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Optimal hematocrit in an artificial microvascular network

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Abstract

BACKGROUND—Higher hematocrit increases oxygen carrying capacity of blood, but also increases blood viscosity, thus decreasing blood flow through the microvasculature and reducing the oxygen delivery to tissues. Therefore, an optimal value of hematocrit that maximizes tissue oxygenation must exist.

STUDY DESIGN AND METHODS—We used viscometry and an artificial microvascular network (AMVN) device to determine optimal hematocrit *in vitro*. Suspensions of fresh red blood cells (RBCs) in plasma, normal saline or a protein-containing buffer, and suspensions of stored RBCs (at week 6 of standard hypothermic storage) in plasma with hematocrits ranging 10 – 80% were evaluated.

RESULTS—For viscometry, optimal hematocrits were 10, 25.2, 31.9, 37.1 and 37.5% for fresh RBCs in plasma at shear rates of ≤3.2, 11.0, 27.7, 69.5, and 128.5 s^{-1}. For the AMVN, optimal hematocrits were 51.1, 55.6, 59.2, 60.9, 62.3 and 64.6% for fresh RBCs in plasma and 46.4, 48.1, 54.8, 61.4, 65.7 and 66.5% for stored RBCs in plasma at pressures of 2.5, 5, 10, 20, 40 and 60 cmH_2O.

CONCLUSION—Although exact values of optimal hematocrit may depend on specific microvascular architecture, our results suggest that optimal hematocrit for oxygen delivery in the microvasculature depends on perfusion pressure. Anemia in chronic disorders may, therefore, represent a beneficial physiological response to reduced perfusion pressure resulting from decreased heart function and/or vascular stenosis. Our results may help explain why therapeutically increasing hematocrit in such conditions with RBC transfusion frequently leads to worse clinical outcomes.

Introduction

Tissue oxygenation is determined by the product of oxygen carrying capacity of blood and the rate of blood flow through the tissue's microvasculature. Because the oxygen carrying capacity of blood depends on the number of red blood cells (RBCs) available to participate in the transport of oxygen (as well as on the ability of individual RBCs to carry oxygen),

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tissue oxygenation should theoretically increase with increasing hematocrit. However, increasing hematocrit also increases the apparent viscosity of blood, which diminishes the flow of blood through the microvasculature and therefore reduces tissue oxygenation. A value of hematocrit for which these two opposite effects balance each other represents the optimal hematocrit that maximizes tissue oxygenation.[1–3]

Under normal physiological conditions, hematocrit is maintained at 35–45% in women and at 40–50% in men.[4] These normal values, however, may not represent the optimal hematocrit, since an increase in hematocrit for healthy young athletes can elevate their exercise performance.[5,6] On the other hand, chronic inflammatory processes, infections, chronic kidney disease, heart failure and cancer are often associated with anemia (i.e. decreased hematocrit), and it is debated if anemia in these conditions is a marker or a mediator of disease.[7–12] Current guidelines recommend a restrictive transfusion strategy with a threshold value for hemoglobin at 7–8 g/dL, corresponding to a hematocrit of 20.6 – 23.5%.[13,14] This discrepancy between the apparently higher than normal optimal hematocrit in healthy young athletes, and lower than normal optimal hematocrit in patients is intriguing and deserves further investigation.

Previous *in vitro* studies have attempted to estimate the optimal value of hematocrit for tissue oxygenation *in vivo* using straight capillary tubes with different diameters, perfused at various shear stresses with whole blood or RBC suspensions.[1,15–20] In these prior studies, the optimal hematocrit was described by the dynamic relationship between hematocrit and apparent blood viscosity in the capillary tubes. Oxygen transport effectiveness, calculated as the quotient of hematocrit and apparent blood viscosity, was plotted against hematocrit, creating a parabolic curve with the maximum at the optimal hematocrit. Several *in vivo* studies have also attempted to determine the optimal hematocrit in short, non-bifurcating segments of microvessels.[21,22] While these studies expanded upon the earlier *in vitro* studies (by introducing factors such as vasodilation/-constriction and RBC-endothelium interactions), they also could not account for the dynamic heterogeneity of hematocrit found in complex networks of bifurcating capillaries[23–25] and thus could not properly describe the optimal hematocrit in the microvasculature.

The ability of the circulatory system to transport oxygen to tissues and organs is determined by both the rheology of blood and the architecture of the microvasculature. Microvascular networks generally consist of relatively short (<400 μm) capillary segments of various diameters interconnected in a branching pattern. Because of plasma skimming, in any non-symmetrical capillary bifurcation the daughter branch with the highest flow rate receives an even higher fraction of all RBCs entering the bifurcation, thus increasing the local hematocrit in this branch. Higher hematocrit increases the apparent viscosity of blood and therefore reduces the rate of blood flow through that branch, which in turn reduces the fraction of RBCs the daughter branch receives from the bifurcation.[26,27] This coupling between plasma skimming in capillary bifurcations and the dependence of blood viscosity on local hematocrit generates spontaneous oscillations of blood flow in capillary segments of microvascular networks, and produces a highly heterogeneous and dynamically changing distribution of hematocrit throughout the microvasculature.[23–25]

