Japan) and viewed on a Sony monitor (PMV-1271Q, Tokyo, Japan). Measurements were made using a 40X (LUMPFL-WIR, NA 0.8, Olympus) water immersion objective. Sites of investigation were chosen based on their visual acuity and location within the microvascular network. Since the large feeding arterioles (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 comparison could be made directly with baseline levels. Red blood cell velocity in A1 arterioles were measured on-line by using the photodiode/cross-correlator system (28)(Photo Diode/Velocity Tracker Model 102 B, Vista Electronics, San Diego, CA). A video image-shearing method was used to measure vessel diameter (29). The centreline velocity was corrected according to vessel size to obtain the mean RBC velocity ($V$) (35). Measurements of vessel diameter and red blood cell velocity were taken by the investigator at every selected time point. The investigator was not blinded as to treatment group and time during the experiment. Blood flow was calculated from the measured values as $Q = V \times \pi (D/2)^2$. Measurements of vessel diameter, red blood cell velocity and blood flow were made at baseline, 30, 60, 90 and 120 minutes after the exchange transfusion with packed red blood cells.

**Microvascular pO2 distribution.** High resolution non-invasive microvascular pO2 measurements were made at baseline in the control group and 120 min after exchange transfusion with packed red blood cells in the increased Hct group using phosphorescence quenching microscopy (PQM) (44). PQM is based on the oxygen dependent quenching of phosphorescence emitted by an albumin-bound metalloporphyrin complex after pulsed light excitation. PQM is independent of the dye concentration within the tissue and is used to measure both, intravascular and extravascular pO2 since the albumin-dye complex continuously extravasates into the interstitial tissue (7, 45). Interstitial pO2 measurements
have been found to be identical to simultaneous measurements made with recessed electrodes (7). Tissue pO₂ was measured in regions between capillaries. The reported values are the average of several determinations in several animals. PQM allows precise localization of the pO₂ measurements without subjecting the tissue to injury. The measurements provide detailed understanding of the microvascular oxygen distribution and show whether oxygen is delivered to interstitial areas.

The system setup has been in detail described elsewhere (30, 44). Animals received a slow intravenous injection of 15mg/kg palladium-meso-tetra (4-carboxyphenyl) Porphyrin (Porphyrin Products, Inc., Logan, UT; concentration 10.1 mg/ml) which is allowed to circulate for 20 minutes. The phosphorescence was excited by pulsed light (30Hz, 4µsec duration) for a period of < 5sec; the measurement site was microscopically vignetted by an adjustable slit. For intravascular measurements an optical rectangular slit, approximately 5 X 35 µm, was positioned longitudinally within the vessel of interest. For interstitial tissue measurements a 15 X 10 µm slit was placed in intercapillary spaces in regions void of large vessels. The phosphorescence decay curves were analyzed off line, using a standard single exponential least squares numerical fitting technique and the resultant time constants were applied to the Stern-Volmer equation to calculate pO₂, using predetermined parameter corrected for this model (30).

**Cardiac output measurements.** Cardiac output (CO) was measured with the modified thermodilution technique described by Cabrales et al. (8). For this procedure a different group of animals not investigated in terms of microvascular parameters has been used due to the complexity of the setup and the difficulty of positioning the instrumented animal on the microscope. After analysis of baseline parameters (Hct, hemoglobin, arterial blood gases, MAP and HR) baseline CO was measured. Exchange transfusion with packed red blood cells
was started 5 - 10 minutes later and CO was measured again after 60 min. Because of volume addition with each cardiac output measurement (150 µl of saline) measurements can maximally be taken twice in the same animal to avoid errors. In addition to the baseline measurement, the 60 min time point was chosen because microvascular flow data showed the maximum increase from baseline 60 min after exchange with packed red blood cells.

