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RESEARCH ARTICLE

A model of anemic tissue perfusion after blood transfusion shows critical role of endothelial response to shear stress stimuli

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Abstract

Although some of the cardiovascular responses to changes in hematocrit (Hct) are not fully quantified experimentally, available information is sufficient to build a mathematical model of the consequences of treating anemia by introducing RBCs into the circulation via blood transfusion. We present such a model, which describes how the treatment of normovolemic anemia with blood transfusion impacts oxygen (O_2) delivery (DO_2, the product of blood O_2 content and arterial blood flow) by the microcirculation. Our analysis accounts for the differential response of the endothelium to the wall shear stress (WSS) stimulus, changes in nitric oxide (NO) production due to modification of blood viscosity caused by alterations of both hematocrit (Hct) and cell free layer thickness, as well as for their combined effects on microvascular blood flow and DO_2. Our model shows that transfusions of 1- and 2-unit of blood have a minimal effect on DO_2 if the microcirculation is unresponsive to the WSS stimulus for NO production that causes vasodilatation increasing blood flow and DO_2. Conversely, in a fully WSS responsive organism, blood transfusion significantly enhances blood flow and DO_2, because increased viscosity stimulates endothelial NO production causing vasodilatation. This finding suggests that evaluation of a patients' pretransfusion endothelial WSS responsiveness should be beneficial in determining the optimal transfusion requirements for treating patients with anemia.

NEW & NOTEWORTHY Transfusion of 1 or 2 units of blood accounts for about 3/4 of the world blood consumption of 119 million units per year, whereas a current world demand deficit is on the order of 100 million units. Therefore, factors supporting the practice of transfusing 1 unit instead of 2 are of interest, given their potential to expand the number of interventions without increasing blood availability. Our mathematical model provides a physiological support for this practice.

blood transfusion; endothelial response; mathematical model

INTRODUCTION

Blood transfusion is widely used to restore/increase oxygen (O_2) delivery (DO_2). The latter is directly proportional to the product of blood flow, i.e., cardiac output and blood hemoglobin (Hb) concentration (1). Addition of red blood cells (RBCs), i.e., increase in hematocrit (Hct), has two countervailing effects on DO_2. On one hand, it increases Hb concentration and the capacity to transport O_2. On the other, it increases blood viscosity and, according to purely hydraulic considerations of flow regulation, decreases cardiac output and the capacity to deliver O_2. This suggests that, for blood transfusion to be effective, changes in Hct must induce significant additional effects, independent of the change in O_2 carrying capacity.

An indication of the existence of such effects was first reported by Martini et al. (2). They showed that, in awake hamsters, the small isovolemic increase of Hct by the exchange-transfusion of packed RBCs (pRBCs) caused a decrease of systemic blood pressure (MAP). As Hct continued to increase, the initial decline of MAP reversed itself after a 10% increase over normal. This change was accompanied by a significant increase in cardiac output, which reversed in synchrony with the changes of MAP as Hct was increased further. Martini et al. (2) attributed these phenomena to changes in the production of endothelial nitric oxide (NO) synthase (eNOS), since the same experiments carried out in nitro-L-arginine methyl ester (L-NAME)-treated hamsters and eNOS-knockout mice, both of which do not produce NO via eNOS, showed neither decrease in MAP nor increase in cardiac output.

The regulation of microvessel diameter by NO bioavailability at the endothelial level results from the balance between two countervailing factors. The increase of shear stress on the endothelial surface, induced by increased blood flow and/or blood viscosity, causes endothelial NO concentration to increase. This phenomenon is counteracted by the increase in NO scavenging due to the increase in Hb concentration. In this context, Hct plays a dual role, as a viscogenic element in regulating blood viscosity and endothelial wall shear stress (WSS), and simultaneously as an NO sink. To complicate matters further, the effective distance between

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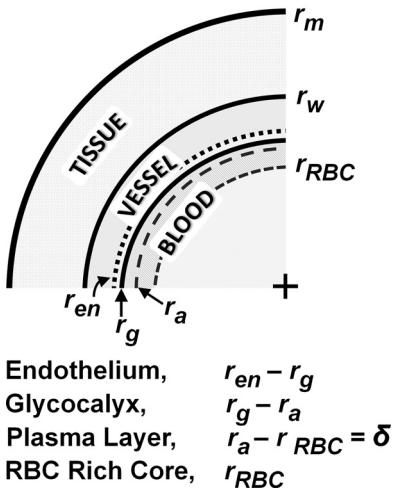

Figure 1. Cross section of an arteriole. A version of the Krogh tissue cylinder model, used in our analysis, consists of RBC-rich core, RBC-free plasma layer, glycocalyx, endothelium, vascular wall, and parenchymal tissue adjacent to the vessel wall. RBC, red blood cells.

RBCs and the endothelial surface, termed the "plasma layer," varies with Hct, vessel diameter, extent of the glycocalyx, and blood vessel flow. This effective distance is also a factor affecting NO bioavailability to smooth muscle.

Although some of the cardiovascular responses to changes in Hct are not fully quantified experimentally, available information is sufficient to build a mathematical model of the consequences of treating anemia by introducing RBCs into the circulation via blood transfusion. Such an analysis serves to identify variables that may be manipulated to increase the rate of DO_2 to the tissues, independently of changing blood O_2 carrying capacity. Our predictive model of DO_2 in anemia describes the interaction between the mechanistic effects and vascular responses. The former are caused by modification in blood-flow properties due to the changes in blood O_2 carrying capacity, whereas the latter stem from alteration in the related vascular NO bioavailability.

Our modeling study strives to elucidate how the treatment of normovolemic anemia with blood transfusion impacts DO_2 by the microcirculation. In other words, it is applicable to the case where the patient blood volume is stable, and presumed normal, and Hct is increased by transfusion of pRBCs. Our model explicitly accounts for the response of blood vessel diameter to changes in the WSS induced by changing Hct and, therefore, blood viscosity. It calculates the WSS for each Hct induced by the transfusion of pRBCs after blood volume returns to the pretransfusion value. The resulting WSS, in turn, induces a diameter response due to mechano-transduction in the endothelium that changes its rate of NO production thus affecting smooth muscle tension and the arteriolar vessel diameter, blood flow, and DO_2.

METHODS

We adapt the modified Krogh tissue model of an arteriole (3) to represent the vessel tissue using several layers (Fig. 1). These layers comprise an RBC-rich core, RBC-free plasma layer, glycocalyx, endothelium, vascular wall, and parenchymal tissue adjacent to the vessel wall. The radius of each part is shown in Table 1.