In this study we hypothesized that optimal hematocrit for capillary networks may differ from that for straight capillary tubes or short, non-bifurcating segments of microvessels, and that its value may depend on the overall pressure differential applied to the capillary network. We tested this hypothesis using the artificial microvascular network (AMVN), a microfluidic device composed of interconnected capillary microchannels arranged in a pattern inspired by the microvasculature of rat mesentery.[28–30] We have used the AMVN previously to measure the impact of RBC deformability,[29,31] shape,[32] aggregation,[33] and osmolality of the suspending medium[34] on perfusion of microvasculature *in vitro*, to demonstrate the occurrence of self-sustaining spontaneous oscillations of capillary blood flow in microvascular networks[25] and to document a significant improvement in rheological properties of RBCs stored anaerobically[35] and after washing in normal saline[30] or in 1% solution of human serum albumin.[36] Here, the AMVN enabled systematic evaluation of a range of hematocrits at a number of precisely controlled pressure differentials in a capillary network with complex architecture (Figure 1).

Materials and Methods

Fabrication of the AMVN devices

The design and fabrication of the AMVN devices has been previously described in detail.[30] Briefly, a patterned silicon wafer was used to mold the AMVN devices from polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning, Midland, MI). Assembled devices were filled with GASP buffer (1.3 mM NaH_2PO_4, 9 mM Na_2HPO_4, 140 mM NaCl, 5.5 mM glucose, 1% bovine serum albumin; osmolality 290 mmol/kg; pH 7.4)[30] containing 1% mPEG-silane (Laysan Bio, Arab, AL) and incubated at ambient temperature for 8 hours.

Sample preparation for viscometry measurements

Whole blood was collected via venipuncture from healthy consenting volunteers (n = 10) into Vacutainer tubes (10 mL, K_2EDTA, BD, Franklin Lakes, NJ). Whole blood was centrifuged at 2500 × g for 5 minutes. Supernatant plasma centrifuged at 2500 × g for 10 minutes to remove any remaining cells. Residual plasma, leukocytes and the uppermost layer of RBCs were removed via aspiration. For suspensions in saline, packed RBCs were washed twice in saline and then re-suspended in saline at 60% hematocrit. For suspensions in autologous plasma, packed RBCs were re-suspended in autologous plasma at 60% hematocrit, verified with a hematology analyzer (XT-1800i, Sysmex, Kobe, Japan). Aliquots with hematocrits of 10, 20, 30, 35, 40, 45 and 50% were then prepared by diluting the stock suspensions with calculated volumes of saline or autologous plasma.

Sample preparation for the AMVN measurements

Whole blood was collected via venipuncture from healthy consenting volunteers (n = 7) into Vacutainer tubes (10 mL, K_2EDTA, BD, Franklin Lakes, NJ). Whole blood was centrifuged at 3000 × g for 5 minutes. Supernatant plasma was aspirated and centrifuged at 3000 × g for 10 minutes to remove any remaining cells. Residual plasma, leukocytes and the uppermost layer of RBCs were removed via aspiration. Packed RBCs were diluted with GASP buffer and leukocyte-depleted with a leukoreduction filter (Purecell NEO, Haemonetics, Braintree, MA). The leukoreduced filtrate was centrifuged at 3000 × g for 5 minutes, and the

supernatant GASP and the uppermost layer of RBCs were removed via aspiration. For suspensions in GASP buffer, packed RBCs were re-suspended at 80% hematocrit, verified with a hematology analyzer (Medonic M-series, Boule Medical, Stockholm, Sweden). For suspensions in plasma, packed RBCs were diluted in autologous plasma and centrifuged at 3000 × g for 5 minutes (to ensure removal of residual GASP buffer), then re-suspended in autologous plasma at 80% hematocrit, verified by microcentrifugation performed in triplicate (PowerSpin BX, Unico, Dayton, NJ). Aliquots with hematocrits of 10, 20, 30, 35, 40, 45, 50, 55, 60, and 70% were then prepared by diluting the stock suspensions with calculated volumes of GASP buffer or autologous plasma.

Units of RBCs (n = 7) stored hypothermically for 6 weeks were purchased from a blood bank (CPD>AS-1, leukoreduced, Gulf Coast Regional Blood Center, Houston, TX). Stored RBCs were centrifuged at 3000 × g for 5 minutes. The supernatant storage solution and the uppermost layer of RBCs were removed via aspiration. Aliquots of stored RBCs in thawed fresh frozen plasma (random donor, group AB, Gulf Coast Regional Blood Center) were then prepared, and the hematocrits were verified as described above. All samples were evaluated within 4 hours.

Measurements of viscometry

A Couette-type coaxial rotational viscometer (Contraves LS30, ProRheo, Althengstedt, Germany) calibrated with H_2O (0.7 mPa·s at 37°C) was used. RBC suspension viscosities were measured at shear rates of 128.5, 69.5, 27.7, 11.0, 3.2, 0.95, 0.28 and 0.11 s^{-1}. All samples were incubated in a water bath at 37°C before completing the measurements, and all viscosity measurements were performed at 37°C.

