Effects of various training modalities on blood volume

W. Schmidt, N. Prommer

Department of Sports Medicine/Sports Physiology, University of Bayreuth, Bayreuth, Germany

Corresponding author: W. Schmidt, Department of Sports Medicine/Sports Physiology, University of Bayreuth, 95440 Bayreuth, Germany. Tel: +49 921 55 3464, Fax: +49 921 55 3468, E-mail: walter.schmidt@uni-bayreuth.de

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It is controversially discussed whether soccer games should be played at moderate (2001–3000 m) and high altitudes (3001–5500 m) or should be restricted to near sea level and low altitude (501–2000 m) conditions. Athletes living at altitude are assumed to have a performance advantage compared with lowlanders. One advantage of altitude adaptation concerns the expansion of total hemoglobin mass (tHb-mass), which is strongly related to endurance performance at sea level. Cross-sectional studies show that elite athletes possess ~35% higher tHb-mass than the normal population, which is further elevated by 14% in athletes native to altitude of 2600 m. Although the impact of this huge tHb-mass expansion on performance is not yet investigated for altitude conditions, lowland athletes seek for possibilities to increase tHb-mass to similar levels. At sea level tHb-mass is only moderately influenced by training and depends more on genetic predisposition. Altitude training in contrast, using either the conventional altitude training or the live high–train low (≥14 h/day in hypoxia) protocol for 3–4 weeks above 2500 m leads to mean increases in tHb-mass of 6.5%. This increase is, however, not sufficient to close the gap in tHb-mass to elite athletes native to altitude, which may be in advantage when tHb-mass has the same strong influence on aerobic performance at altitude as it has on sea level.

Hypotheses

Altitude residents are characterized by higher oxygen transport capacity of the blood than non-adapted subjects from lowlands. This improved oxygen capacity is due to the adaptation of the erythropoietic system which compensates reduced oxygen availability by increasing total hemoglobin mass (tHb-mass). It, therefore, is assumed that this adaptation process is one main cause for the higher endurance performance of altitude natives in hypoxia. In athletic competitions (e.g., soccer games) conducted at altitude it is assumed that this fact may put lowlanders which do not have sufficient time to adapt their erythropoietic system at a disadvantage. However, the differences in endurance performance at altitude due the advantage of improved oxygen capacity have not yet been quantified. This fact is of special importance regarding the current discussion whether soccer games should be played at moderate (2001–3000 m) and high altitude (3001–5500 m). To answer this question the present review addresses the following hypotheses:

1. Maximal endurance performance directly depends on the oxygen transport capacity and therefore on tHb-mass
2. Athletes living at altitude posses more tHb-mass and do therefore perform better at altitude than non-adapted sea-level subjects
3. Training at sea level of professional athletes does not increase tHb-mass to the same level as it occurs in athletes native to altitude.
4. Altitude training of athletes from lowlands may close the gap in tHb-mass between sea level and altitude athletes and may therefore lead to equal chances regarding aerobic performance.

In addition, this review addresses (i) the hypoxic threshold to elevate tHb-mass in a sea-level athlete and (ii) the magnitude of tHb-mass expansion which can be expected by different hypoxic measures.

Overview

Influence of tHb-mass on endurance performance

Maximal endurance performance expressed as VO₂max depends on muscular oxygen consumption and oxygen transport of the blood. In elite endurance athletes muscular oxidative capacity is well adapted whereas oxygen transport is the main limiting factor (Wagner, 2000). The impact of blood on VO₂max is demonstrated in studies describing the effect of phlebotomy, of blood re-transfusion, and of erythropoietin (EPO) application. One day after drawing 550 mL of blood VO₂max decreased by 255 mL/min in moderately trained subjects (Prommer et al., 2007b). After phlebotomy and subsequent re-transfusion of 1800 mL
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blood VO$_{2\text{max}}$ differed by 740 mL/min (Celsing et al., 1987), and in case of a 4-week lasting EPO application, a raise in VO$_{2\text{max}}$ by 307 mL/min is reported (Parisotto et al., 2000). Best correlations between changes in VO$_{2\text{max}}$ and blood constituents were found for tHb-mass and red cell mass (RCM). The change of 1 g of hemoglobin, either by reduction or by expansion, is associated with a change in VO$_{2\text{max}}$ by ~3 mL/min (Parisotto et al., 2000; Prommer et al., 2007b). This review, therefore, focuses on tHb-mass/RCM rather than on blood or plasma volume although both are the main performance limiting factors for elite athletes under physiological conditions.