The following physiological assumptions underpin our model development:

1) Transport phenomena are one-dimensional (in the radial direction) and in steady-state conditions (3).
2) Blood is treated as a single-phase immiscible, incompressible Newtonian fluid (4).
3) The rate of NO scavenging by RBCs increases linearly with Hct (3, 4, 5, 6).
4) Each individual layer is homogeneous and isotropic (7).
5) Dependence of plasma layer thickness on discharge hematocrit H_d is described by a quadratic polynomial (7).
6) Blood-vessel diameter changes in response to vasodilation are induced by changes in NO availability (8).
7) Pressure gradient in arterioles is constant (9).
8) NO production rate in the endothelium is dependent on WSS (10).
9) O_2 availability in the RBC core increases linearly with Hct (3).
10) Diffusion coefficients of NO and O_2 in the solid layers are half of those in the liquid layers (11, 12).
11) Blood viscosity is a parameter described by a quadratic function of Hct (1).

Table 1. *Model parameters*

Parameter	Symbol	Value	Reference(s)
Solubility of O_2	A	1.34 μM/Torr	3, 15
Diffusivity of O_2 in fluid layers	D_{O2}	2,800 $\mu m^2/s$	3, 15
Diffusivity of NO in fluid layers	D_{NO}	3,300 $\mu m^2/s$	3, 15
Diffusivity of O_2 in solid layers	D_{O2}	1,400 $\mu m^2/s$	3, 11
Diffusivity of NO in solid layers	D_{NO}	1,650 $\mu m^2/s$	3, 11
Maximum O_2 consumption rate in parenchymal tissue	$R_{O2,max}$	6 μM/s	16
Maximum O_2 consumption rate in vascular wall	$R_{O2,max}$	1 μM/s	4
Scavenging rare of NO in blood at 45% Hct	λ_b	382.5 s^{-1}	3, 15
Rate of consumption of NO in tissue	λ_t	1 s^{-1}	3, 15
Michaelis-Menten constant for NO in endothelium	K_M	4.7	17
Initial arteriole radius before blood transfusion	R	20 μm	3, 15
Endothelial thickness	$r_{en} - r_g$	1 μm	15
Glycocalyx thickness	$r_g - r_a$	0.25^{-1} μm	15
Vascular wall thickness	$r_w - r_{en}$	9 μm	15
Parenchymal tissue thickness	$r_t - r_w$	80 μm	3

Due to the effect first proposed by Khayutin et al. (13), we postulate that these changes occur while MAP remains constant. We assume it to be so throughout the whole range of Hct changes, a simplification justified by the study of Martini et al. (2), which showed that in hamsters undergoing isovolemic Hct changes in the range proposed by the present model the range of MAP changes was ±10%. We also assume blood volume to remain constant, and changes in the relative volumes of RBCs and plasma to be adjusted so that this condition is satisfied for each Hct analyzed. These assumptions imply that our model of the effects of Hct changes on DO_2 is applicable to conditions of stable normovolemic anemia. The baseline state is a normal human, characterized by cardiac output of 5 L/min, MAP of 120/80 mmHg, Hct = 45%, and blood viscosity of 5.0 cP.

The relation between WSS and vessel wall NO concentration and vessel diameter was reported in Sriram et al. (10), where plasma viscosity was varied and different Hct levels were induced by isovolemic hemodilution in the awake hamster window preparation. The resulting blood and plasma viscosities were measured in blood samples, and blood flow velocity and diameter changes of arterioles were measured in vivo. The NO concentrations in the arteriolar walls were measured by an amperometric technique using nafion-coated carbon electrodes (14). We assume that the similarity of the principal microhemodynamic parameters (e.g., capillary and RBC dimensions, capillary Hct, flow velocities, and blood O_2 carrying capacity, etc.) between mammalian species leads to the similarity of general biochemical phenomena between mammalian species.

Our model accounts for the physiological and biochemical processes collated in Fig. 2 (a mathematical representation of these phenomena is given in the Appendix). It predicts concentration of NO and partial pressure of O_2 in a region where NO is scavenged by RBCs, produced by the endothelium, and consumed in the vascular wall and the parenchymal tissue. Since the plasma layer thickness depends on Hct, the NO production rate is controlled by WSS. Therefore, these effects are coupled and constrained by mass conservation across the interfaces between adjacent layers. The model comprises a system of 12 equations for 12 unknowns, yielding the NO concentration and P_{O_2}, from the center of the blood vessel to the tissue outer boundary at radius around 110 μm. The model was solved for the full range of endothelial responses, ranging from the complete lack of response to shear stress stimulation due to blood viscosity increase (0%) to the full response, i.e., maximal or 100% production of NO. Values of the parameters used in our simulation are presented in Table 1.

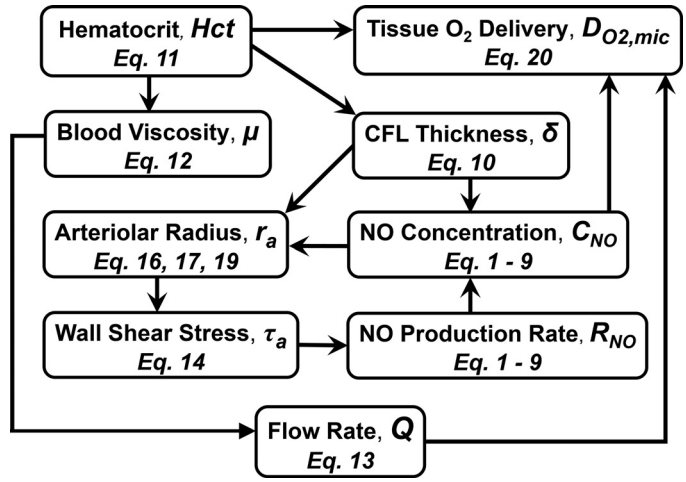

Figure 2. The physiological and biochemical processes accounted for by our model. The notations for each variable and the associated equations are also included.

RESULTS

We use our model to explore the effects of blood transfusion on DO_2 in anemia by simulating a series of Hct states, from 10% to 50%, and then transfusing 0, 0.0625, 0.125, 0.25, 0.5, 1 and 2 units of pRBCs.[1] The principal findings of this study are summarized in Fig. 3. It shows that the increase of DO_2 due to transfusion of up to 2 units of blood is comparatively small for an inert (constant blood vessel diameter) circulation characterized by endothelial dysfunction. Most experimental data on hemodilution and hemoconcentration show that MAP remains approximately constant following Hct changes (9). Therefore, in the inert circulation, changes in DO_2 are caused by changes in flow due to changes in blood viscosity (1). Figure 3 highlights the importance of vasoactivity, in which the blood vessel diameter responds to changes in blood flow. In our model, the normalcy or health condition of the endothelium manifests itself via the sensitivity of the endothelial NO production to WSS changes. This sensitivity may be impaired due to injury and disease conditions or altered by the introduction of pharmacological agents such as sildenafil citrate and L-NAME.