Measurements of the AMVN perfusion rate

The measurements of the AMVN perfusion rate were performed as previously described in detail.[30,33] A water reservoir (30 mL syringe, BD) fixed to a linear motion stage (Series A40 UniSlides, Velmex, Bloomfield, NY) and connected to the AMVN outlet by flexible tubing was used to modulate the perfusion pressure differential across the AMVN. To perform the AMVN measurement, an AMVN device was secured to the stage of the microscope, connected to the water reservoir, and flushed with GASP buffer. A sample of 25 μL of RBC suspension was then pipetted into each AMVN inlet port, and a 2 mm magnetic stir bar (Spinbar, Bel-Art, Wayne, NJ) was inserted into each inlet. A magnetic stirrer (Model 1060, Instech Laboratories, Plymouth Meeting, PA) was used to actuate the stir bars. The RBC samples were allowed to fully fill the network, then the driving pressure was set to 0 cmH_2O, and imaging of the 70 μm wide “venule” channels downstream of the AMVN was initiated as described below. Three bursts of images were collected at 0 cmH_2O, then 18 bursts were collected at driving pressures of 60, 40, 20, 10, 5 and 2.5 cmH_2O, respectively.

Images of the AMVN were acquired with an inverted bright-field microscope (IX73, Olympus, Center Valley, PA) equipped with a high-speed CMOS camera (Flea3, Point Grey Research, Richmond, Canada). A band-pass blue filter (B-390, Hoya, Fremont, CA) was used to improve image contrast. Image sequences were acquired at 10× magnification in bursts of 10 frames (at 150 fps) every 10 seconds for the duration of each measurement.

Image sequences were analyzed offline with a custom algorithm implemented in MATLAB (MathWorks, Natick, MA).

Statistical analysis

Statistical analysis was performed using built-in functions of MATLAB 2014b Statistics Toolbox (MathWorks). Two-way repeated measures analysis of variance (ANOVA) was used to evaluate interactions between groups. A p-value of less than 0.05 was considered significant. Oxygen transport effectiveness datasets from viscometry were fit with second degree polynomials and those obtained from AMVN measurements were fit with third degree polynomials.

Results

Figure 2 shows the viscosities of fresh RBC suspensions in saline (Fig. 2a) and in plasma (Fig. 2b) measured across a range of shear rates (0.3 – 128 s^{-1}) for various hematocrits. For all hematocrits, suspension viscosity increased with decreasing shear rate. Increases in suspension viscosity at decreasing shear rates were larger for higher hematocrits. The viscosity of RBC suspensions in plasma were higher than that of RBC suspensions in saline, which reflected higher viscosity of plasma and the ability of plasma proteins to induce RBC aggregation. Oxygen transport effectiveness (defined as sample hematocrit, Hct, divided by sample viscosity, η) increased with increasing shear rate and was generally higher for RBC suspensions in saline (Fig. 2c) than in plasma (Fig. 2d). In saline, oxygen transport effectiveness decreased almost linearly for all shear rates, while in plasma, oxygen transport effectiveness plateaued at intermediate hematocrits for high shear rates (11 – 128 s^{-1}) and decreased almost linearly for low shear rates (0.3 – 3.2 s^{-1}). The interaction between Hct and viscosity as well as the interaction between Hct and oxygen transport effectiveness (assessed via viscometry) were found to be statistically significant ($p \ll 0.001$) across all shear rates evaluated.

Figure 3 shows the AMVN perfusion rate for fresh RBCs suspended in GASP buffer (Fig. 3a) and in plasma (Fig. 3b) evaluated across a range of driving pressures (2.5 – 60 cmH_2O) for various sample hematocrits. The AMVN perfusion rate for RBC suspensions in GASP buffer (Fig. 3a) and in plasma (Fig. 3b) decreased approximately linearly with increasing hematocrit across all driving pressures evaluated, and was generally lower for RBCs suspended in plasma than in GASP buffer, for all corresponding driving pressures and hematocrits. We defined oxygen transport effectiveness as the product of the AMVN perfusion rate, Q_{AMVN}, and sample hematocrit, Hct, divided by the driving pressure, ΔP. The oxygen transport effectiveness reached maximum at intermediate hematocrits for both RBC suspensions in GASP buffer (Fig. 3c) and in plasma (Fig. 3d) across all driving pressures. The gradual shift of the maximal oxygen transport effectiveness towards higher hematocrits was more pronounced for suspensions in plasma than in GASP buffer (compare Fig. 3d and Fig. 3c). The oxygen transport effectiveness was generally lowest at the lowest hematocrit evaluated (10%) for both types of suspensions. The interaction between Hct and AMVN perfusion rate as well as the interaction between Hct and oxygen transport

effectiveness (assessed via AMVN measurements) were found to be statistically significant ($p \ll 0.001$) across all driving pressures evaluated.

Figure 4 illustrates the dependence of the AMVN perfusion rate (Fig. 4a) and of the oxygen transport effectiveness (Fig. 4b) on hematocrit for suspensions of stored RBCs (6 week storage duration) in plasma (ABO Rh-matched) at various driving pressures. Similarly to fresh RBCs (Fig. 3b and d), the AMVN perfusion rate for stored RBCs suspended in plasma declined approximately linearly with increasing hematocrit for all driving pressures (Fig. 4a), and the oxygen transport effectiveness was maximized at intermediate hematocrits with the maximum shifting towards higher hematocrits for higher pressures (Fig. 4b). The differences between AMVN perfusion rates for fresh RBCs in plasma and stored RBCs in plasma were found not to be statistically significant ($p > 0.073$) across all driving pressures evaluated. The differences between oxygen transport effectivenesses for fresh RBCs in plasma and stored RBCs in plasma were statistically significant ($p < 0.042$) for driving pressures greater than or equal to 10 cmH_2O, but were not statistically significant ($p > 0.158$) for driving pressures of 5 cmH_2O or below.