Measurement of tHb-mass

Until 1990 only few data on tHb-mass and RCM for training and altitude conditions were available because the prevailing direct determination methods at that time were based on radioactive markers and were, therefore, associated with considerable side effects. The CO re-breathing method first described by Grehan and Quinquard (1882) and modified by Thomsen et al. (1991), Burge and Skinner (1995) and Schmidt and Prommer (2005) provided the possibility of collecting substantial amounts of data. It was, however, at first criticized by, e.g., Sawka et al. (2000) as to overestimate tHb-mass by about 20% due to the assumption that CO diffuses to myoglobin and muscular cytochromes. This argument was recently rebutted by Prommer and Schmidt (2007) demonstrating that this effect is negligible because the duration of the test (7 min) is too short for large diffusion rates. Complete mixing of CO in the blood including also those red cells being located in the spleen (Prommer et al., 2007a) is guaranteed 7 min after inhalation of the CO bolus. In an extensive meta-analysis Gore et al. (2005) demonstrated the CO-re-breathing method as the superior technique [coefficient of variance (CV) 2.2%] compared with the hitherto gold standard using radioactive markers ($^{51}$chromium labeled red cells, CV 2.8%) and to Evans Blue dilution (CV 6.7%). The actual optimized CO-re-breathing technique with a typical error between 1.1% (Gore et al., 2006) and 1.7% (Schmidt & Prommer, 2005) allows the identification of even small changes in tHb-mass (95% confidence limit: between ±2.2% and ±3.3%, respectively) occurring with endurance training and adaptation to hypoxia.

**tHb-mass at altitude**

It is a well-known fact since decades that altitude hypoxia stimulates erythropoiesis and increases tHb-mass and blood volume (BV). Weil et al. (1968) described a threshold at an arterial PO$_2$ of 70 mmHg (1600 m) corresponding to an oxygen saturation of 95%, below which RCM continuously increases with declining SaO$_2$. Residents from 2600 m (Bogota, Colombia; Böning et al., 2001) and 3550 m (Putre, Chile; Heinicke et al., 2003) are therefore characterized by 11% and 14% higher tHb-mass values, respectively. In subjects from 4390 m even 83% elevated values were found (Sánchez et al., 1970). It has, however, to be noted that all of these data were collected from South and North American inhabitants. Whether this polycythemia is also prevalent in residents from the Himalayas is questionable because they possess much lower [Hb] (Beall, 2000) than the Andean population residing at similar altitudes, indicating either higher plasma volume or lower hemoglobin mass.

In this context, data are not only rare for chronic hypoxia but also for transient stays at altitude. No changes in tHb-mass are reported for up to 3-week periods below 4000 m (Sawka et al., 2000) while modest increases were described after 6 months long-term intermittent hypoxia at 3550 m (11%; Heinicke et al., 2003; Prommer et al., 2007c), and after a 6-week Himalaya expedition at a mean altitude of 5000 m (14%; Böning et al., 1997). Strong changes by more than 40% were shown by Pugh (1964) after 126 days above 5500 m and by Reynafarje et al. (1959) after 1 year at 4550 m. Available data, therefore, clearly demonstrate that hypoxia improves oxygen transport capacity in case time of exposure and altitude pass a certain threshold.

**tHb-mass and performance – cross-sectional studies in normoxia**

Beside altitude exposure the state of endurance performance is the main factor associated with tHb-mass and RCM. Kjellberg et al. (1949) were the first investigators describing the relationship between BV, tHb-mass, and aerobic performance. They reported a training-dependent level of BV and tHb-mass of 75.0 mL/kg and of 11.5 g/kg for untrained male subjects, 90.1 mL/kg (+20%) and 13.6 g/kg (+18%) for moderately trained athletes, as well as 103.4 mL/kg (+38%) and 15.7 g/kg (+37%) for elite athletes, respectively. A similar picture on a 10% lower absolute level was demonstrated for females. In a second pioneer study, Astrand (1952) confirmed this close relationship between tHb-mass and VO$_{2\text{max}}$ ($r = 0.97$) for a broad range of tHb-mass (between 100 and 900 g) for all age groups between 7 and 30 years. The differences in BV between trained and untrained subjects were confirmed later by Dill et al. (1974) and Brotherhood et al. (1975), while other investigators (e.g., Green et al., 1999) showed only marginal differences in both,
tHb-mass and BV. In 2001 Heinicke et al. differentiated BV and tHb-mass in male elite athletes of different disciplines and reported about 40% higher values in endurance athletes (running, cycling, triathlon, and cross country skiing) than in untrained subjects, but only moderately elevated values in athletes practicing anaerobic disciplines (+12%, down hill skiers) and in swimmers (+20%). In the latter group, the supine position during training and the immersion effects may counteract the BV and tHb-mass expansion. Data from football players are not yet available. From the results of Gore et al. (1997) and Heinicke et al. (2001), however, we may assume a BV of about 100 mL/kg and tHb-mass of 14.0 g/kg in elite football players (VO2max 65 mL/kg).