Different levels of anemia in Fig. 3, simulated by lowering Hct while maintaining constant MAP, are in the qualitative agreement with the observations of Messmer et al. (9).[2] We Fn2
used our model to predict the effect of blood transfusion on DO_2 for varying degrees of anemia at constant blood volume, i.e., for transfusion of 0.0625 to 2.0 units of blood. Figure 4 depicts our predictions of flow changes in a circulatory system that either fully responds or does not respond to the WSS vasodilator stimulus. It reveals that, if the endothelium is not responsive, treating patient with anemia with a 2-unit transfusion (the most frequently used transfusion quantity) would reduce flow relative to its level without transfusion, regardless of the state of anemia. This figure also illustrates the highly nonlinear effects of blood transfusion: the comparatively large changes in DO_2 are obtained with the initial introduction of 1/16 to 1/4 units of blood.

This finding stems from the fact that transfusion always increases blood viscosity and, therefore, the resistance to flow. For the fully responsive endothelium, the WSS-induced Fn1

[1]Throughout this presentation, a unit of blood (or pRBCs) has a volume of 300 mL and 65% Hct.
[2]This comprehensive study was conducted in anesthetized dogs to obtain a DO_2 vs. Hct response curve that is virtually identical to its counterpart in Fig. 3. A principal difference is that our maximal DO_2 for a person normally occurs at Hct 25%, whereas experiments in anesthetized dogs showed a maximal DO_2 at 30% Hct.

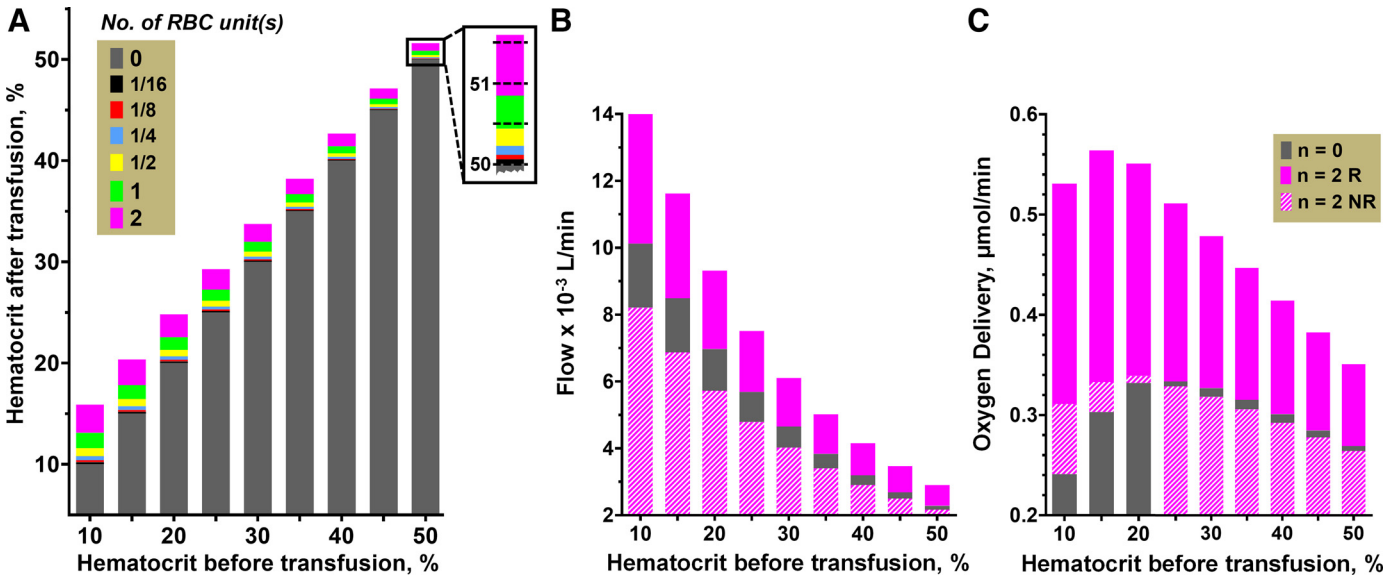

Figure 3. *A*: hematocrit before and after transfusion of n = 0, 0.0625, 0.125, 0.25, 0.5, 1 and 2 units of pRBCs in anemic individuals whose Hct ranges from 10% to 50%; the posttransfusion Hct is evaluated by *Eq. 11*. *B* and *C*: flow and DO_2 in a person with either responsive (R) or nonresponsive (NR) vasculature, after 2-unit transfusion. If the endothelium is not responsive, 2-unit transfusion always lowers flow rate and leads to the relatively small increase of DO_2, reaching its maximum at Hct 25%. In the responsive vasculature, the change of Hct increases WSS and the production of the vasodilator NO, overwhelming the negative effect of increased viscosity. In these graphs, the values of Hct, flow, and DO_2 are those given by the *top* color line limit of each condition, i.e., if Hct before transfusion is 30% it remains at 30% after the transfusion of zero units and rises to 34% after the transfusion of n = 2 units of blood. Similarly, blood in the responsive vasculature is 10^{-2} L/min when Hct is 10% and 0 units are transfused, and becomes 14×10^{-3} L/min when Hct is 10% and 2 units are transfused. DO_2, oxygen delivery; Hct, hematocrit; pRBCs, packed red blood cells; RBC, red blood cell; WSS, wall shear stress.

vessel dilatation increases flow rates as Hct increases, since the resistance to flow decreases in proportion to the fourth power of the change of vessel diameter. Oxygen delivery for each of these cases is shown in Fig. 5. For the nonresponsive endothelium, DO_2 increase of ~29%, relative to no transfusion, is obtained only when two units are transfused to correct an RBC loss of around 80%. At this low Hct, DO_2 falls below normal only when Hct < 14%.[3]

These results suggest that, for levels of endothelial responsiveness ranging from zero to full, the organism at rest receives sufficient O_2 down to Hct levels that are significantly lower than those indicated by the commonly used transfusion trigger of 50% Hb deficiency.[4] This finding is in part supported by the commonly accepted observation that the O_2 supply-and-demand relation according to which the circulatory O_2 delivery is 2/3 in excess of the metabolic demand of the organism at rest (18).

Figure 6 provides another illustration of how the endothelium's physiological condition affects changes in DO_2 due to the transfusion of 1 or 2 units of pRBC in a 50% anemic subject. It provides an explanation of the general practice to transfuse 2 units of blood when Hct falls to 50% of normal, a common "transfusion trigger." Transfusing 1 unit of pRBCs would only be effective in restoring DO_2 if 80% of the endothelium were fully responsive to the induced stimulus. This information is usually unavailable at the time of transfusion. Figure 6 also explains why transfusion of 2 units of pRBCs is normally used in patients with disease, trauma, or surgical blood loss, when blood Hb reaches 50% of normal. Such patients would be at risk of ischemia in critical organs if their endothelial responsiveness were impaired, a condition that is seldom known.