**Oxygen release.** Oxygen release to the microcirculation was calculated by means of the following equation

\[ O_2 \text{ release} = \text{Hct} \times \Delta S_{A-V} \times \gamma \times \text{MCHC} \times Q \quad (1) \]

where \( \Delta S_{A-V} \) is the difference in \( O_2 \) saturation between arterioles and venules given by the \( O_2 \) dissociation curve, \( \gamma \) is the oxygen carrying capacity of hemoglobin (Hb) at 100% saturation (1.34 ml \( O_2 \)/g Hb), MCHC is the amount of hemoglobin in grams per unit RBC volumetric concentration (32.2 g/dl of RBCs) and \( Q \) is the average flow (relative to baseline) through arteriolar and venular vessels 120 min after exchange transfusion with packed RBCs.

**Statistical analysis.** Results are presented as means ± SD unless otherwise noted. Data in the Microhemodynamics section are presented as relative to levels at baseline. A ratio of 1.0 signifies no change from baseline while lower or higher ratios are indicative of changes proportionally higher or lower than baseline. Comparison of baseline values from the three different groups of animals was performed using one way analysis of variance (ANOVA) and post hoc analyses were performed with the Bonferroni’s multiple comparison test. Data within each group were analyzed using ANOVA for repeated measures and followed by the Bonferroni’s multiple comparison test. Changes were considered statistically significant if \( P < 0.05 \).
RESULTS

**Microhemodynamics.** 6 hamsters were used to study microcirculatory parameters in 25 A1 arterioles and 24 large venules (Vl) at 30, 60, 90 and 120 min after exchange transfusion with packed red blood cells. Since the effect of a wide range of Hct increases (4 - 25% of baseline) has already been extensively studied by Martini et al., (36) in this study we focused on Hct increases between 7-13% of baseline, which have been shown to cause the maximum blood pressure drop, to increase microvascular vessel diameters, red blood cell velocities and blood flow compared to baseline.

**Increased Hct group (n = 6):**

*Arterioles.* A1 arterioles (baseline diameter 51.4 ± 14.9 µm) showed a significant vasodilation from baseline at 30 and 60 min after exchange transfusion (1.09 ± 0.16 and 1.10 ± 0.20 respectively; P < 0.05). The maximum vasodilation occurred at 60 min whereas diameters returned toward baseline levels at 90 and 120 min after exchange transfusion (1.05 ± 0.13 and 1.02 ± 0.15 respectively) (Fig. 1). Red blood cell velocities (baseline values 5.3 ± 2.8 mm/s) were significantly increased compared to baseline at 60 and 120 min after exchange transfusion with packed red blood cells (1.24 ± 0.44 and 1.29 ± 0.55 respectively; P < 0.05) whereas at 30 and 90 min there was no significant change from baseline velocity (1.14 ± 0.36 and 1.21 ± 0.48 respectively). The maximum velocity increase occurred 120 min after exchange transfusion (Fig. 2). Microvascular blood flow (baseline values: 14.05 ± 15.10 nl/s) showed no significant change from baseline at 30 min (1.32 ± 0.61) but was significantly increased at all following time points, showing the maximum increase 60 min after the exchange transfusion (60 min: 1.57 ± 0.79; 90 min: 1.46 ± 0.70 and 120 min: 1.40 ± 0.64; P < 0.05) (Fig. 3). All microvascular data is normalized to baseline.

*Venules.* Large venules (baseline diameter 63.03 ± 28.14 µm) dilated from baseline at 60 and 120 min after exchange transfusion, though statistically not significant (60 min: 1.02 ±
0.15 and 120 min: 1.03 ± 0.13). At 30 min diameters showed no difference from baseline and at 90 min large venules showed a slight, statistically not significant vasoconstriction (0.98 ± 0.15) (Fig. 1). Red blood cell velocities (baseline values 1.2 ± 0.4 mm/s) increased from baseline at all time points though statistically not significant (30 min: 1.14 ± 0.36, 60 min: 1.19 ± 0.4, 90 min: 1.12 ± 0.41 and 120 min: 1.12 ± 0.41) (Fig. 2). Venular blood flow (baseline values 5.8 ± 11.3 nl/s) increased at all time points compared to baseline (30 min: 1.15 ± 0.42, 60 min: 120 ± 0.40, 90 min: 1.03 ± 0.39) showing a significant increase only at 120 min (1.25 ± 0.52; P < 0.05) (Fig. 3).