The strong positive relationship between VO2max, BV, and tHb-mass for absolute and relative values was proved in several cross-sectional studies with small numbers of subjects (Astrand, 1952; Convertino, 1991; Gore et al., 1997; Schmidt et al., 1999; Heinicke et al., 2001).

To obtain a generally accepted base for the dependency of VO2max on tHb-mass and BV, we performed the following meta-analysis: In total 611 subjects, 393 males and 218 females, living at sea level \( n = 490 \), 2600 m \( n = 82 \), or 3500 m \( n = 39 \) were included. The studies were carried out by two laboratories [Berlin (\( n = 212 \)) and Bayreuth (\( n = 399 \))] and most of the data are published in peer-reviewed journals (Schmidt, 1999, 2002; Heinicke et al., 2001; Böning et al., 2001; Schmidt et al., 2002; Prommer et al., 2007c).

All subjects performed a vita maxima test on a treadmill or on a cycle ergometer with a similar protocol (for detailed description see original publications). In order to obtain comparable VO2max for the cycle ergometer and the treadmill, values achieved with the latter test were reduced by 7% (e.g. Dill et al., 1974). VO2max which was examined at altitude was calculated to sea-level conditions according to Fulco et al. (1998). In all subjects tHb-mass and BV were determined by the CO re-breathing technique as described by Heinicke et al. (2001, \( n = 507 \)) or by Schmidt and Prommer (2005, \( n = 104 \)). Both methods yielded identical results (Schmidt & Prommer, 2005). Furthermore, hemoglobin concentration and hematocrit \( n = 593 \) as well as serum concentrations of EPO \( n = 496 \) and transferrin receptor \( n = 370 \) were included into the meta-analysis. All of the data, except for EPO, were normally distributed and showed no systematic difference between the two laboratories. No dependencies on age were observed. The athletes were classified into four performance categories according to their relative sea-level VO2max values (for classification see Table 1).

### Total hemoglobin mass and altitude training

The results of this meta-analysis are in accordance with the tHb-mass and BV data of Kjellberg et al. (1949) showing the lowest values for non-exercising subjects (NP) and about 30% higher values for elite athletes (EP, Fig. 1, Table 1). It has to be noted that the NP-group was not completely sedentary. In all of the athletic performance groups, plasma volume was likewise expanded as red cell volume leading to similar hemoglobin concentrations in all subgroups of the same altitude.

Despite a broad scattering of VO2max (e.g., with a prevailing tHb-mass of 12.0 g/kg VO2max values between \( ~40 \) and \( ~60 \) mL/kg/min can be attained), there is a close relationship between these two parameters \( r = 0.79 \), Fig. 2). The slope of the regression line (at 0 m: 4.4), which is similar for males (4.2) and females (4.6) indicates that a change in tHb-mass by 1 g/kg is associated with a change in VO2max by 4.4 mL/kg/min. A similar close dependency is obtained between BV and VO2max \( r = 0.762 \) where a change in 1 mL blood/kg is related to a change in VO2max by 0.7 mL/kg/min.

These data are well in accordance with the results of previous studies involving smaller sample size. Gore et al. (1997) described a slope of 4.4 for relative tHb-mass and Convertino (1991) 0.66 for relative BV and VO2max.

While the effects of tHb-mass and BV on performance can be precisely quantified by all these cross-sectional studies, we did not find any significant dependency of VO2max on hemoglobin concentration (males \( r = 0.03 \), females \( r = 0.12 \)) or hematocrit (males \( r = 0.08 \), females \( r = 0.11 \)). We, therefore, conclude that under physiological, non-anemic conditions at sea level the oxygen transport to the muscle tissue is regulated by changes in BV with normal hemoglobin concentration rather than by changes in hemoglobin concentration, itself.

### tHb-mass and performance – cross-sectional studies in hypoxia

There is no doubt that altitude residents possess higher tHb-mass than comparable inhabitants from lowlands (see “tHb-mass at altitude”). If tHb mass is the major determinant of performance, one could assume that endurance performance is also increased which would give this population an advantage in competitions at altitude (e.g., soccer games). Whether the strong relationship between the oxygen transport system and VO2max is also true for natives to altitude, however, still has to be evaluated.

To address this question, we also included subjects from altitude (2600 m, \( n = 82 \); 3500 m, \( n = 39 \)) into the meta-analysis and classified them according to the criteria for lowlanders (see Table 1). Their VO2max determined at altitude was, therefore, cor-
rected to sea-level conditions (see Fulco et al., 1998). Because the relationship between tHb-mass and VO$_{2\text{max}}$ was not different in both altitude groups (Fig. 2) they were combined to one group (2600 m) in order to increase statistical power.