DISCUSSION

The consequences of using super plasma expanders, depicted in Fig. 6, point to potential strategies for enhancing the effectiveness of blood transfusion, while improving outcome and decreasing blood usage. This possibility arises because increase in the NO production, leading to vasodilatation and increased blood flow, can be significantly enhanced by increasing WSS. This can be achieved by increasing the viscosity of blood plasma, which would significantly increase the viscosity of blood in the capillary system that has the largest endothelial surface in the circulation. This effect is not attainable by increasing blood viscosity through increasing systemic Hct, which principally affects central blood viscosity and only minimally affects capillary Hct. That is because blood Hct progressively decreases from its central systemic value to half of this value in the capillary circulation (19). According to *Eq. 12*, changes in blood viscosity due to changes in central Hct are minimal in the capillaries, since capillary Hct is ~1/3 of central Hct (18). The area of active endothelium differs by a factor of 100 between capillary and central circulation. Consequently, the Hct-dependent blood viscosity

[3]These extreme conditions are somewhat mitigated because plasma carries some oxygen in solution, to the extent that at normal Hct RBCs carry 98% of O_2, and plasma carries the remainder 2%, which becomes about 4% for ultralow Hct conditions.
[4]Following Zimmerman et al. (1), we use the expression Hct = 0.45·(1 − Hb_{def}) to relate Hct to Hb_{def} = Hb deficiency (RBC loss). Here, 0.45 refers to the "normal" Hct level of 45%.

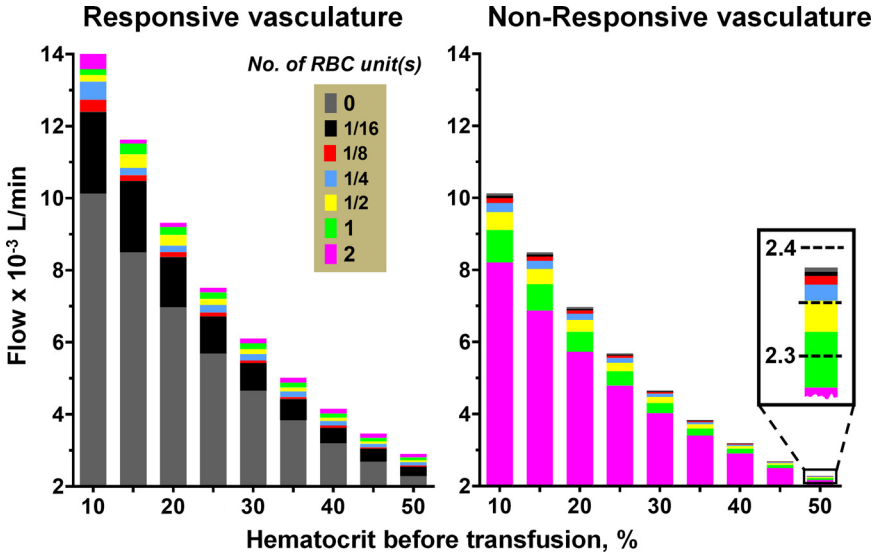

Figure 4. Flow in responsive and nonresponsive vasculature before and after transfusion, as Hct varies from 10% to 50% when transfusing 0.0625, 0.125, 0.25, 0.5 1.0 and 2.0 units of pRBCs. In nonresponsive vasculature, transfusing an anemic patient lowers flow, regardless of the state of anemia. Conversely, transfusion always increases flow in patients with a WSS-responsive vasculature. Hct, hematocrit; pRBCs, packed red blood cells; RBC, red blood cell; WSS, wall shear stress.

effects on endothelial function are minimal in the central circulation, wherein the NO producing endothelium surface area is smallest, and maximal in the capillaries whose endothelial surface is ~100 times that of the central circulation.

Our model supports this experimentally observed effect. The crosshatched region in Fig. 6 represents DO_2 that could be achieved by the hypothetical expansion of the vasodilator response by 50% in addition to the maximal 100% obtained solely from blood transfusion. This approach would increase the efficacy of transfusing either 1 or 2 units of blood, producing significant increases in DO_2 without transfusing blood. The beneficial effect of increasing plasma viscosity on increasing DO_2 in anemia is also evidenced in the experiments of Messmer et al. (9).

Our model predicts how Hct changes due to blood loss or transfusion would alter the blood flow properties. These alterations are significant because blood viscosity, although a direct function of plasma viscosity, is a quadratic function of Hct (1). Although these changes are generally recognized, they are not taken into consideration when assessing the number of blood units needed to restore DO_2 after blood loss. Instead, the decision-making process in such an intervention is based on measurements of either blood Hct, Hb content, or extracorporeal P_{O_2}, rather than on determination of the specific DO_2 deficit. That is because the determination of DO_2 requires an additional measurement of cardiac output. This complex and hitherto invasive procedure can currently be done by noninvasive ultrasound. However, for such measurements to be meaningful, they would have to be done before and after the procedure, requiring two interventions.

Our findings suggest that treatment of oxygenation deficiency should directly address its objective, focusing on the rate of DO_2 rather than on O_2 transport capacity without the simultaneous consideration of transport

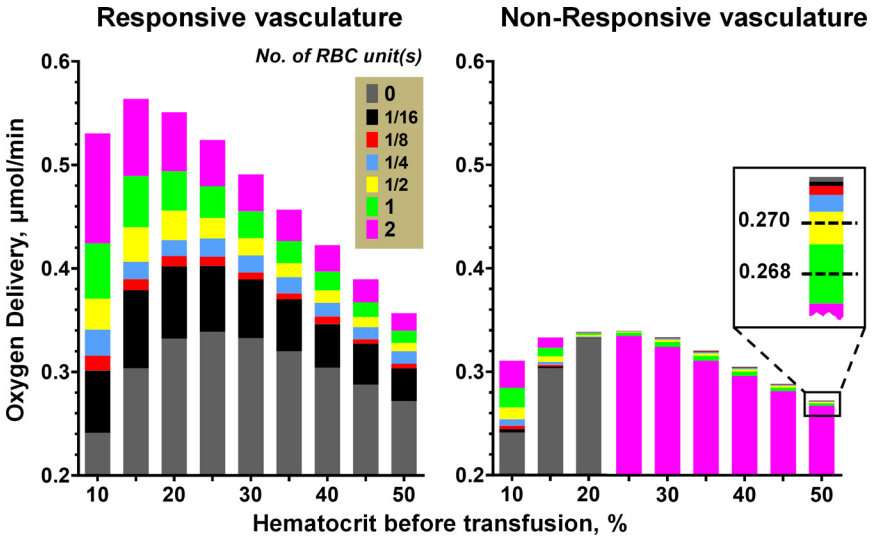

Figure 5. Oxygen delivery in responsive and nonresponsive vasculature before and after transfusion, as Hct varies from 10% to 50% when transfusing 0.0625, 0.125, 0.25, 0.5 1.0 and 2.0 units of pRBCs. If the endothelium is not responsive, an increase of about 29% in DO_2 is obtained only when 2 units are transfused to correct an RBC loss of around 80%. On the contrary, for a fully responsive endothelium, DO_2 doubles relative to normal. DO_2, oxygen delivery; Hct, hematocrit; pRBCs, packed red blood cells; RBC, red blood cell.