**Control Group** (n = 5):

5 hamsters were used to study microcirculatory parameters in 18 A1 arterioles (diameter 55.96 ± 11.42 µm, red blood cell velocity 6.29 ± 2.61 mm/s, blood flow 14.84 ± 9.46 nl/s) and 25 large venules (diameter 63.83 ± 29.99 µm, red blood cell velocity 1.37 ± 0.29, blood flow 6.0 ±7.33 nl/s) without increasing Hct. Microvessel diameter, red blood cell velocity and blood flow was not statistically different from baseline values of the Hct augmented group.

**Blood pressure.** We have previously shown that after isovolemic exchange transfusion with packed red blood cells, blood pressure decreased in proportion to the percentage rise in Hct, a behavior that was best described by a second order polynomial (36). In the current study blood pressure values of Hct augmented animals followed the previously observed trend: Increasing Hct to the range of 7 - 13% of baseline produced the maximum blood pressure drop (6 - 13 mmHg of baseline). Increasing Hct further (up to 20% of baseline) made blood pressure return towards baseline levels. We have previously shown that Hct increases greater than 19% of baseline increased systemic blood pressure above baseline (1 - 15 mmHg) (36). In the current study we omitted repeating Hct increases above 20% of
baseline, since this study was designed to examine the effect of small Hct increases on oxygen transport parameters and cardiac output.

**Microvascular oxygen distribution.** Animals of the control group (n = 5) were used to acquire baseline oxygen tensions. No statistical differences were found of systemic and microvascular data at baseline compared to the Hct augmented group so that comparison could be made.

As previously reported by Martini et al. (36) at the end of the experiment Hct was approximately 50% lower than immediately after exchange transfusion. At this time point Hcts of the previously exchanged transfused animal group were not significantly different from the Hcts of the control group (48.8% ± 1.5 vs. 46.2% ± 3.1). Consequently, microvascular pO2 measurements in arterioles, venules and tissue were not different between these groups (arteriolar pO2 in Hct augmented animals averaged 44 ± 4 mmHg vs. 45 ± 5 mmHg in control animals; venular pO2 in Hct augmented animals averaged 34 ± 5 mmHg vs. 36 ± 7 mmHg in control animals; tissue pO2 was 24 ± 6 mmHg in Hct augmented animals and 25 ± 4 mmHg in control animals).

**Oxygen release.** Table 1. shows the calculated values for oxygen release to the microcirculation for Hct augmented and the control animals. Hct augmented animals showed a 20% increase in oxygen release to the chamber microcirculation; however this increases was not statistically significant due to cumulative effect of the errors in each measurement. This effect was mainly attributed to the significantly increased microvascular blood flow (P < 0.05). A factor contributing to the variability of the oxygen delivery data was the fact, that volumetric flow was in general not conserved between arterioles and venules because of the few vessels present in the chamber, and the dispersion of sizes and flow velocities. As a consequence we used the average of the arteriolar and venular flows in the calculations. The
increase in oxygen delivery at the Hct increase where systemic effects were maximal although not statistically significant, suggests that there may be a weak parabolic trend in the microvascular oxygen delivery data; however this would not appear to be physiologically significant in the tissue studied.

**Cardiac output.** Cardiac output was measured in 13 hamsters at baseline and 60 min after exchange transfusion with packed red blood cells. In order to account for weight differences between the animals, cardiac output data were converted into cardiac index (cardiac output/body weight). The 60 min time point was chosen because arteriolar flow data showed the maximum increase from baseline 60 min after the exchange with red blood cells. In this animal group a larger range of Hct increases (2 - 20% of baseline) than in the microcirculation group (7 – 13% of baseline) was induced in order to explore the effect on cardiac output within the entire range of Hct increases that were previously (36) shown to decrease blood pressure, induce vasodilation and increase microvascular blood flow.