In all male performance groups tHb-mass was between 9% and 14% higher in the altitude than in the sea-level groups (Fig. 1). A similar picture with slightly lower differences was found for the female groups. This higher RCM was compensated by reduced plasma volume leading in turn to a similar BV as in lowlanders (Table 1). Only in the elite athletes from altitude tHb-mass and BV were disproportionately high which explains their very high VO$_{2\text{max}}$ values. Also hemoglobin concentration of the altitude subgroups was considerably increased due to lower plasma volume and elevated tHb-mass.

The regression line demonstrating the relationship between tHb-mass and VO$_{2\text{max}}$ is displaced to the right for the altitude residents indicating the need of higher tHb-mass for a similar aerobic performance as achieved by lowlanders. From these data, we conclude that increased tHb-mass in altitude residents does not result in higher VO$_{2\text{max}}$ values at sea level. This is supported by Prommer et al. (2007c) describing even lower VO$_{2\text{max}}$ at sea level for well-adapted subjects to 3550 m with markedly increased tHb-mass compared with lowlanders.

Hence, the question is whether altitude residents and hypoxia-adapted subjects can at least benefit from their hematological adaptation in hypoxia. Again Prommer et al. (2007c) found no differences in VO$_{2\text{max}}$ obtained at 3550 m in altitude-adapted soldiers and newcomers being the first day at that altitude despite a difference in tHb-mass of 11%. Furthermore, data of Heinicke et al. (2003) indicate lower VO$_{2\text{max}}$ in residents living permanently at altitude compared with newcomers.

### Table 1. VO$_{2\text{max}}$, blood volumes, and hematological indices of subjects from lowlands and altitude classified according to their aerobic performance

<table>
<thead>
<tr>
<th></th>
<th>NP (n=70)</th>
<th>MP (n=121)</th>
<th>HP (n=110)</th>
<th>EP (n=92)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VO$_{2\text{max}}$ (mL/kg/min)</strong></td>
<td></td>
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<tr>
<td><strong>Hb (g dL)</strong></td>
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<tr>
<td><strong>BV (mL/kg)</strong></td>
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<td><strong>PV (mL/kg)</strong></td>
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<td><strong>EV (mL/kg)</strong></td>
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<td><strong>n = 514</strong></td>
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<tr>
<td><strong>n = 79</strong></td>
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<td><strong>n = 176</strong></td>
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<td><strong>n = 42</strong></td>
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</tbody>
</table>

The subjects were divided into four groups according to their VO$_{2\text{max}}$ at sea level. Males: normal performance (NP, n=70) <48 mL/kg/min, moderate performance (MP, n=121) 48–57 mL/kg/min, high performance (HP, n=110) 58–67 mL/kg/min, and elite performance (EP, n=92) >67 mL/kg/min. Females: NP (n=52) <37 mL/kg/min, MP (n=83) 38–47 mL/kg/min, HP (n=45) 48–57 mL/kg/min, and EP (n=38) >57 mL/kg/min.

0 m indicates near sea level condition, 2600 m combines data from natives to 2600 m and 3550 m as well as long-term intermittently adapted subjects to 3550 m (adaptation period between 6 months and 22 years). Differences between altitudes:

- *P<0.05,
- **P<0.01,
- ***P<0.001.

Except for haemoglobin concentration all differences between MP, HP, and EP to NP are highly significant.
To evaluate the effect of tHb-mass on performance at altitude directly Young et al. (1996) infused 700 mL autologous erythrocytes in saline (≈97 g hemoglobin) before ascending to 4300 m. VO\textsubscript{2max} measured the day before re-infusion, and on day 1, and day 9 at altitude did not differ from the values of a control group. At altitude it dropped by 26% and remained on the same low level in both groups. These results were confirmed by Pandolf et al. (1998) showing no significant differences in 3.2 km race time at 4300 m when 700 mL autologous erythrocytes were re-transfused.

All these studies clearly state that increased tHb-mass does not necessarily lead to higher aerobic performance at altitude. It has, however, to be noted that all studies were conducted with relatively un-trained subjects and that the results may not mirror the behavior of elite athletes. According to Wagner (2000), the oxygen transport system gains importance with increasing training state whereas in un-trained subjects the metabolic capacity of the muscle is the performance-limiting factor. We, therefore, cannot exclude that like at sea level the erythrocytic system is the determining factor for endurance performance in elite athletes. To answer this question more data are necessary.

### Strategies to increase tHb-mass

#### Effect of training in normoxia on tHb-mass

High tHb-mass and BV in elite endurance athletes are frequently assumed to be due to an erythropoietic adaptation to the training process. Sawka et al. (2000) postulated no effect on RCM in case the training period is <11 days, but showed a marked increase by about 8% when 21 days are exceeded. Original data from the literature are, however, inconsistent. In most training studies lasting 4–12 weeks on untrained or trained subjects no effect on tHb-mass or RCM was found using direct methods for determination like radioactive labeling (Ray et al., 1990; Green et al., 1991; Shoemaker et al., 1996) or CO re-breathing (Gore et al., 1997). Only Remes (1979) described a small increase by 4.1% in RCM in a group of 30 soldiers after 6 months of military training. Two other studies, however, using the indirect determination by Evans Blue dilution and subsequent calculation of RCM reported an increase by ~8% after 3 weeks (Schmidt et al., 1988) and by ~12% after 12 weeks of endurance training (Warburton et al., 2004). Because it cannot be excluded that the cell factor (ratio body Hct/venous Hct) used in these studies for calculation of tHb-mass changes during the training period as it occurs e.g., at altitude (Sánchez et al., 1970) the positive erythropoietic response to training has to be confirmed by direct measurements.