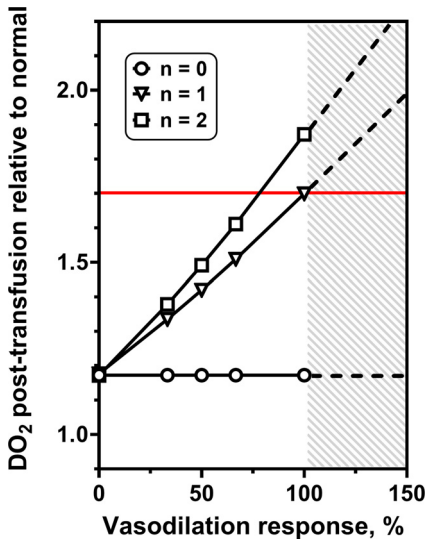

Figure 6. Increase of DO_2 after transfusion in 50% Hb deficit condition as a function of vasodilation response to WSS. The 70% DO_2 augmentation relative to normal condition requires transfusion of 1 unit of pRBCs if the endothelium is 100% responsive to mechanical stimulation, and of 2 units of pRBCs if the endothelium is at least of 75% responsive. The shadowed part of the graph corresponds to the level of DO_2 augmentation attained using 1 or 2 units of pRBCs in combination with the infusion of fluids with "super plasma expansion capacity," yielding a 95% DO_2 augmentation when using 1 unit of pRBCs, and a 120% DO_2 augmentation when using 2 units of pRBC. DO_2, oxygen delivery; Hb, blood hemoglobin; pRBCs, packed red blood cells; WSS, wall shear stress.

rates per se. With exception of encapsulated Hb suspensions (20), all molecular Hb solutions proposed as blood substitutes are vasoactive due to NO scavenging, causing various degrees of vasoconstriction. Notable exceptions are PEG-Hb (14) and polymerized Hbs (21). These large macromolecules scavenge NO, but also produce it by increasing endothelial shear stress as a consequence of increasing plasma viscosity due to their molecular configuration (22).

Another intriguing intervention strategy stems from the observation that the NO concentration at the wall is not limited by the endothelial NO production capacity. Instead, it can be introduced using NO carriers, e.g., nano-particles currently being developed (23). Hence, management of blood flow velocity by the NO system can significantly influence DO_2 and a method for routine evaluation of NO endothelial responsiveness would be useful in lowering the quantity of transfused blood.

Conclusions

We explored analytically the effect of blood transfusion on O_2 delivery in an anemic subject. We found that outcome varies significantly, depending on the ability of the endothelium to respond to the vasodilatation stimulus arising from the changes in NO production, which overcomes the increased flow hindrance due to increased blood viscosity which increases Hct. Lacking the endothelial NO as stimulus, blood transfusion has small effects and may lower DO_2, since the increased Hct increases blood viscosity. Our findings also suggest shifting the focus of blood transfusion, from that of maintaining O_2 carrying capacity, to the restoration of O_2 delivery, which is directly related to survival.

We illustrate the fundamental role of the endothelial function in regulating arteriolar diameter in maintaining DO_2 in conditions of blood loss. Our study addressed the effect of NO mechano-transduction in the control of arteriolar vessel diameter, assuming that it is either whole (i.e., maximal, and commensurate to that of a healthy normal individual), intermediate down to nonexistent due to injury or disease. The NO concentration at the wall is not limited by the endothelial NO production capacity, since NO can be introduced using NO carriers, particularly nano-particles currently being developed (23), and potentially presently available and usable high viscosity plasma expanders (24). Hence, management of blood flow velocity by the NO system with existing plasma expanders can significantly influence DO_2. Furthermore, the extent of the variability of endothelial responsiveness to biomechanical stimulation is not known. Therefore, a method for routine evaluation of NO endothelial responsiveness would be useful in lowering the quantity of transfused blood.

Although there are significant differences between circulatory DO_2 associated with an endothelium that is either fully responsive or nonresponsive to WSS changes, this is a characteristic mostly prevalent in young, healthy individuals whose hemoglobin deficits might be most frequently due to accidental injuries, rather than systemic disease and age. Most persons exhibit some degree of endothelial impairment; determination of its extent in an individual remains an unrecognized challenge in deciding whether and how much to transfuse in individuals with obvious blood loss.

To date, there is no clear information on the relation between changes in Hct, blood flow, and DO_2 before and after transfusion. This type of data, coupled with a quantitative evaluation of endothelial function in individuals presumed to require blood transfusion, has the potential to significantly reduce the number of blood units used. In this context, we note the existence of multiple methods to evaluate endothelia-dependent changes in regional ultrasound blood flow measurement in response to pharmacological interventions (25). They, however, are mostly applied as cardiac perfusion diagnostic, primarily in research settings, and not as a tool for evaluation of tissue perfusion as a function of blood rheological properties related to blood transfusion. Specific development of this methodology for evaluating endothelial responsiveness to blood viscosity changes could produce highly positive outcomes given the risks associated with transfusion (26), and the health and longevity issues related with the number of units transfused (27, 28).

Transfusion of 1 or 2 units of blood may account for as much as ¾ of the world blood consumption of 119 million units per year (29), whereas there is a current world demand deficit on the order of 100 million units when medicine is practiced according to current standards (30). Therefore, factors that support shifting the practice of transfusion from 2 units to 1 unit are of interest, given their potential for expanding the number of interventions independently of changes in blood availability.

APPENDIX: MATHEMATICAL MODEL OF O_2 DELIVERY

Transport Equations

RBC-rich core ($0 \le r \le r_a - \delta$, where δ is the plasma layer thickness). O_2 partial pressure P_{O2} is assumed to be constant and NO concentration, C_{NO}, to satisfy a steady-state reaction-diffusion equation[5]

$$\frac{D_{NO}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_{NO}}{\partial r}\right) - \lambda_b C_{NO} = 0 \ , \tag{1}$$

where D_{NO} is the diffusion coefficient in the RBC-rich core, $\lambda_b = \lambda_0 \mathrm{Hct}/0.45$ is the reaction rate constant of NO scavenging by RBCs that varies linearly with Hct (4), and λ_0 is the value of λ_b at the reference value of Hct (45%).

