Optimal hematocrit for maximal exercise performance in acute and chronic erythropoietin-treated mice

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Erythropoietin (Epo) treatment increases hematocrit (Htc) and, consequently, arterial O\textsubscript{2} content. This in turn improves exercise performance. However, because elevated blood viscosity associated with increasing Htc levels may limit cardiac performance, it was suggested that the highest attainable Htc may not necessarily be associated with the highest attainable exercise capacity. To test the proposed hypothesis that an optimal Htc in acute and chronic Epo-treated mice exists—i.e., the Htc that facilitates the greatest O\textsubscript{2} flux during maximal exercise—Htc levels of wild-type mice were acutely elevated by administering novel erythropoiesis-stimulating protein (NESP; wtNESP). Furthermore, in the transgenic mouse line tg6 that reaches Htc levels of up to 0.9 because of constitutive overexpression of human Epo, the Htc was gradually reduced by application of the hemolysis-inducing compound phenylhydrazine (PHZ; tg6PHZ). Maximal cardiovascular performance was measured by using telemetry in all exercising mice. Highest maximal O\textsubscript{2} uptake (VO\textsubscript{2max}) and maximal time to exhaustion at submaximal exercise intensities were reached at Htc values of 0.58 and 0.57 for wtNESP, and 0.68 and 0.66 for tg6PHZ, respectively. Rate pressure product, and thus also maximal working capacity of the heart, increased with elevated Htc values. Blood viscosity correlated with VO\textsubscript{2max}. Apart from the confirmation of the Htc hypothesis, we conclude that tg6PHZ adapted better to varying Htc values than wtNESP because of the higher optimal Htc of tg6PHZ compared to wtNESP. Of note, blood viscosity plays a critical role in limiting exercise capacity.

Results

Male wild-type and tg6 mice were approximately 8 and 9 weeks old, respectively, when beginning their respective treatments. As expected, differences in Htc were initially observed (Table S1): whereas wild-type males had an Htc of 0.46 ± 0.03, the constitutively Epo-overexpressing transgenic tg6 males suffered from excessive erythrocytosis expressed Htc values of 0.78 ± 0.06. No differences in age, resting mean arterial blood pressure, heart rate, O\textsubscript{2} uptake (VO\textsubscript{2}), or respiratory exchange ratio (RER) were observed between wild-type and tg6 animals at the beginning of the incremental exercise test.

The optimal Htc hypothesis is in disagreement with several studies (17, 18). All of these authors provide evidence that O\textsubscript{2} delivery and thus exercise performance, including VO\textsubscript{2max}, remained relatively constant with chronic excessive erythrocytosis. These findings indicate that adaptive mechanisms to excessive erythrocytosis exist.

To explore the consequences of excessive erythrocytosis in vivo we developed a transgenic mouse line (termed tg6) that reaches Htc values of 0.8–0.9 as a result of a constitutive overexpression of human Epo cDNA (19). Adaptive mechanisms to excessive erythrocytosis include increased plasma nitric oxide levels and enhanced erythrocyte flexibility (19, 20). These data suggest that a shift of the optimal Htc for a maximal endurance performance to a higher Htc value occurs in tg6 mice.

The present study tested the hypothesis that there is an optimal Htc value that allows for maximal systemic endurance performance. The effect of chronically elevated Htc on exercise capacity may differ from that of acutely elevated Htc. We propose that mice with excessive erythrocytosis adapt better to varying Htc levels than animals that experience an acute increase in Htc level. For this purpose, wild-type mice were injected with novel erythropoiesis stimulating protein (NESP; wtNESP) to increase Htc, tg6 mice were treated with the hemolysis-inducing compound phenylhydrazine (PHZ; tg6PHZ), and both wild-type and tg6 mice that did not receive treatment served as controls. Metabolic and cardiovascular measurements were obtained at rest and during endurance performance, whereas whole-blood analysis including rheology was carried out at rest.