Figure 5 shows the dependence of optimal hematocrit (defined as the hematocrit at which oxygen transport effectiveness reached its maximum) on shear rate (Fig. 5a, based on data from Fig. 2c–d) and on driving pressure (Fig. 5b, based on data from Fig. 3c–d and Fig. 4b) for all RBC suspensions studied. In case of viscometry, suspensions of fresh RBCs in saline had the optimal hematocrit consistently near the lowest value evaluated (20%). For suspensions of fresh RBCs in plasma, at low shear rates the optimal hematocrit was also near the lowest studied (10%), but then increased logarithmically with increasing shear rate, plateauing around 35% for the highest shear rates evaluated (10, 25.2, 31.9, 37.1 and 37.5% for shear rates of ≤3.2, 11.0, 27.7, 69.5, and 128.5 s^{-1}, respectively; Fig. 5a). For perfusion of the artificial microvascular network (AMVN), optimal hematocrit generally increased with increasing driving pressure (Fig. 5b): from 42.6% at 2.5 cmH_2O to 52.2% at 60 cmH_2O for fresh RBCs suspended in GASP buffer, from 51.1% at 2.5 cmH_2O to 64.5% at 60 cmH_2O for fresh RBCs suspended in plasma (51.1, 55.6, 59.2, 60.9, 62.3 and 64.6% for driving pressures of 2.5, 5, 10, 20, 40 and 60 cmH_2O, respectively), and from 46.4% at 2.5 cmH_2O to 66.5% at 60 cmH_2O for stored RBCs suspended in plasma (46.4, 48.1, 54.8, 61.4, 65.7 and 66.5% for driving pressures of 2.5, 5, 10, 20, 40 and 60 cmH_2O, respectively).

Discussion

Prior *in vitro* studies of optimal hematocrit in straight capillary tubes found that the capacity to deliver oxygen was maximized at intermediate hematocrit values, while low hematocrit suspensions lacked sufficient RBCs to effectively transport oxygen and high hematocrit suspensions were too viscous to effectively perfuse the capillary.[1,15–20] The optimum hematocrit values for tube diameters of 500 μm, 100 μm and 50 μm were found to be 38%, 44% and 51% respectively.[17] In this study, we first performed analysis of the dynamic relationship between hematocrit and apparent suspension viscosity using a Couette-type viscometer[37,38] for RBCs suspended in either plasma (which induces RBC aggregation), or in saline (which does not have that effect). Using the viscometric data we found that increasing hematocrits increased RBC suspension viscosities exponentially and that

suspension viscosities were inversely related to applied shear rates (Fig. 2a–b). At high shear (129 s^{-1}), an increase in hematocrit from 20% to 60% increased the viscosity 3.5-fold for RBCs suspended in plasma and 4-fold for RBCs suspended saline. At the lowest shear rate investigated (0.3 s^{-1}), the dependence of viscosity on hematocrit was even more pronounced; viscosity increased 17.5-fold (plasma) and 14.3-fold (saline) between 20 and 60% hematocrit. The shear rate dependence of suspension viscosity was greater for RBCs suspended in plasma than in saline due to RBC aggregation in plasma; at 60% hematocrit, suspension viscosity increased 11.1-fold (plasma) and only 4.4-fold (saline) between shear rates of 129 and 0.3 s^{-1}.

Similar to prior *in vitro* studies, we calculated the capacity to deliver oxygen to the microvasculature (oxygen transport effectiveness) as the quotient of hematocrit and apparent suspension viscosity. Oxygen transport effectiveness increased with increasing shear rate, and was generally higher for RBCs suspended in saline than in plasma (Fig. 2c–d). In saline, oxygen transport effectiveness decreased almost linearly as hematocrit increased from 30 to 60%, and reached its maxima for optimal hematocrits ranging between 20% (0.3–27 s^{-1}) and 22.4% (128 s^{-1}) (Fig. 2c). For RBCs in plasma, at the highest shear rate (129 s^{-1}), the oxygen transport effectiveness plateaued between 20 and 50% hematocrit, with an optimal hematocrit of 37.5% (Fig. 2d). At a shear rate of 69 s^{-1}, a plateau between 20 and 50% also existed, but the optimal hematocrit was at 37.1%. At a shear rate of 27 s^{-1} a plateau was visible between 20 and 35%, and the optimal hematocrit was at 31.9%. At lower shear rates the plateau disappeared and optimal hematocrit shifted from 25.2% at 11 s^{-1} to 10% for all lower hematocrits. In other words, the value of optimal hematocrit assessed viscometrically shifted towards lower hematocrits with decreasing shear rates.

The estimates of optimal hematocrit derived from viscometric data are relevant primarily for blood flow in larger vessels. Our data suggest that for RBCs in plasma, at high shear rates hematocrit is optimal over a wide range with a plateau between 30 and 50% (Fig. 5a). With decreasing shear rates (that is decreasing blood pressure and hence blood flow, as seen in shock *in vivo*), the plateau effect may be lost and optimal hematocrits may become shifted towards lower values. This shift of the optimal hematocrit to lower values may even be aggravated *in vivo* by the infusion of large volumes of saline (so-called fluid resuscitation), which is the cornerstone of shock therapy. In our study, the optimal hematocrit for RBCs in saline was about 20% (Fig. 5a), suggesting that dilution of plasma with saline could potentially shift the optimal hematocrit to even lower values.