Plasma layer ($r_a - \delta \le r \le r_a$) and *glycocalyx* ($r_a \le r \le r_g$, where r_g is the radial distance from the center to the outer edge of the glycocalyx). No biochemical reactions occur in both regions due to the absence of RBCs. Hence, the radial variability of C_{NO} and P_{O2} is described by steady-state diffusion equations

$$\begin{aligned} \frac{D_{NO}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_{NO}}{\partial r}\right) &= 0 \\ \frac{\alpha D_{O2}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial P_{O2}}{\partial r}\right) &= 0 \end{aligned} \ , \tag{2}$$

where α is the O_2 solubility. The diffusion coefficients of both NO (D_{NO}) and O_2 (D_{O2}) in the fluid (i.e., in the RBC core and the plasma layer) are larger than their counterparts in the porous media [glycocalyx, endothelium, vascular wall, and parenchymal tissue] (31). To be concrete, we assume D_{NO} and D_{O2} in the solid compartments to be half of those in the liquid compartments (3).

Endothelium ($r_g \le r \le r_{en}$, where r_{en} is the radial distance from the center to the outer edge of the endothelium). Following Refs. 3, 4, and 15, we assume the O_2 consumption rate to be twice the rate of NO production. The variability of C_{NO} and P_{O2} is described by a system of coupled steady-state reaction-diffusion equations

$$\begin{aligned} \frac{D_{NO}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_{NO}}{\partial r}\right) + R_e &= 0 \\ \frac{\alpha D_{O2}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial P_{O2}}{\partial r}\right) - 2R_e &= 0 \end{aligned} \ . \tag{3}$$

The reaction rate R_e follows Michaelis-Menten kinetics (17, 32)

$$R_e = \frac{R_{NO} P_{O2}}{P_{O2} + K_M} \ , \tag{4}$$

where K_M is the Michaelis-Menten constant, and R_{NO} is the shear-stress-dependent NO production rate (10).

Vascular wall ($r_{en} \le r \le r_w$, where r_w is the radial distance from the center to the outer edge of the vascular wall) and *parenchymal tissue* ($r_w \le r \le r_t$, where r_t is the radial distance from the center to the outer edge of the parenchymal tissue). We assume that NO undergoes a pseudo first-order reaction, and O_2 consumption is inhibited by NO and corresponds to Michaelis-Menten kinetics. The transport equations for C_{NO} and P_{O2} are

$$\begin{aligned} \frac{D_{NO}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_{NO}}{\partial r}\right) - \lambda_t C_{NO} &= 0 \\ \frac{\alpha D_{O2}}{r}\frac{\partial}{\partial r}\left(r\frac{\partial P_{O2}}{\partial r}\right) - R_m &= 0 \end{aligned} \ , \tag{5}$$

where λ_t is the NO consumption rate by tissue; and O_2 consumption rate is given by

$$\begin{aligned} R_m &= \frac{R_{O2,max} P_{O2}}{P_{O2} + K_M^m} \\ K_M^m &= 1 + \frac{C_{NO}}{27 \ \mathrm{nM}} \end{aligned} \tag{6}$$

with the maximum O_2 consumption rate $R_{O2,max}$ being lower in the vascular wall than in the parenchymal tissue (11).

Boundary Conditions

As C_{NO} and P_{O2} are both axisymmetric about the arteriole center $r = 0$, the transport equations are subject to the following boundary conditions

$$\begin{aligned} \frac{\partial C_{NO}}{\partial r}(r=0) &= 0 \\ \frac{\partial P_{O2}}{\partial r}(r=0) &= 0 \end{aligned} \ . \tag{7}$$

Following Lamkin-Kennard et al. (15), we assume that neither NO nor O_2 can diffuse through the outer boundary of the parenchymal tissue ($r = r_t$), i.e., that

$$\begin{aligned} \frac{\partial C_{NO}}{\partial r}(r=r_t) &= 0 \\ \frac{\partial P_{O2}}{\partial r}(r=r_t) &= 0 \end{aligned} \ . \tag{8}$$

Finally, mass conservation across the interfaces between the adjacent layers implies

$$\begin{aligned} C_{NO}^- = C_{NO}^+, \ P_{O2}^- &= P_{O2}^+ \\ \left(D_{NO}\frac{\partial C_{NO}}{\partial r}\right)^- &= \left(D_{NO}\frac{\partial C_{NO}}{\partial r}\right)^+ \\ \left(D_{O2}\frac{\partial P_{O2}}{\partial r}\right)^- &= \left(D_{O2}\frac{\partial P_{O2}}{\partial r}\right)^+ \end{aligned} \ , \tag{9}$$

where the superscripts $-$ and $+$ indicate that the corresponding quantities are computed from the left and the right of each interface shown in Fig. 1.

Plasma Layer Thickness

The second-degree polynomial model (7)

$$\delta = -2.2650 H_d^2 - 1.4377 H_d + 3.2131 \tag{10}$$

provides an accurate representation of the relationship between CFL thickness δ and discharge hematocrit H_d for a wide range of microvessel radii.

Posttransfusion Hct, Blood Viscosity, and Flow Rate

The posttransfusion Hct is evaluated according to

[5]The NO effects are not limited to those included in our model. An additional factor that may eventually be included is that the NO tissue concentration affects tissue O_2 consumption (34).

$$\mathrm{Hct}_{\mathrm{posttransfusion}} = \frac{\mathrm{Hct} \times bv + n \times 0.65 \times 0.3}{bv + n \times 0.3}, \quad (11)$$

where n is the units of pRBCs transfused, a unit of pRBCs has a volume of 300 mL and 65% Hct, bv = 5 L is the patient's initial blood volume.

The blood viscosity is a quadratic function of Hct (1)

$$\mu = 1.22 + 0.00675 \times Hct \times 10^2 + 0.00208 \times Hct^2 \times 10^4. \quad (12)$$

The blood flow rate is given by the Poiseuille law,

$$Q = \frac{\pi J r_a^4}{8\mu}, \quad (13)$$

where J is the pressure gradient.

WSS-Dependent NO Production

The rate of NO production depends on the wall shear stress,

$$\tau_a \equiv \tau(r_a) = \mathrm{J}r_a/2. \quad (14)$$

This dependence is captured by Kirby et al. (33) and Sriram et al. (10), both including a nonlinear relationship between wall shear stress and NO production rate. To accelerate the computations, we utilized a third-degree polynomial,

$$\bar{R}_{NO} = 0.0010{\tau_a}^3 - 0.0374{\tau_a}^2 + 0.6089\tau_a + 1.000 \quad (15)$$

fitted to the model's predictions in Sriram et al. (10) with χ^2 = 0.99. $\bar{R}_{NO}$ is the NO production rate normalized by the NO production rate at zero shear stress or 1.6158 μM/s (10). *Equation 15* is for the fully functional endothelium NO production, i.e., for fully responsive vasculature. With an impaired endothelium (nonresponsive vasculature), the NO production rate is zero.