We, therefore, documented the effect of a 9 months lasting (December–September) endurance training for a marathon competition on tHb-mass and VO\textsubscript{2max} in 16 leisure sportsmen (six without marathon experience). As demonstrated in Table 2, tHb-mass significantly increased by 6.4% (60 g) and BV by 10.3% (690 mL) the latter being mostly due to the well-known plasma volume expansion (+11.6%, 470 mL). As a consequence [Hb] significantly decreased by 0.5 g/dL. VO\textsubscript{2max} continuously increased during the training period by 5.9% (250 ± 213 mL/ min) and was closely related to the increase in tHb-mass. The slope of the regression line is equal to that after phlebotomy and blood re-transfusion confirming again that the change in tHb-mass by 1 g is...
correlated with a change in VO2max by \( \sim 3 \text{ mL/min} \) (Fig. 3).

It has to be noted that this moderate increase in tHb-mass by 6.4% was observed in relatively untrained subjects. In highly trained athletes, Prommer et al. (2005) showed in contrast no systematic changes in five repetitive measurements over a whole training year. The mean individual oscillation (difference between the lowest and highest value) during the year was 4.6%, which is just slightly above the noise of the applied CO-re-breathing method. From all available data, we may therefore conclude that training has only small effects on tHb-mass and that genetic predisposition should be considered to be responsible for high tHb-mass in elite athletes and subsequently for their high endurance performance.

Whenever the impact of special genetic alterations, as e.g., ACE gene polymorphism (e.g. Joyner, 2001) is still unclear, our opinion is supported by Martino et al. (2002) describing high BV (92.3 mL/kg) and high tHb-mass (13.8 g/min) in subjects with high VO2max (65.0 mL/kg/min) without any training history. Also our results from a longitudinal study determining tHb-mass twice with an interval of 9 years (1998–2007, \( n = 11 \)) showed unchanged values (tHb-mass in 1998: 1028 ± 184 g, in 2007: 1023 ± 196 g, TEM = 4.0%) despite most of the previously competitive athletes had quit their carrier or markedly reduced their training volume (unpublished results).

### Effect of training in hypoxia on tHb-mass (Table 3)

The effect of conventional altitude training (Live High–Train High, LH–TH) on tHb-mass or RCM is still discussed controversially. Some authors are of the opinion that the erythropoietic capacity of elite endurance athletes has already reached an upper limit which cannot be exceeded by using physiological measures (see Gore et al., 1998). On the other hand the very high tHb-mass values of elite athletes native to altitude (+14%, Fig. 1) let us assume that the erythropoietic capacity has not reached its maximum in lowlanders. Altitude training or hypoxic training may, therefore, serve as an additional stimulus to increase tHb-mass. The literature, however, provides inconsistent results. No effects were found after training camps performed below 2000 m (1760 m, Friedmann et al., 1999; 1900 m, Svedenhag et al., 1997) and also at 2690 m when athletes fell ill (Gore et al., 1998). An alternative explanation is that the accuracy of the method used in these studies was not high enough to detect small changes. The majority of studies, however, found an increase in tHb-mass. Substantial increases after a 3- or 4-week lasting training camp at altitudes between 2100 and 2500 m are shown by Rusko et al. (2004+6%), Friedmann et al. (2005+6.3%), Heinicke et al. (2005+9.3%), and Levine and Stray-Gunderson (1997+10.5%).

During the last years the concept “LH–TL” introduced by Drs. Levine and Stray-Gunderson (1992) and by Finnish scientists from Dr. Rusko’s group (Laitinen et al., 1995), became more and more popular. Most investigators affirm that this form of training achieves positive effects on aerobic performance even though the mechanisms involved are not clear yet. Some attribute increased performance after a LH–TL protocol mainly to augmented RCM (Levine & Stray-Gundersen, 2005), while the others consider increased efficiency and buffer capacity most relevant mechanisms because they did not find substantial increases in tHb-mass (Gore &

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**Table 2. VO2max, blood volumes, and hematological indices during a 9-month lasting endurance training of 16 male leisure sportsmen**