We further investigated the relationship between hematocrit, driving pressure and perfusion rate in an artificial microvascular network (AMVN). We found that the AMVN perfusion rate decreased approximately linearly with increasing hematocrit and that the AMVN perfusion rate was proportional to applied driving pressure (Fig. 3a–b). At high driving pressures (60 cmH_2O), a decrease in hematocrit from 80% to 10% increased the AMVN perfusion rate 2.3-fold for RBCs suspended in plasma and 3.7-fold for RBCs suspended in GASP buffer. At the lowest driving pressure investigated (2.5 cmH_2O), the dependence of the AMVN perfusion rate on hematocrit was even more pronounced; as hematocrit decreased from 80 to 10%, the perfusion rate increased 8.5-fold (plasma) and 7.7-fold (GASP buffer). The dependence of the AMVN perfusion rate on driving pressure was

substantially stronger for RBCs suspended in plasma than in GASP; at 80% hematocrit, as driving pressure increased from 2.5 to 60 cmH_2O the AMVN perfusion rate increased 200-fold for plasma and only 90-fold for GASP buffer. (For reference, under normal conditions the pressure drop from small arterioles to venules across the microcirculation *in vivo* is on the order of 40 cmH_2O.[39])

Oxygen transport effectiveness in the AMVN increased with increasing driving pressure and was generally higher for RBC suspensions in GASP than in plasma. In GASP buffer, oxygen transport effectiveness had a parabolic relationship with hematocrit, reaching its maxima for optimal hematocrit values ranging between 42.6% (for 2.5 cmH_2O) and 52.2% (for 60 cmH_2O) (Fig. 3c and Fig. 5b). In plasma, oxygen transport effectiveness also had a parabolic relationship with hematocrit, however the highest values of oxygen transport effectiveness generally occurred at higher hematocrits than for GASP buffer – optimal hematocrit values for RBCs suspended in plasma ranged between 51.1% (for 2.5 cmH_2O) and 64.6% (for 60 cmH_2O) (Fig. 3d and Fig. 5b). Overall, the optimal hematocrit for oxygen transport in the artificial microvascular network shifted towards lower values for lower driving pressures. In addition, stored RBCs had a lower optimal hematocrit at low perfusion pressures (≤10 cmH_2O) than fresh RBCs. A possible explanation for this result is that differences in RBC deformability are inversely related to applied shear forces, i.e. become more prominent at lower perfusion pressures. Our data suggests that plasma (as compared to GASP buffer) has the capacity to increase the optimum hematocrit and that optimum hematocrit, as measured by perfusion of the AMVN, is higher than that estimated by viscometric techniques.

Interestingly, when the AMVN was perfused with RBCs which had been stored hypothermically for 6 weeks and re-suspended in ABO-matched plasma, optimal hematocrit values ranged from 46.4% (for 2.5 cmH_2O) to 66.5% (for 60 cmH_2O), a wider range with the lower bound extending well below what we measured for *fresh* RBCs suspended in plasma (Fig. 5). The practical implications of these differences between fresh and stored RBCs are particularly important to the field of transfusion medicine. Because RBCs stored for a prolonged period of time shift the optimal hematocrit for oxygen transport effectiveness to lower values, massive transfusion of stored RBCs up to a physiologic value may not be beneficial, and lower than normal values should be the optimal target. This hypothesis is supported by studies in critically ill patients with sepsis[40,41] and acute upper gastrointestinal beeding.[42] In sickle cell disease, which is characterized by less deformable RBCs, it has been shown that increasing fractions of sickle cells progressively decrease the optimal hematocrit.[43] Oxygen transport effectiveness at low shear rates is adversely affected by RBC transfusions, which may explain why transfusion therapy may actually worsen complications in patients with sickle cell disease.[37]

There are several inherent limitations of the AMVN system. The AMVN is constructed of PDMS, which is inert and stiff, and therefore does not reproduce the biochemical or mechanical properties of microvasculature *in vivo*.[25,30,31] It has been shown *in vivo* that an increase in hematocrit increases the endothelial production of nitric oxide,[44–46] which leads to arteriolar vasodilation,[46] reduced microcirculatory driving pressure and hence reduced perfusion rate. A parabolic ‘U’-shaped relationship exists between hematocrit and mean arterial pressure,[44] defining an optimal hematocrit for vasodilation. The relationship between

this optimal hematocrit for vasodilation and the optimal hematocrit for oxygen transport effectiveness remains elusive and could not be addressed in our AMVN experiments. Additionally, the channels of the AMVN have rectangular cross-sections, which is not physiological, as microvessels *in vivo* have circular cross-sections.[47] Finally, the specific layout of the AMVN, which was inspired by the architecture of rat mesentery microvasculature, is only one possible configuration of microchannels and is not necessarily representative of all human microvasculature.[29,30] Therefore, the specific values of optimal hematocrit we found using the AMVN may not be universally applicable across different microvascular networks and should not be directly extrapolated to all microvascular networks *in vivo*. Nevertheless, the AMVN has previously been used to investigate various rheological and hemodynamic phenomena and has proven to be a robust *in vitro* model for microvascular perfusion.[25,29–36]