Blood Vessel Vasodilation and Autoregulation

The change of plasma layer thickness with changing Hct affects both the NO diffusion flux into the RBC-rich core and NO scavenging by RBCs. These changes affect NO concentration in the vessel wall, vessel wall radius (8), and vessel wall shear stress. The interactions of these physiological and biochemical processes, described in Fig. 2, ultimately determine blood flow.

Numerical Algorithm for Calculating Posttransfusion Vessel Radius r_a

We rely on the measurements of NO concentration and blood vessel radius (14) to calculate vessel radius in steady state. This is done as follows:

1) Take J = 40,000 dyn/cm^3 and $r_a = R$ = 20 μm at H_d = 0.45 as reference point; calculate the reference NO concentration, C_{NO_0}, at the vessel outer radius by solving *Eqs. 1–9*.
2) Calculate the posttransfusion Hct by *Eq. 11* and apply a small change to the vessel radius:

$$r'_a = R + \Delta R. \quad (16)$$

Here, we set ΔR = 2 μm if the endothelium is fully functioning and the blood vessel dilates, and ΔR = 0 μm if the vasculature is nonresponsive.

3) Set the counter to n = 0, the algorithm tolerance to $\varepsilon = 10^{-4}$, and the iteration factor to k = 0.3.
4) Set $r_a^{(n)} = r'_a$.
5) Solve for the NO concentration, $C_{NO}^{(n)}$, based on new vessel radius, $r_a^{(n)}$.
6) Compute the vessel radius that corresponds to the updated NO concentration, $C_{NO}^{(n)}$

$$r'_a = \mathrm{R}\left[1 - \beta\left(1 - \frac{C_{NO}^{(n)}}{C_{NO_0}}\right)\right] \quad (17)$$

according to the r_a and C_{NO} relationship provided in Tsai et al. (14), β is chosen to be 0.3.
7) If

$$\left|\frac{r'_a - r_a^{(n)}}{r'_a}\right| \le \varepsilon \quad (18)$$

then output $r_a^{(n)}$. Otherwise, modify the value of the vessel radius according to

$$r_a^{(n+1)} = \left[1 - k\frac{r'_a - r_a^{(n)}}{r'_a}\right] r_a^{(n)}. \quad (19)$$

8) Set $n = n + 1$. Go to Step 4.

Once this updated vessel radius is obtained, we solve coupled reaction–diffusion *Eqs. 1–9* with a finite-difference numerical method (3).

Oxygen Delivery to the Microcirculation

A mass balance analysis suggests that O_2 delivery to the microcirculation, $DO_{2,\mathrm{mic}}$, is the amount of oxygen per unit time delivered by the arterioles minus the diffusional loss (1),

$$DO_{2,\mathrm{mic}} = \alpha \times P_{O2,r_a-\delta} \times Q - D_{O2} \times 2\pi R \times \alpha \times (P_{O2,r_a-\delta} - P_{O2,r_w}), \quad (20)$$

where Q is the blood flow rate, $P_{O2,r_a-\delta}$ is the O_2 partial pressure in the RBC-core, and P_{O2,r_w} is the O_2 partial pressure before entering the parenchymal tissue.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.I. and D.M.T. conceived and designed research; W.L. performed experiments; W.L. and A.G.T. analyzed data; W.L., M.I., and D.M.T. interpreted results of experiments; W.L. and A.G.T. prepared figures; W.L. drafted manuscript; A.G.T., M.I., and D.M.T. edited and revised manuscript; W.L., A.G.T., M.I., and D.M.T. approved final version of manuscript.

REFERENCES

1. **Zimmerman R, Tsai AG, Salazar Vázquez BY, Cabrales P, Hofmann A, Meier J, Shander A, Spahn DR, Friedman JM,**