We found that increasing Hct up to 20% of baseline increases cardiac index between 5 - 31% of baseline with the maximum effect when Hct is increased 8 - 13% of baseline. If Hct is augmented above 20%, cardiac index decreases. This relationship is best described by a second order polynomial ($R^2 = 0.84; P < 0.05$) (**Fig. 4**). During the entire experiment animals were monitored in terms of blood pressure showing that the maximum increase in cardiac index corresponded with the maximum blood pressure decrease. Blood pressure changes after exchange transfusion with packed red blood cells are best fitted by a second order polynomial ($R^2 = 0.62; P < 0.05$) (**Fig. 4**).

**Vascular resistance.** Vascular resistance was calculated by dividing the animals MAP by the cardiac index value, measured 60 min after exchange transfusion with packed red blood cells. **Fig. 5** shows that Hct increases up to 20% from baseline exert a biphasic effect
on vascular resistance causing vascular resistance to decrease to 70% of baseline following Hct increases up to 10% of baseline. Further increases in Hct produce an increase in vascular resistance which reaches values above baseline if Hct is increased more than 20% from baseline ($R^2 = 0.83; P < 0.05$).
DISCUSSION

The principal finding of this study is the observation of a parabolic relationship between Hct and cardiac index. Progressively increasing Hct up to 10% from baseline increased cardiac index by 31% from baseline. Further increases in Hct lead cardiac index return to baseline in a step wise manner at 20% increase in Hct. The augmented Hct caused an increase in microvascular blood flow and a trend toward a slight increase in oxygen delivery and extraction in the tissue of the hamster window chamber; however the variability in the calculation due to the cumulative effect of the errors in each measuring method, precluded obtaining statistical significance for this result.

Exchange transfusions from donor animals, even when these are virtually identical specimens, may cause anaphylactic reactions resulting in hypotension, tachypnea, tachycardia, fever, chills, hemoglobinemia and hemoglobinuria. In this context we note that: 1) there was no change in the animals’ body temperature which was measured continuously as a requirement for the cardiac output measurements (37.7 ± 0.47 ºC at baseline vs. 37.72 ± 0.29 ºC at the end of the experiment); 2) none of the exchange transfused animals showed an acute cardiovascular decompensation with hypotension and tachycardia, and the decrease in blood pressure after exchange transfusion occurred slowly and was not accompanied by an increase in heart rate; 3) we didn’t detect any signs of hemolytic transfusion reactions which would colour the plasma red. Therefore on the basis of these observations, we ruled out that our findings may in part be due to the occurrence of anaphylactic reactions.

The concept of “optimal hematocrit” has been extensively discussed during the past 40 years (13, 14, 41). Variations in hematocrit markedly influence the ability of the cardiovascular system to function at its maximum capacity. Anemia impairs the ability of the
circulatory system to deliver oxygen to tissues because of the reduced oxygen carrying capacity of blood. Chronic anemia may result in increased cardiac output secondary to decreased afterload, increased preload and increased chronotropic and inotropic effects, which may lead to left ventricular hypertrophy and congestive heart failure (21, 40). On the other hand the opposite condition, polycythemia, impairs oxygen transport to tissues because of an excessive increase in whole blood viscosity. Conditions associated with high Hcts such as Polycythemia vera or adaptation to high altitudes are reported to increase MAP and to decreased cardiac output due to a viscosity dependant increase in vascular resistance (27, 38). In conditions of a compromised circulatory system, where vessels walls have lost their elasticity due to atherosclerotic lesions and their response to vasoactive vasodilatory transmitters is reduced, high Hct values may further impair organ perfusion. Many studies show that high Hcts may be associated with coronary heart disease (42), cerebrovascular disease (43) and peripheral vascular disease (17).