The AMVN data suggest that the optimal hematocrit for tissue oxygenation could be higher than the physiological range, which is in contrast to the viscometric results. This exemplifies that blood flow properties in the circulation are complex and cannot be defined by any single measurement. The high optimal hematocrit seen in AMVN experiments is in agreement with *in vivo* observations in healthy young athletes, but not with clinical data in patients.[6] Both of our *in vitro* methods indicated that for high shear rates or perfusion pressures (and hence higher rate of blood flow), the optimal hematocrit is shifted towards higher values. Well-trained athletes meet such conditions, which could explain why blood doping and erythropoietin administration improve exercise performance.[5] Higher than normal hematocrits also exist in polycythemia vera, but hematocrit reduction to values <45% is recommended because it reduces cardiovascular morbidity and mortality.[48] Lower than normal hematocrits frequently accompany chronic diseases such as kidney disease, heart failure, critical illness, sepsis and cancer. Many of these patients are older and may have decreased blood flow and thus decreased microvascular perfusion pressures, either systemically, by an impaired pump function of the heart (e.g. in chronic heart failure), or locally, distal of arterial stenosis (e.g. in peripheral arterial disease). Our data suggest that at lower pressure differentials across the microcirculation, the optimal hematocrit would be shifted towards lower values. Anemia in chronic disorders may therefore be a beneficial response with regard to tissue oxygenation. This hypothetical notion allows a better understanding of why many clinical studies, which attempted to correct such anemias, yielded disappointing results. A normalization of anemia in chronic kidney disease did not reduce the risk of cardiovascular events and did not improve quality of life,[7,8,10,49] but increased the risk of stroke and death.[11,50] In septic shock,[40,51] critical care,[41,52] and acute upper gastrointestinal bleeding,[42] a restrictive transfusion regimen was as effective or even superior to a liberal transfusion regimen. Treatment of anemia in chronic heart failure had no clinical benefit.[12,53] Although preoperative anemia was associated with postoperative morbidity and mortality in cardiac and non-cardiac surgery,[54,55] a preoperative correction of anemia did not result in improved clinical outcomes.[56–58] Correction of anemia in cancer patients was associated with increased mortality.[9,59] Current guidelines, therefore, recommend a restrictive RBC transfusion strategy with a hemoglobin threshold of 7–8 g/dL.[13] In locally decreased perfusion pressure, such as in peripheral arterial disease, a lower than normal hematocrit has been shown to be beneficial.[60–62]

We conclude that the optimal hematocrit for oxygen transport *in vitro* decreases with decreasing flow rate. For flow in larger vessels, viscometry suggests that it is lower than the normal hematocrit, while for microvascular perfusion it may be higher than the normal value. By balancing those opposing effects, the normal physiological hematocrit may represent an optimal hematocrit for normal conditions, whereas it may be lower in diseases with low systemic or local blood flow. Our observations thus shed light on an old question; suggesting that a universally valid optimal hematocrit value may not exist, but may rather depend on underlying pathophysiological conditions.

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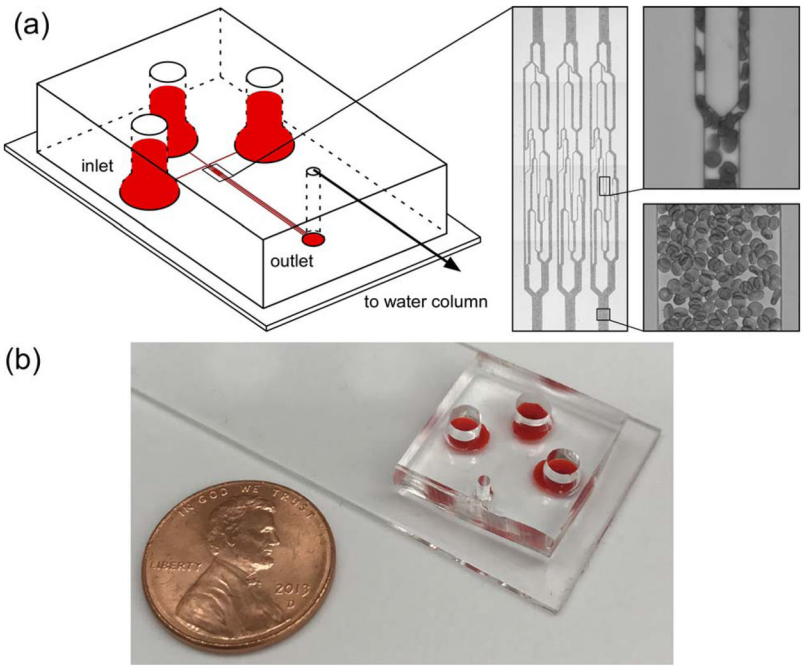

Figure 1.

Artificial microvascular network (AMVN). (**a**) Schematic illustration of the AMVN. Each AMVN device contained three parallel, identical capillary networks (**inset**) with separate inlets and a shared outlet. Arrow indicates direction of flow through the device. (**b**) Photograph of the assembled AMVN device (one-cent US coin is shown for overall size reference).