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In a normal physiological hematocrit (Htc) range, erythropoietin (Epo) treatment or red blood cell retransfusion that increases hemoglobin concentration ([Hb]) improves maximal O\textsubscript{2} uptake (VO\textsubscript{2max}) and enhances endurance performance (1–6). Little, however, is known regarding the impact of Htc alteration over a wide-ranging Htc. Augmented [Hb] values are associated with a rise in blood viscosity and, consequently, with a higher peripheral vascular resistance that may reduce VO\textsubscript{2max} because of the falling cardiac output (7, 8). Accordingly, the exercise capacity of polycythemic patients with chronic obstructive pulmonary disease (COPD) is improved after hemodilution (9). Because of these counteracting effects, it was suggested that the optimal Htc may be lower than expected because of limitations induced by a higher blood viscosity (8, 10–12). Although the role of Htc on exercise performance seems obvious, there is only one ex vivo study available addressing the above hypothesis in isolated higher vertebrate muscles (11). It should be noted, however, that this may vary under different circumstances because of the blood's non-Newtonian behavior (13). Factors affecting this variation include the species, the organs involved, and whether the organism is in resting or exercising conditions (11, 12, 14–16). Thus, the above mentioned observations do not always reflect the general situation in exercising mammals and humans.
Optimal Htc for Maximal Endurance Performance. Endurance performance consists of the product of an individual’s VO$_{2\text{max}}$ and duration of exercise at a certain percentage of that VO$_{2\text{max}}$ until exhaustion (21). Thus, to investigate the impact of varying Htc levels on endurance performance, individual data of VO$_{2\text{max}}$ (Fig. 1) and time to exhaustion (Fig. 2) were plotted against Htc. To study pulse., regression plot of tg6/tg6PH at a higher blood viscosity. Fig. S3

Fig. S2 www.pnas.org/cgi/doi/10.1073/pnas.0912924107

Increasing Mean Arterial Blood Pressure, Constant Heart Rate, and Altered Stroke Volume with Rising Htc Levels at VO$_{2\text{max}}$. Mean arterial blood pressure, heart rate, and stroke volume were all quantified at VO$_{2\text{max}}$. Mean arterial blood pressure rise with increasing Htc levels in wtNESP and tg6PHZ (Fig. S3 a). Overall, wtNESP reached higher mean arterial blood pressure values compared to those in tg6PHZ. Heart rate did not change with increasing Htc levels and also was not different from group to group (Fig. S3 b).

Previous studies have identified a correlation between Htc and maximal VO$_{2\text{max}}$. Single prints represent individual values. —, regression plot of wild-type (wt) wtNESP; —, regression plot of tg6/tg6PHZ. Also depicted are maximal VO$_{2\text{max}}$ values of wt wtNESP (Max. wt wtNESP) and tg6/tg6PHZ (Max. tg6/tg6PHZ).

Increasing Myocardial VO$_{2}$ with Rising Htc Levels at VO$_{2\text{max}}$. To study the impact of myocardial VO$_{2}$ on VO$_{2\text{max}}$, the rate pressure product was also correlated with Htc levels. Myocardial VO$_{2}$

Fig. 1. Relationship between hematocrit (Htc) and maximal O$_{2}$ uptake (VO$_{2\text{max}}$) in wtNESP and tg6PHZ mice. Single prints represent individual values. —, regression plot of wild-type (wt) wtNESP; —, regression plot of tg6/tg6PHZ. Also depicted are maximal VO$_{2\text{max}}$ values of wt wtNESP (Max. wt wtNESP) and tg6/tg6PHZ (Max. tg6/tg6PHZ).
increased with increasing Htc values in both groups (Fig. 5). Both graphs showed a similar slope, but wtNESP mice had higher rate pressure product values at corresponding Htc levels than did tg6PHZ, indicating that the hearts of wtNESP mice had a higher requirement of myocardial O\textsubscript{2} supply.

**Discussion**

The present study demonstrates that optimal Htc values maximizing systemic endurance performance in mammals exist. In doing so we observed that NESP-treated wild-type animals maximized exercise performance at a lower optimal Htc than hemolysed tg6PHZ mice constitutively overexpressing Epo. Furthermore, the data demonstrated that (i) the optimal Htc levels for maximal systemic exercise performance and maximal stroke volume were similar; and (ii) blood volumes were dramatically increased at higher Htc levels.