<table>
<thead>
<tr>
<th></th>
<th>December/January</th>
<th>March/April</th>
<th>August/September</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>80.0 ± 9.5</td>
<td>80.2 ± 9.2</td>
<td>79.1 ± 9.3</td>
</tr>
<tr>
<td>[Hb] (g dL)</td>
<td>15.2 ± 0.8</td>
<td>15.1 ± 0.7</td>
<td>14.7 ± 0.5**</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>43.9 ± 1.7</td>
<td>44.1 ± 1.9</td>
<td>43.1 ± 1.5</td>
</tr>
<tr>
<td>tHb-mass (g)</td>
<td>932 ± 112</td>
<td>967 ± 110*</td>
<td>992 ± 103***</td>
</tr>
<tr>
<td>Red cell mass (mL)</td>
<td>2683 ± 299</td>
<td>2823 ± 313**</td>
<td>2906 ± 273**</td>
</tr>
<tr>
<td>Blood volume (mL)</td>
<td>4729 ± 705</td>
<td>7027 ± 664*</td>
<td>7410 ± 627***</td>
</tr>
<tr>
<td>Plasma volume (mL)</td>
<td>4037 ± 439</td>
<td>4204 ± 413</td>
<td>4504 ± 390***</td>
</tr>
<tr>
<td>VO2max (mL/min)</td>
<td>4250 ± 381</td>
<td>4426 ± 480**</td>
<td>4500 ± 444***</td>
</tr>
</tbody>
</table>

The initial anthropometric and performance data are: age 41.2 ± 5.9 years, body mass 80.0 ± 9.5 kg, height 177.3 ± 5.9 cm, VO2max 53.5 ± 4.5 mL/kg/min. Mean training volume from December to April: 320 ± 98 min/week, from April to September 410 ± 119 min/week.

Difference from baseline:

\*\( P < 0.05 \)

\**\( P > 0.01 \)

\***\( P < 0.001 \)

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**Fig. 3. Relationship between changes in tHb-mass and VO2max after a 9-month lasting marathon training. THe-mass, Total hemoglobin mass.**
<table>
<thead>
<tr>
<th>Author</th>
<th>Year of publication</th>
<th>Athletic discipline</th>
<th>Number of athletes</th>
<th>Sex</th>
<th>Mode of hypoxia</th>
<th>Days/nights in hypoxia</th>
<th>Altitude/artificial altitude in meters</th>
<th>Daily exposure to hypoxia (h/day)</th>
<th>Total hours in hypoxia</th>
<th>Change in tHb-mass/RCM (%)</th>
<th>Significance</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional altitude training</strong></td>
<td></td>
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</tr>
<tr>
<td>Dill et al.</td>
<td>1974</td>
<td>Runners</td>
<td>12</td>
<td>m</td>
<td>Natural</td>
<td>20</td>
<td>2300</td>
<td>24</td>
<td>480</td>
<td>0.5</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Telford et al.</td>
<td>1996</td>
<td>Runners</td>
<td>9</td>
<td>m</td>
<td>Natural</td>
<td>28</td>
<td>1760</td>
<td>24</td>
<td>672</td>
<td>0</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Svedenhag et al.</td>
<td>1997</td>
<td>Cross country skiers</td>
<td>7</td>
<td>m+f</td>
<td>Natural</td>
<td>30</td>
<td>1900</td>
<td>24</td>
<td>720</td>
<td>3.1</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Friedmann et al.</td>
<td>1999</td>
<td>Boxers</td>
<td>16</td>
<td>m</td>
<td>Natural</td>
<td>18</td>
<td>1800</td>
<td>24</td>
<td>432</td>
<td>-5</td>
<td>+</td>
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<tr>
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<td>1998</td>
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At the first glance, the effect of LH–TL on tHb-mass is confusing (Table 3) reaching from no effect (e.g., Ashenden et al., 1999a, b) to increases by 10.1% (Brugniaux et al., 2006). When, however, the changes in tHb-mass are analyzed precisely according to the daily time of hypoxic exposure a clear statement is possible (Fig. 4): In case of 3–4 weeks lasting LH–TL protocols above 2100 m there is no substantial effect on tHb-mass when daily hypoxic exposure is \( < 14 \text{ h} \) (4.5 ± 1.9%). Once this threshold is exceeded similar erythropoietic effects as described for continuous altitude training are achieved (6.5 ± 1.8%). These results agree with recommendations of Rusko et al. (2004) of spending more than 12 h/day above a natural or simulated altitude of 2000 m for 3 weeks and the findings of Wilber et al. (2007) that more than 22 h/day at 2000–2500 m or 12–16 h/day at higher altitude (2500–3000 m) serve as potent erythropoietic stimuli.