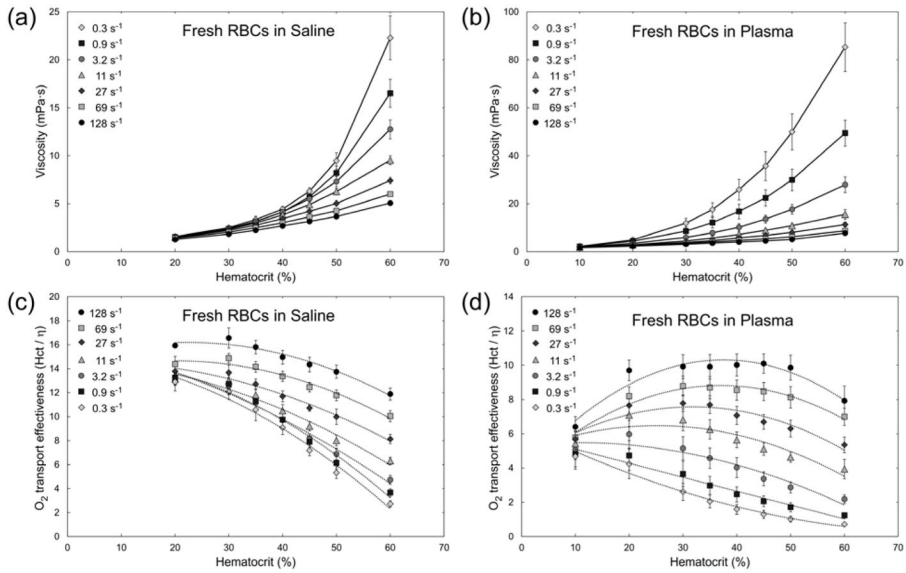

Figure 2.

Estimation of optimal hematocrit for fresh red blood cells (RBCs) based on viscometry. (**a**) Viscosity of RBC suspensions in saline and (**b**) in plasma at ambient temperature for a range of hematocrits (20 – 60%) measured at various shear rates (0.3 – 128 s^{-1}) with a Couette viscometer. (**c**) Oxygen transport effectiveness (defined as sample hematocrit divided by sample viscosity) for RBC suspensions in saline and (**d**) in plasma for each shear rate. Mean values ± standard deviation, n = 10.

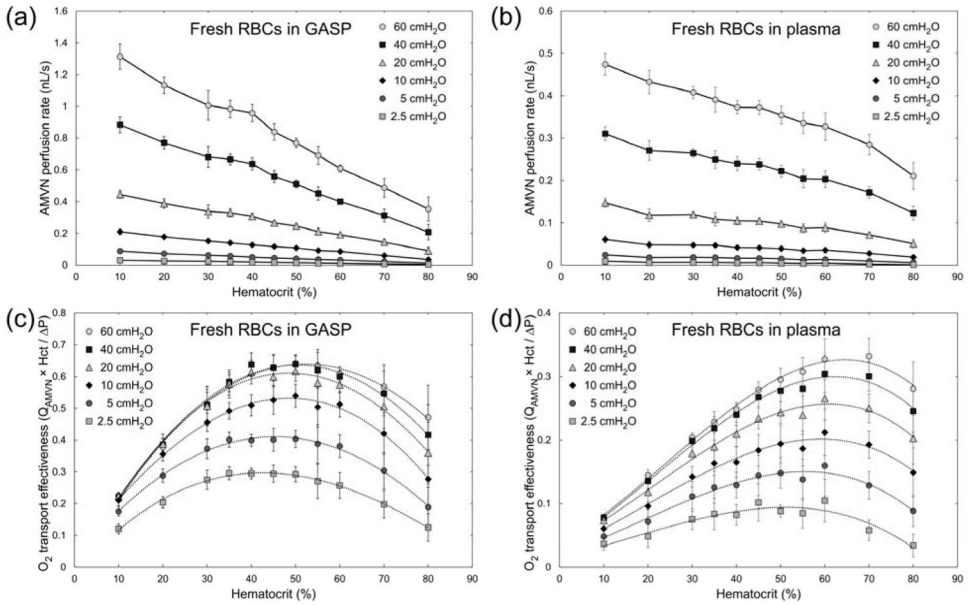

Figure 3.

Estimation of optimal hematocrit for oxygen transport effectiveness for fresh red blood cells (RBCs) based on perfusion of an artificial microvascular network (AMVN). (**a**) AMVN perfusion rate for RBC suspensions in GASP buffer and (**b**) in plasma for a range of hematocrits (10 – 80%) measured at various driving pressures (2.5 – 60 cmH_2O). (**c**) Oxygen transport effectiveness (defined as the product of the AMVN perfusion rate and sample hematocrit divided by driving pressure) for RBC suspensions in GASP buffer and (**d**) in plasma for each driving pressure. Mean values ± standard deviation, n = 5 (RBC suspensions in GASP buffer), n = 7 (RBC suspensions in plasma).