Tartakovsky DM, **Intaglietta M.** Posttransfusion increase of hematocrit per se does not improve circulatory oxygen delivery due to increased blood viscosity. *Anesth Analg* 124: 1547–1554, 2017. doi:10.1213/ANE.0000000000002008.
2. **Martini J**, **Carpentier B**, **Negrete AC**, **Frangos JA**, **Intaglietta M.** Paradoxical hypotension following increased hematocrit and blood viscosity. *Am J Physiol Heart Circ Physiol* 289: H2136–H2143, 2005. doi:10.1152/ajpheart.00490.2005.
3. **Sriram K**, **Vázquez BY**, **Yalcin O**, **Johnson PC**, **Intaglietta M**, **Tartakovsky DM.** The effect of small changes in hematocrit on nitric oxide transport in arterioles. *Antioxid Redox Signal* 14: 175–185, 2011. doi:10.1089/ars.2010.3266.
4. **Chen X**, **Jaron D**, **Barbee KA**, **Buerk DG.** The influence of radial RBC distribution, blood velocity profiles, and glycocalyx on coupled NO/O_2 transport. *J Appl Physiol (1985)* 100: 482–492, 2006. doi:10.1152/japplphysiol.00633.2005.
5. **Gundersen SI**, **Chen G**, **Palmer AF.** Mathematical model of NO and O_2 transport in an arteriole facilitated by hemoglobin based O_2 carriers. *Biophys Chem* 143: 1–17, 2009. doi:10.1016/j.bpc.2009.02.005.
6. **Tsoukias NM**, **Popel AS.** Erythrocyte consumption of nitric oxide in presence and absence of plasma-based hemoglobin. *Am J Physiol Heart Circ Physiol* 282: H2265–H2277, 2002. doi:10.1152/ajpheart.01080.2001.
7. **Sriram K**, **Intaglietta M**, **Tartakovsky DM.** Non-Newtonian flow of blood in arterioles: consequences for wall shear stress measurements. *Microcirculation* 21: 628–639, 2014. doi:10.1111/micc.12141.
8. **Sriram K**, **Salazar Vázquez BY**, **Tsai AG**, **Cabrales P**, **Intaglietta M**, **Tartakovsky DM.** Autoregulation and mechanotransduction control the arteriolar response to small changes in hematocrit. *Am J Physiol Heart Circ Physiol* 303: H1096–H1106, 2012. doi:10.1152/ajpheart.00438.2012.
9. **Messmer K**, **Sunder-Plasmann L**, **Klövekorn WP**, **Holper K.** Circulatory significance of hemodilution: rheological changes and limitations. *Adv Microcirc* 4: 1–77, 1972.
10. **Sriram K**, **Laughlin JG**, **Rangamani P**, **Tartakovsky DM.** Shear-induced nitric oxide production by endothelial cells. *Biophys J* 111: 208–221, 2016. doi:10.1016/j.bpj.2016.05.034.
11. **Fischkoff S**, **Vanderkooi JM.** Oxygen diffusion in biological and artificial membranes determined by the fluorochrome pyrene. *J Gen Physiol* 65: 663–676, 1975. doi:10.1085/jgp.65.5.663.
12. **Mahler M**, **Louy C**, **Homsher E**, **Peskoff A.** Reappraisal of diffusion, solubility, and consumption of oxygen in frog skeletal muscle, with applications to muscle energy balance. *J Gen Physiol* 86: 105–134, 1985. doi:10.1085/jgp.86.1.105.
13. **Khayutin VM**, **Melkumyants AM**, **Rogoza AN**, **Veselova ES**, **Balashov SA**, **Nikolsky VP.** Flow-induced control of arterial lumen. *Acta Physiol Hung* 68: 241–251, 1986.
14. **Tsai AG**, **Cabrales P**, **Manjula BN**, **Acharya SA**, **Winslow RM**, **Intaglietta M.** Dissociation of local nitric oxide concentration and vasoconstriction in the presence of cell-free hemoglobin oxygen carriers. *Blood* 108: 3603–3610, 2006. doi:10.1182/blood-2006-02-005272.
15. **Lamkin-Kennard KA**, **Buerk DG**, **Jaron D.** Interactions between NO and O_2 in the microcirculation: a mathematical analysis. *Microvasc Res* 68: 38–50, 2004. doi:10.1016/j.mvr.2004.03.001.
16. **Davis BN**, **Yen R**, **Prasad V**, **Truskey GA.** Oxygen consumption in human, tissue-engineered myobundles during basal and electrical stimulation conditions. *APL Bioeng* 3: 026103, 2019. doi:10.1063/1.5093417.
17. **Buerk DG.** Can we model nitric oxide biotransport? A survey of mathematical models for a simple diatomic molecule with surprisingly complex biological activities. *Annu Rev Biomed Eng* 3: 109–143, 2001. doi:10.1146/annurev.bioeng.3.1.109.
18. **Leach RM**, **Treacher DF.** The pulmonary physician in critical care · 2: oxygen delivery and consumption in the critically ill. *Thorax* 57: 170–177, 2002. doi:10.1136/thorax.57.2.170.
19. **Lipowsky HH**, **Firrell JC.** Microvascular hemodynamics during systemic hemodilution and hemoconcentration. *Am J Physiol* 250: H908–H922, 1986. doi:10.1152/ajpheart.1986.250.6.H908.
20. **Sakai H**, **Hara H**, **Yuasa M**, **Tsai AG**, **Takeoka S**, **Tsuchida E**, **Intaglietta M.** Molecular dimensions of Hb-based O_2 carriers determine constriction of resistance arteries and hypertension. *Am J Physiol Heart Circ Physiol* 279: H908–H915, 2000.doi:10.1152/ajpheart.2000.279.3.H908.
21. **Belcher DA**, **Lucas A**, **Cabrales P**, **Palmer AF.** Tumor vascular status controls oxygen delivery facilitated by infused polymerized hemoglobins with varying oxygen affinity. *PLoS Comput Biol* 16: e1008157, 2000. doi:10.1371/journal.pcbi.1008157.
22. **Zhou Y**, **Cabrales P**, **Palmer AF.** Simulation of NO and O_2 transport facilitated by polymerized hemoglobin solutions in an arteriole that takes into account wall shear stress-induced NO production. *Biophys Chem* 162: 45–60, 2012. doi:10.1016/j.bpc.2011.12.006.
23. **Cabrales P**, **Ortiz D**, **Friedman JM.** NO supplementation for transfusion medicine and cardiovascular applications. *Futur Sci OA* 1: FS051, 2015. doi:10.4155/fso.15.51.
24. **Tsai AG**, **Vázquez BYS**, **Hofmann A**, **Acharya SA**, **Intaglietta M.** Supra-plasma expanders: the future of treating blood loss and anemia without red cell transfusions? *J Infus Nurs* 38: 217–222, 2015. doi:10.1097/NAN.0000000000000103.
25. **Flammer AJ**, **Anderson T**, **Celermajer DS**, **Creager MA**, **Deanfield J**, **Ganz P**, **Hamburg NM**, **Lüscher TF**, **Shechter M**, **Taddei S**, **Vita JA**, **Lerman A.** The assessment of endothelial function: from research into clinical practice. *Circulation* 126: 753–767, 2012. doi:10.1161/CIRCULATIONAHA.112.093245.
26. **Shander A**, **Javidroozi M**, **Ozawa S**, **Hare GMT.** What is really dangerous: anaemia or transfusion? *Br J Anaesth* 107, *Suppl* 1: i41–i59, 2011. doi:10.1093/bja/aer350.
27. **Engoren MC**, **Habib RH**, **Zacharias A**, **Schwann TA**, **Riordan CJ**, **Durham SJ.** Effect of blood transfusion on long-term survival after cardiac operation. *Ann Thorac Surg* 74: 1180–1186, 2002. doi:10.1016/s0003-4975(02)03766-9.
28. **Koch CG**, **Li L**, **Duncan AI**, **Mihaljevic T**, **Loop FD**, **Starr NJ**, **Blackstone EH.** Transfusion in coronary artery bypass grafting is associated with reduced long-term survival. *Ann Thorac Surg* 81: 1650–1657, 2006. doi:10.1016/j.athoracsur.2005.12.037.
29. Blood safety and availability (Online). https://www.who.int/news-room/fact-sheets/detail/blood-safety-and-availability [2021 Oct 23].
30. **Roberts N**, **James S**, **Delaney M**, **Fitzmaurice C.** The global need and availability of blood products: a modelling study. *Lancet Haematol* 6: e606–e615, 2019. doi:10.1016/S2352-3026(19)30200-5.
31. **Sharan M**, **Popel AS.** A two-phase model for flow of blood in narrow tubes with increased effective viscosity near the wall. *Biorheology* 38: 415–428, 2001.
32. **Liao JC**, **Hein TW**, **Vaughn MW**, **Huang KT**, **Kuo L.** Intravascular flow decreases erythrocyte consumption of nitric oxide. *Proc Natl Acad Sci USA* 96: 8757–8761, 1999. doi:10.1073/pnas.96.15.8757.
33. **Kirby PL**, **Buerk DG**, **Parikh J**, **Barbee KA**, **Jaron D.** Mathematical model for shear stress dependent NO and adenine nucleotide production from endothelial cells. *Nitric Oxide* 52: 1–15, 2016. doi:10.1016/j.niox.2015.10.004.
34. **Shen W**, **Xu X**, **Ochoa M**, **Zhao G**, **Wolin MS**, **Hintze TH.** Role of nitric oxide in the regulation of oxygen consumption in conscious dogs. *Circ Res* 75: 1086–1095, 1994. doi:10.1161/01.RES.75.6.1086.