To test the conclusions of Wilber, we performed an analysis of variance including the variables “daily time of hypoxic exposure (h/day),” “altitude/degree of hypoxia” (in meters), “type of hypoxia” (altitude or artificial hypoxia), and “total days of LH–TL protocol” of all the studies having performed LH–TL protocol of 12–29 days above 2100 m. We found a strong effect on tHb-mass of daily time of hypoxic exposure \( (P < 0.001) \), a small effect of “total days of LH–TL protocol” \( (P < 0.05) \), and no significant effects of altitude or the type of exposure. Including all these four variables into a multiple linear regression analysis a strong relationship to tHb-mass was found \( (r = 0.864, P < 0.001) \) allowing to anticipate the effect of LH–TL on the erythropoietic response.

Concerning short-term hypoxic exposure, there is no evidence that normoxic/hypoxic intervals (e.g., 5 min/5 min; Julian et al., 2004) or longer regular (training) sessions in hypoxia for 1–3 h have any positive effect on tHb-mass (Rodriguez et al., 2007).

We conclude that training at altitude either conventional or LH–TL can increase tHb-mass in the mean as long as certain requirements, i.e. more than 14 h/day above 2100 m for at least 3 weeks are complied. However, the effects of chronic hypoxia on tHb-mass as demonstrated for athletes native to altitude cannot be achieved by the usually performed altitude/hypoxic training measures.

Mechanisms of regulation of tHb-mass

The regulation of tHb-mass is mainly under control of the hormone EPO, which is produced in peritubular cells of the kidney. Serum EPO concentration is regulated by the HIF-1 system mediating the signal of reduced renal PO\(_2\) (for detailed descriptions see e.g. Caro, 2001). When analyzing the resting EPO concentration of the cross-sectional studies described above only small influence of altitude and training status occur. Serum transferrin receptor concentration, in contrast, was positively influenced by altitude and training status proving the induction of an accelerated erythropoiesis.

During acute exercise one may assume reduced systemic PO\(_2\) and therefore the induction of the HIF-1a cascade. Data concerning the intra-renal PO\(_2\) during exercise are, however, not yet available. Exercise induced lower renal perfusion and reduced oxygen transport to the kidney may be compensated by decreased sodium re-absorption and therefore lower oxygen consumption as well as by enhanced oxygen delivery due to a right shifted oxygen dissociation curve. It is, therefore, no surprise that plasma EPO concentration does not increase during and some hours after exercise (e.g. Schmidt et al., 1991), while it increases some days after long-lasting exercise (Schwandt et al., 1991) probably as a result of hemodilution and lower hemoglobin concentration. For training at sea level, we therefore state no direct influence on the erythropoietic system but an indirect adaptation of tHb-mass by regulating hemoglobin concentration up to individually normal values after plasma volume expansion. In contrast to exercise at sea level renal PO\(_2\) decreases in hypoxia resulting in an inversely proportional increase of plasma EPO concentration (Ge et al., 2002).

When adapting to altitude EPO initially increases for about 2 days depending primarily on the decline in hemoglobin–oxygen saturation, which is associated with the degree of altitude. After staying some days at altitude, EPO again decreases reaching a constant level slightly above the original base line.
It is not known, whether this decline reflects enhanced EPO-binding to its receptor (Rusko et al., 2004) or a decrease in EPO production due to a functional feedback loop limiting the hypoxic response by a HIF-regulated expression of prolyl-4-hydroxylase (PHD2 and PHD3) (Stiehl et al., 2006).

Interestingly, the EPO-system completely recovers within 2 or 3 days after returning to sea level. Subjects commuting weekly between altitudes show oscillations in plasma-EPO for more than 20 years (Heinicke et al., 2003). Daily changes of altitudes may increase the sensitivity of the EPO system and may therefore explain higher morning plasma EPO concentration when applying the LH–TL compared with the LH–TH protocol (Rusko et al., 2004).

When exercise is performed under acute hypoxia, EPO increases in a similar magnitude as under resting hypoxic conditions. Exercise in chronic hypoxia (natives to 3500 m) even results in a significant decrease in plasma EPO (Schmidt et al., 1993). We, therefore, conclude that exercise under hypoxic conditions has no direct effect on red cell production.

Originally, RCM was assumed to be exclusively regulated by the erythropoietic activity of the bone marrow and the red cell survival time to be relatively constant (~100 days) in healthy subjects. This dogma was disproved by Alfrey et al. (1997) and Rice et al. (2001) showing a selective destruction of young red cells (age lower than 12 days) in cases of plethora induced by space flight or descend from high altitude. Changing the altitude from 4380 m to sea level resulted in a decrease of RCM by about 10% within 7 days in well-acclimatized subjects (Rice et al., 2001). This reaction called neocytolysis is triggered by a strongly reduced plasma EPO concentration whereas daily administration of a small dose of rhEPO completely prevents the hemolysis. Neocytolysis probably occurs within the spleen, where endothelial cells respond to the withdrawal of EPO by influencing the interaction of phagocytes with young red cells which are targeted by surface adhesion molecules (Trial et al., 2001; Rice & Alfrey, 2005; Risso et al., 2007).