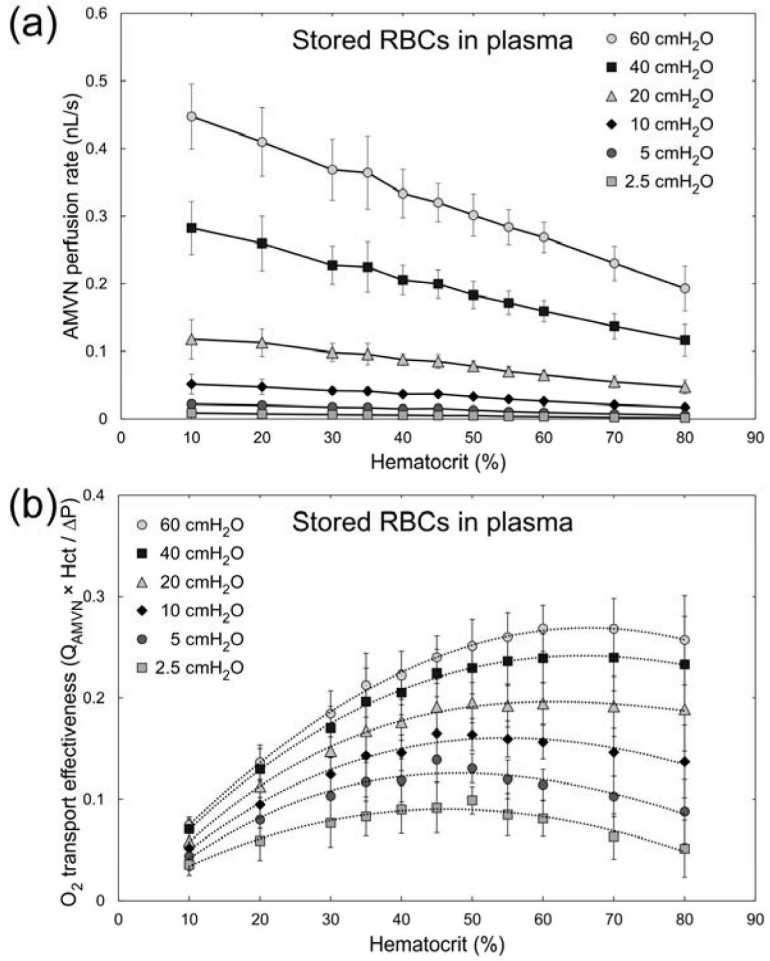

Figure 4.

Estimation of optimal hematocrit for oxygen transport effectiveness for 6 week old, stored red blood cells (RBCs) re-suspended in plasma based on perfusion of an artificial microvascular network (AMVN). (**a**) AMVN perfusion rate for a range of hematocrits (10 – 80%) measured at various driving pressures (2.5 – 60 cmH_2O). (**b**) Oxygen transport effectiveness (defined as the product of the AMVN perfusion rate and sample hematocrit divided by driving pressure) for each driving pressure. Mean values ± standard deviation, n = 7.

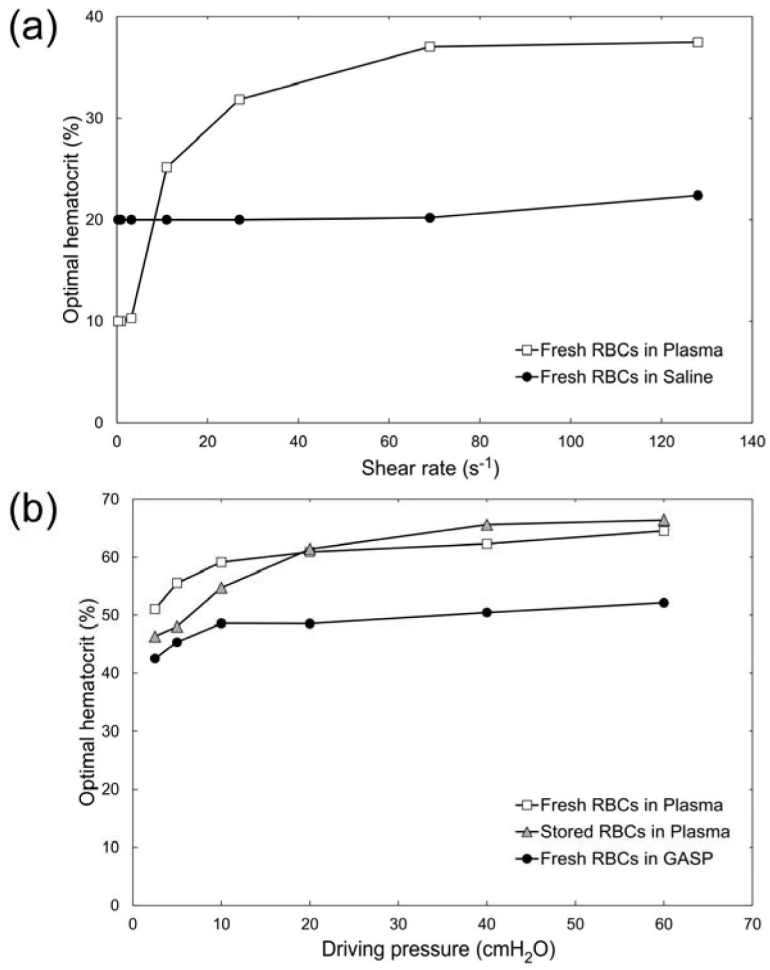

Figure 5.

Dependence of optimal hematocrit on shear rate or on driving pressure. (**a**) Optimal hematocrit based on viscometry (defined as the hematocrit at which oxygen transport effectiveness was maximized) for each shear rate. (**b**) Optimal hematocrit based on perfusion of an artificial microvascular network (AMVN) (defined as the hematocrit at which oxygen transport effectiveness was maximized) for each driving pressure.