\( \text{VO}_{2\text{max}} \) as well as time to exhaustion as well as initially rose, reached a maximum value, and then decreased with increasing Htc values. Maximal \( \text{VO}_{2\text{max}} \) and time to exhaustion values were found at Htc levels of 0.58 and 0.57 for wtNESP, and 0.68 and 0.66 for tg6PHZ, respectively. Our data are in agreement with the classical optimal Htc hypothesis (8, 10–12, 25), where it was speculated that the optimal systemic Htc of mammals would be much higher than the commonly observed 0.45. These calculations were based on the conditions present in the circulatory system at rest, as exercise induces changes in vessel diameters, blood flow, internal temperature, and blood distribution. However, before this study there was no experimental proof of this concept in living mammals. Gaethgens and coworkers (11) studied the effect of blood perfusion creating various Htc levels in isolated dog muscle during rhythmic isotonic exercise. Maximal \( \text{VO}_{2} \) and contractile power reached a plateau at Htc levels between 0.4 and 0.7. The authors concluded that these results may not be transferable to whole-body systemic exercise because each organ may present with its own individual optimal Htc (11, 16). Therefore, the systemic Htc value must be interpreted as an average of all organ-specific optimal Htc values, thereby providing the whole organism with adequate O\textsubscript{2} supply. During exercise more O\textsubscript{2} is required by the working skeletal muscles and thus a shift from the optimal physiological to the optimal Htc value for maximal endurance performance is observed. To cover their high O\textsubscript{2} demand during strenuous exercise, some terrestrial vertebrate species, such as horses and dogs, but not mice, release stored erythrocytes into the circulation by splenic contraction (26, 27). This mechanism elevates the blood O\textsubscript{2}-carrying capacity during exercise within seconds. As a consequence, Htc increases from ≈0.4 at rest to ≈0.6 at exercise (28, 29) facilitating an improvement in exercise performance. These hematopoietic parameters return quickly to resting values when the animals stop exercising, preventing a constant overload of the cardiovascular system (28–30).

In light of our data, it is tempting to speculate that horses and dogs temporarily elevate their Htc levels close to the optimal value Htc to reach maximal systemic endurance performance. Indeed, splenic contraction has also been observed in humans during performing a maximal exercise test, as evidenced by a reduction in its volume and two-thirds decrease in splenic erythrocyte content, but Htc levels increased only a very small percentage from rest to maximal working levels (31). At first glance, the observed relationship between \( \text{VO}_{2\text{max}} \) and Htc in our study is in contradiction to others described in human studies. Several investigators have shown that there is a strong correlation between \( \text{VO}_{2\text{max}} \) and total hemoglobin mass or blood volume (2, 3, 22), but not between \( \text{VO}_{2\text{max}} \) and Htc (32). It is of note, however, that most human studies are carried out with Htc values from 0.4 to 0.5. Thus, the ergogenic effect of total hemoglobin mass and blood volume as the primary limiting factors of performance in endurance sports seems only to be valid in the physiologically occurring Htc range. At higher Htc levels, other...
Blood volume

![Graph A](image)

\[ y = -0.0022x^2 + 0.6703x + 93.356 \]

\[ R^2 = 0.6891; P < 0.0001 \]

\[ y = -0.001t + 0.5474x + 76.706 \]

\[ R^2 = 0.4472; P < 0.0001 \]

Total hemoglobin mass

![Graph B](image)

\[ y = -40.341x^2 + 61.123x + 122.03 \]

\[ R^2 = 0.635; P < 0.0001 \]

\[ y = -14.648x + 48.38x + 108.38 \]

\[ R^2 = 0.4006; P < 0.0001 \]

**Fig. 3.** Relationship between maximal O\(_2\) uptake (VO\(_{2}\)\(_{\text{max}}\)) and blood volume (a) and total hemoglobin mass (b) during terminal determination in wtNESP and tg6PHZ mice. Singles prints represent individual values. —, regression plot of wild-type (wt)/wtNESP; —, regression plot of tg6/tg6PHZ.

factors, such as blood viscosity, may counteract the positive effects of enhanced arterial O\(_2\) content because the total hemoglobin mass, unlike to Htc or [Hb], cannot be masked by other factors. In agreement with this hypothesis, we found that the closest relationship between VO\(_{2}\)\(_{\text{max}}\) and total hemoglobin mass in wtNESP animals is within the Htc range of 0.4 and 0.55 (\(R^2 = 0.513; P < 0.001\)).

The augmented blood volume at higher Htc values observed in tg6 mice is caused by the dramatically increased number of erythrocytes, whereas plasma volume remains unchanged (19). An elevation in blood volume enhances end-diastolic volume (preload) and results in increased stroke volume via the Starling mechanism (33), which will lead to the enhancement of VO\(_{2}\)\(_{\text{max}}\) as long as heart rate is not altered. However, after reaching a maximum of 0.57 for wtNESP and 0.68 for tg6PHZ, the blood viscosity may have a negative contribution to cardiac performance. High blood viscosity increases arterial blood pressure and diminishes venous return because of the increased peripheral resistance (12, 34), a fact that may reduce stroke volume and thus exercise performance. A converse observation is made in patients suffering from polycythemic COPD after phlebotomy (9). In these patients, an improvement in exercise tolerance appears to be due to an improvement in cardiac function as evidenced, primarily, by an increased stroke volume. The close correlation between VO\(_{2}\)\(_{\text{max}}\) and blood viscosity, as well as between stroke volume, arterial blood pressure, and Htc, confirms this explanation. Moreover, the fact that rate pressure product and arterial blood pressures were increasing with incremental elevation of Htc levels shows that VO\(_{2}\)\(_{\text{max}}\) was unaffected by the maximal work capacity of the heart at optimal Htc.