This mechanism can also be considered as cause of the above-described missing response of tHb-mass to LH–TL protocols with <14 h/day in hypoxia. In these cases, the daily occurring increase of EPO may induce a high production of reticulocytes, which, however, do not survive due to the strong oscillating EPO-levels (Schmidt, 2006).

Also the effects of conventional altitude training may be modified by neocytolytic processes. That is that under practical aspects a longer stay at altitude may be more efficient, because more of the red cells produced at altitude exceed the critical age of ~10 days and will therefore not be subject to neocytolytic processes after the athletes return to sea level.

The different individual responses to altitude (Chapman et al., 1998) have been discussed on a genetic and physiological background. In a first study, Witkowski et al. (2002) showed a specific allele of the EPO gene (D7S477 allele) discriminating between subjects reacting with high or low erythropoietin response to 2800 m altitude. This result, however, was not confirmed in follow-up studies. One theory for the existence of responders and non-responders is based on the individual hypoxic ventilatory response influencing arterial Hb–O₂-saturation and subsequently renal EPO production. In the study of Heinicke et al. (2005) an example for a responder is provided. His increase in tHb-mass by 18.3% within 3 weeks at 2100 m (mean of the group +7.2%) was accompanied by lower Hb–O₂-saturation, right shifted oxygen dissociation curve, and higher EPO concentration. Reasons for a missing response to altitude may be a high hypoxic ventilatory response (HVR) as it is typical for females rather than for males (Böning et al., 2004) as well as the inhibition of erythropoiesis due to inflammatory diseases or a lack of iron availability.

Conclusions

VO₂max directly depends on tHb-mass under normoxic conditions. Hence, a change in tHb-mass of 1 g results in a change of VO₂max of ~3 mL/min. Elite endurance athletes show in the mean 35% higher values than the normal population, whereas athletes native to altitude possess even 14% more. The altitude induced higher O₂-transport capacity does neither augment VO₂max at sea level nor at altitude in relatively untrained subjects. Whether it increases endurance performance in elite athletes native to altitude still remains questionable.

Training at sea level has only small direct impact on the erythropoietic system. Genetic predisposition seems to be a prerequisite for high tHb-mass and high endurance performance. Conventional altitude training as well as the concept LH–TL similarly increases tHb-mass as long as the following guidelines are followed: altitude above 2100 m, duration about 3–4 weeks, daily time of hypoxic exposure not <14 h/day (>14 h/day – tHb-mass +6.5%; <14 h/day – tHb-mass +0.7%). The mean level of tHb-mass in elite athletes native to altitude (+14%) can, however, not be achieved by these altitude training measures.

Recommendations

1. Organizers of athletic competitions should be aware that athletes of endurance disciplines from lowlands may be discriminated due to
insufficient time for hematological adaptation and ventilatory acclimatization.

2. Regarding the general strong relationship between tHb-mass and VO$_{2max}$ athletes from lowlands may improve their aerobic capacity at altitude by increasing their tHb-mass. tHb-mass in highly trained athletes can be augmented by either conventional altitude training and/or LH–TL at altitudes above 2100 m. Both modalities of altitude training should be performed for at least 3 weeks and in case of LH–TL procedures the daily hypoxic exposure should not be $\leq$14 h/day at natural altitude or corresponding normobaric or hypobaric hypoxia.

3. Whether an athlete can increase his tHb-mass by altitude or hypoxia and to which extent this may occur is individually different. In case various stays at altitude or various hypoxic measures are scheduled the erythropoietic response has to be evaluated to distinguish responders from non-responders.

4. Athletes of endurance disciplines are advised to make use of the hematological adaptation process and arrive at least 1 week at moderate and 2 weeks at high altitude before the start.

5. Since 3 weeks after an altitude training tHb-mass is reduced again by 50% the time lag between descent from altitude training and competition should be $\leq$20 days.

6. Filled iron stores (we recommend Ferritin levels of $\geq$35 ng/mL) are necessary to obtain the optimal effect of altitude or hypoxia exposure on erythropoiesis.

Further investigation

1. Further investigations are needed to clarify the role tHb-mass is playing for maximum performance at altitude. It has to be investigated whether the strong relationship between tHb-mass and VO$_{2max}$ at sea level is also true for hypoxic conditions.

2. tHb-mass and BV of football players native to different altitudes should be compared placing special emphasis on ethnic origin since the form of adaptation to hypoxia may be different.

3. The effects of neocytolysis on RCM have to be evaluated when well-adapted athletes descend from altitude and when sea-level athletes live high and train low.

Key words: VO$_{2max}$, total hemoglobin mass, red cell mass, altitude, live high–train low.

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Conflict of interest: The author has declared that they have no conflict of interests.

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