The regulation of cardiac output was first studied by Frank and Starling who found that the output of the heart is mainly determined by the inflow into the heart except when this inflow is excessively increased or when the pumping capacity of the heart is decreased (26). Guyton et al. showed that an increase in venous resistance significantly decreases cardiac output while an increase in arterial resistance has very little effect on cardiac output (25). This finding is mainly attributed to the fact that the venous portion of the vascular system has a much greater distensibility and volume than the arterial portion. It follows that increasing the resistance from the venous system to the heart can cause ample storage of blood in the veins. Based on this fact Richardson and Guyton (38) hypothesized that high Hcts produce sluggishness in the venous return and would therefore decrease cardiac output.
We have shown (36) that increasing Hct between 7 - 13% from baseline significantly increased vascular shear stress which induced the release of NO, leading to vasodilation and a significantly increased flow in A1 arterioles. In line with these results, in the current study we found that venular blood flow also significantly increased under conditions of small increases in Hct. Following Richardson’s and Guyton’s hypothesis, the increased flow on the venous side might cause an augmented venous return, increased pre-load of the heart and therefore an increased cardiac output.

It is plausible to assume that the microcirculatory flow increases also occur in the heart leading to improved oxygen delivery to myocytes and enhanced cardiac function. It has been shown that increased coronary perfusion increases myocardial contraction and oxygen consumption, a phenomenon known as the Gregg effect (1, 15, 24, 32). It could be hypothesized that the increased oxygen consumption by the myocardium and the resulting increased contractility contributes to the increase in cardiac output found in our experiments. However this hypothesis remains to be further investigated.

Resting left ventricular myocardial oxygen consumption is very high (~ 60µl/min/g) and is accomplished by a high oxygen extraction rate (75%). Under conditions of increased myocardial oxygen consumption, such as exercise, the need for increased oxygen delivery to myocytes can only be produced by enhanced coronary blood flow. The balance between myocardial oxygen consumption and oxygen delivery through coronary flow is described by the coronary venous oxygen content. It has been shown that inhibition of NO synthesis decreases coronary venous oxygen content (2, 5), indicating that NO mediated vasodilation plays an important role in increasing oxygen delivery to the myocardium. Numerous studies suggest that the vasodilator effect of NO takes place mainly in the large coronary arteries (4, 10, 11).
The increase in cardiac index is clearly a reflection of the over all lowering of peripheral vascular resistance, in the presence of an increase of viscosity dependant vascular resistance. This is significant because it shows that there is a range of Hct increase where the NO mediated mechanism responsible for this effect described by Martini et al. (36), outweighs the purely mechanical effects due to increased blood viscosity which are linearly related to changes in vascular resistance. This is in part due to anatomical dependant vascular resistance being related to vessel diameter to the fourth power, which magnifies any small consistent variation of vessels diameter.

Our results indicate the existence of a range in which Hct increases from baseline are beneficial in terms of cardiac output, systemic blood pressure regulation, microvascular blood flow and oxygen release to peripheral tissues. Increasing Hct over 20% from baseline would appear to be the limit that is compensated for by vasodilation and greater augmentations lead to an increase in peripheral vascular resistance, decreased microcirculatory blood flow, and decreased venous return. The combination of decreased pre-load, increased after-load and reduced perfusion of the heart microcirculation would cause cardiac output to drop. It would appear that the experiments of Richardson and Guyton (38) and Lindenfeld et al. (33) were carried out at a hematocrit increase > 35% from baseline and therefore beyond the range in which compensatory vasodilatation counteracts for increased vascular resistance due to increased blood viscosity. In fact it is possible that at such high hematocrits there may also be present an autoregulatory reaction due to increased oxygen delivery tending to lower blood flow (33).