The different optimal Htc levels obtained by increasing the normal physiological Htc of wild-type mice and reducing the elevated Htc of tg6 mice could be resolved by at least two mechanisms: (i) enhancement of endothelial nitric oxide synthase activity, which results in peripheral vasodilation despite concomitant increased endothelial-1 levels (19, 35), and (ii) regulated elevation of blood viscosity by increasing erythrocyte flexibility in tg6 mice compared to their controls (20). Both mechanisms would induce a shift to a higher optimal Htc value in the tg6 mice. Thus, tg6PHZ might be able to adapt better to varying Htc levels than wtNESP. Physiological adaptations to excessive erythrocytosis are also observed in dogs and humans (17, 18). Moreover, one case report in sport medicine describes a successful Finnish cross-country skier with an autosomal dominant mutation in Epo receptor that resulted in increased sensitivity of erythroid progenitors to Epo that ultimately led to Htc levels of 0.68 (17). That endurance athlete won several Olympic gold medals. Based on our study, we conclude that his Htc may be very close to the optimal Htc for maximal endurance performance.

In summary, the results of the present study confirm the optimal Htc hypothesis during systemic exercise in mice. The reason for this is that blood viscosity increases with rising Htc levels, limiting the blood's O\(_2\) transport capacity. Furthermore, animals with chronic excessive erythrocytosis adapted better to different Htc levels than did acutely NESP-injected animals. At normoxia, the heart can tolerate higher rate pressure product at higher Htc levels. Thus, the optimal Htc values for maximal endurance performance were not caused by heart failure or attainment of the maximal working capacity of the heart, VO\(_{2}\)\(_{\text{max}}\) is mainly limited by O\(_2\) delivery.

Blood viscosity

![Graph C](image)

\[ y = -1.2907x^2 + 12.664x + 113.88 \]

\[ R^2 = 0.4339; P < 0.0001 \]

\[ y = -1.7469x^2 + 24.414x + 64.448 \]

\[ R^2 = 0.5419; P < 0.0001 \]

**Fig. 4.** Relationship between maximal O\(_2\) uptake (VO\(_{2}\)\(_{\text{max}}\)) and blood viscosity during terminal determination in wtNESP and tg6PHZ mice. Singles prints represent individual values. —, regression plot of wild-type (wt)/wtNESP; —, regression plot of tg6/tg6PHZ. Also depicted are maximal VO\(_{2}\)\(_{\text{max}}\) values of wt/ wtNESP (Max. wt/wtNESP) and tg6/tg6PHZ (Max. tg6/tg6PHZ).
Materials and Methods
Experimental Animals and Set-up. The constitutively Epo-overexpressing tg6 mouse line was generated as described in ref. 19. Compared to wild-type control, the tg6 mouse line had a 10- to 12-fold increase in plasma Epo-levels, resulting in Htc levels of up to 0.9 (19, 20). Approximately half of the offspring were hemizygous for the transgene and were used for the hemolysis-inducing experiments, whereas the other half were used as wild type for the homoconcentration experiments. Males were 12 weeks old during the first exercise test (Table S1). In total, 41 wild-type mice and 40 tg6 mice were analyzed. No weight loss occurred during the study period. Mice were kept in standard rodent cages (T3) with food and water supplied ad libitum. Other detailed methods are provided in SI Materials and Methods.

The experimental design is shown in Fig. S4. At an age of 3 weeks, only tg6 mice were splenectomized to keep Htc levels low, because extramedullary erythropoiesis occurs in the spleen (20). One week later, telemetric blood pressure transmitters were implanted in 20 wild-type mice and 19 tg6 mice that were 4 weeks old. In the remaining animals, dummy transmitters were implanted (wild type, n = 21; tg6, n = 21). Adjustments of the Htc levels were started in 8- and 9-week-old animals, respectively. At an age of 12 weeks, the main experiments were conducted, including incremental as well as constant workload exercise tests (see below) followed by measurements of the SaO2, Htc, blood viscosity, plasma, and blood volume. To exclude the impact of circadian rhythm, all measurements were performed at the identical time of day.

Other detailed methods are provided in SI Materials and Methods.

Statistics. All data were analyzed by using StatView software (Version 4.57; Abacus Concepts). The relationship between the two parameters was analyzed with linear or polynomial regression. Significances were performed by a one-way analysis of variance (ANOVA). Results are expressed as mean ± SD. Statistical difference was set at P < 0.05.

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