Data from the literature supports the concept that small hematocrit increases may lower vascular resistance. Lipowsky and Firrell (34) made direct measurements of regional
pressure drop and flow in arterioles during slight hemoconcentration (~10% increase in packed RBC fraction) and found that regional vascular hindrance decreased, when normalizing resistance for viscosity increases. These investigators performed hemoconcentration experiments in cats and observed a plateau in arteriovenous resistance at moderate Hct increases which was attributed to the occurrence of vasodilation. Crowell et al. (13) found that in dogs at the optimal Hct of 42% peripheral resistance shows a plateau for small Hct changes and increases drastically at Hct values higher than the optimal one.

The presented data provide a new insight in the physiology of the cardiovascular system, showing the existence of a mechanism that allows Hct to vary within a specific range without becoming pathological. This mechanism may be basic for adaptation to varying physiological and environmental conditions. For instance polycythemia may be a mechanism for augmenting shear stress mediated NO release in high mountain dwellers for preventing hypoxic pulmonary vasoconstriction (3) and thus pulmonary hypertension. The same mechanism may prevent hypertension in polyglobulic mice overexpressing the gene for erythropoietin (39).

The results presented in this paper are relevant to the use of erythropoietin (EPO) as a performance enhancer in sports. EPO stimulates the proliferation and differentiation of erythroid progenitor cells in the bone marrow, thus increasing red blood cell mass and alleviating tissue hypoxia (6, 23). The most important factor for physical performance in endurance sports is the aerobic metabolism or the so called maximal aerobic power (VO$_{2\text{max}}$) (18). VO$_{2\text{max}}$ is mainly dependent on cardiac output and stroke volume and it has been shown that reinfusion of whole blood as well as EPO administration increases VO$_{2\text{max}}$. Our results suggest that the mechanism underlying this phenomenon could take place in the microcirculation, where the increased Hct results in increased shear stress and thus NO
production with the direct consequence of improved microvascular blood flow and oxygen delivery. An important consideration is that the effects seen in our study take place in the organism at rest, when the vascular vasodilatory capacity is still available. This situation is markedly different in an organism striving to attain maximal performance, when the vasodilatory capacity is no longer available. In this situation increased Hct and shear stress will not elicit the additional non linear flow augmentation deployed in the organism at rest, and the increase in oxygen carrying capacity due to increased Hct is negated by the increased peripheral vascular resistance due to the increased viscosity.

The concept of “optimal Hct” may be relevant also in terms of cardiovascular morbidity and mortality. Gagnon et al. (22) examined the data of 5209 men and women who had been followed for 34 years for the development of cardiovascular diseases such as stroke or TIA, congestive heart failure, intermittent claudication, coronary heart disease, myocardial infarction and angina pectoris. They found a J- or U-shaped relationship between Hct and cardiovascular disease, indicating that the optimal Hct for women was 42 - 43% and 45 - 46% for men. Lower and higher Hct groups showed significantly elevated incidence of cardiovascular disease compared to the mid Hct group.

The finding that vascular resistance decreases to 70% of baseline if Hct is increased by 10% of baseline is remarkable. It has been recently shown by de Simone et al. (16) that lower whole blood viscosity and lower Hct were related to higher pulse pressure (P < 0.01). These and our findings suggest that whole blood viscosity and thus Hct plays an important role in regulation of vascular resistance. Since a disproportional increase in vascular resistance seems to be a main factor in promoting cardiovascular diseases such as essential hypertension or myocardial hypertrophy, the beneficial effect of small increases in Hct and thus whole blood viscosity on vascular resistance should be recognized.
Lowering blood viscosity has been considered to be beneficial since the beginning of the practice of medicine and is presently embodied in the practice of hemodilution. However, our results suggest that the concept of a beneficial effect due to lowered blood viscosity on cardiovascular health should be evaluated. A specific level of blood viscosity appears to be necessary for generating shear stress and stimulating the release of vasoregulatory substances such as NO and prostacyclin. These effects have been demonstrated in acute models of extreme hemodilution (46) and shock resuscitation (9). We presently show that small increases in Hct and thus whole blood viscosity have a beneficial effect on cardiac function increasing cardiac output. We therefore conclude that maintenance of blood viscosity, to the present ignored as a homeostatic mechanism, is a major factor in promoting normal cardiovascular function.

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REFERENCES


Fig. 1. Changes in arteriolar and venular diameter 30, 60, 90 and 120 min after increasing Hct 8 - 13% from baseline. Arterioles dilated significantly 30 and 60 min after exchange transfusion with packed red blood cells. Venular diameters didn’t statistically change from baseline.

*, P < 0.05 compared to baseline

Fig. 2. Changes in arteriolar and venular red blood cell (RBC) velocity after increasing Hct 8 - 13% from baseline. Arteriolar red blood cell velocities significantly increased from baseline 60 and 120 min after exchange transfusion showing the maximum increase at 120 min. Venular velocities increased from baseline at all time points though statistically not significant.

*, P < 0.05 compared to baseline

Fig. 3. Changes in arteriolar and venular microvascular blood flow after increasing Hct 8 – 13% from baseline. Arteriolar flow was significantly increased from control at 60, 90 and 120 min and showed the maximum increase at 60 min after the exchange transfusion. Venular blood flow was significantly increased 120 min after the exchange transfusion.

*, P < 0.05 compared to baseline

Fig. 4. Relationship between Hct, blood pressure and cardiac index. Increasing Hct between 8-13% from baseline results in the maximum blood pressure drop (~ 10 mmHg from baseline) and is associated with the maximum increase in cardiac index compared to baseline (31%). Increasing Hct more than 20% from baseline causes blood pressure to increase above baseline levels and cardiac output to decrease below baseline levels. Both relationships (Hct vs. blood
pressure and Hct vs. cardiac index) are best described by a second order polynomial ($R^2 = 0.62$ and $P < 0.05$ and $R^2 = 0.84$ and $P < 0.05$ respectively).

**Fig. 5.** Relationship between vascular resistance and percentage of Hct increase. Increasing Hct up to 20% from baseline has a biphasic effect on vascular resistance: Increasing Hct by 10% from baseline shows the maximum drop in vascular resistance (~70% from baseline); further increases in Hct bring vascular resistance back towards baseline values and eventually, if Hct is increased over 20% from baseline, increase vascular resistance above baseline levels. This relationship is best described by a second order polynomial ($R^2 = 0.83; P < 0.05$).

**Table 1.** Oxygen release between A1 arterioles and large venules (V1) in the hamster window chamber. Increasing Hct between 8 - 13% results in a 20% increase in oxygen delivery to tissues, mainly through significantly increased microvascular blood flow.
FIGURES

**Figure 1**

Arterioles (n = 25)

Venules (n = 24)

Arteriolar and venular diameter (normalized to baseline)

- 30 min
- 60 min
- 90 min
- 120 min

* *
Arteriolar and venular velocity (normalized to baseline)

Fig. 2

- Arterioles (n = 25)
- Venules (n = 24)
Arteriolar and venular flow (normalized to baseline)

- Arterioles (n = 25)
- Venules (n = 24)

Fig. 3
Fig. 4

Blood pressure change
(mmHg from baseline)

Cardiac index change
(% from baseline)

Blood pressure

Hct increase
(% from baseline)

Cardiac index
Fig. 5

Vascular resistance (normalized to baseline)

Hct increase (% from baseline)
After 8 – 13% increase in hematocrit

<table>
<thead>
<tr>
<th>Control</th>
<th>After 8 – 13% increase in hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ release =</td>
<td>Flow (relative to baseline)</td>
</tr>
<tr>
<td>Hct × ΔSₘ₋ᵥ × γ × MCHC × Q</td>
<td>O₂ release</td>
</tr>
<tr>
<td>A1 - V1</td>
<td>4.0 ml O₂ / s</td>
</tr>
</tbody>
</table>

Table 1