Metabolic and vascular support for the role of myoglobin in humans: a multiparametric NMR study


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Duteil, S., C. Bourrillon, J. S. Raynaud, C. Wary, R. S. Richardson, A. Leroy-Willig, J. C. Jouanin, C. Y. Guezennec, and P. G. Carlier. Metabolic and vascular support for the role of myoglobin in humans: a multiparametric NMR study. Am J Physiol Regul Integr Comp Physiol 287: R1441–R1449, 2004; doi:10.1152/ajpregu.00242.2004.—In human muscle the role of myoglobin (Mb) and its relationship to factors such as muscle perfusion and metabolic capacity are not well understood. We utilized nuclear magnetic resonance (NMR) to simultaneously study the Mb concentration ([Mb]), perfusion, and metabolic characteristics in calf muscles of athletes trained long term for either sprint or endurance running after plantar flexion exercise and cuff ischemia. The acquisitions for 1H assessment of Mb desaturation and concentration, arterial spin labeling measurement of muscle perfusion, and 31P spectroscopy to monitor high-energy phosphate metabolites were interleaved in a 4-T magnet. The endurance-trained runners had a significantly elevated [Mb] (0.28 ± 0.06 vs. 0.20 ± 0.03 mmol/kg). The time constant of creatine rephosphorylation (τPCR), an indicator of oxidative capacity, was both shorter in the endurance-trained group (34 ± 6 vs. 64 ± 20 s) and negatively correlated with [Mb] across all subjects (r = 0.58). The time to reach maximal perfusion after cuff release was also both shorter in the endurance-trained group (306 ± 74 vs. 560 ± 240 s) and negatively correlated with [Mb] (r = 0.56). Finally, Mb reoxygenation rate tended to be higher in the endurance-trained group and was positively correlated with τPCR (r = 0.75). In summary, these NMR data reveal that [Mb] is increased in human muscle with a high oxidative capacity and a highly responsive vasculature, and the rate at which Mb resaturates is well correlated with the rephosphorylation rate of Cr, each of which support a teleological role for Mb in O2 transport within highly oxidative human skeletal muscle.

postexercise hyperemia; skeletal muscle; perfusion; oxygenation; phosphocreatine

THE RECOGNITION that the ligand affinity for myoglobin (Mb) to O2 falls between that of hemoglobin and cytochrome oxidase, both of which have integral roles in O2 transport, implicates Mb in the flux of O2 from blood to aerobically active muscle: breast muscle of the nonflying chicken is white and lacks this protein, whereas the breast muscle of ducks and geese (which can fly) is red and rich in Mb (28). Interventional studies that have attempted to increase [Mb] with endurance training have been successful in rats (37) but have been unsuccessful in humans (25, 33, 50).

Indeed, in species other than humans there is mounting evidence against a primary role of Mb-mediated O2 diffusion. Specifically, in terrestrial mammals variations in [Mb] within fibers and between fibers has been found to be inconsistent with the varied aerobic capacity of the tissue (3). Additionally, although Mb has, in fact, been documented to diffuse in vivo (perhaps an important criteria for a significant contribution to O2 flux), its recorded diffusivity is comparatively low and has been interpreted to indicate only a minor role in intracellular O2 transport (27). Most recently and perhaps most significantly, the successful development of mice without Mb, generated by gene knockout, but with normal exercise capacity, initially implied that Mb is not essential to meet the requirements of exercise in this mammal (14, 17). However, followup studies have clearly indicated that there are many compensatory changes to the O2 transport system that must accompany this loss of Mb to sustain life, let alone facilitate normal exercise capacity (17, 18). Ironically, in light of the tone of the first publication with this approach (17), the mouse Mb gene knockout is highly supportive of an important role of Mb in O2 transport.

Nuclear magnetic resonance (NMR) can noninvasively measure both [Mb] (53) and Mb deoxygenation (45, 47) and so is ideally suited to human research in this area. The study of Mb O2 saturation in humans has revealed that Mb becomes ≈50% deoxygenated in heavily exercising muscle (45, 47, 53), a criteria necessary for the models that support the facilitative role of Mb in the transport of O2 from blood to cell (11, 12, 19, 35, 39). In addition to being capable of accurately measuring [Mb] and Mb desaturation, NMR can assess muscle perfusion (6, 16, 42) and both metabolism and metabolic capacity (21, 43, 46).

Consequently, in an effort to better understand the role of Mb within human skeletal muscle, we utilized NMR to study the Mb concentration, perfusion, and metabolic characteristics of the calf muscles from a spectrum of human athletes trained long term for either sprint or endurance running. Utilizing exercise and cuff ischemia as the paradigm, we tested four...
general hypotheses: the endurance-trained muscle compared with the sprint-trained muscle would have 1) the greatest [Mb], 2) the greatest metabolic capacity, and 3) the greatest vascular responsiveness, and 4) there would be a strong relationship between the assessment of Mb and these important parameters of O2 transport and utilization.

MATERIALS AND METHODS

Study Population

The protocol received unrestricted approval from the Cochin University Hospital Ethics Committee (Paris, France), and the athletes who had volunteered to participate in the study gave written informed consent. All 13 subjects were elite male runners undergoing specific endurance (ER; n = 7) or sprint (SR; n = 6) training. The actual sports in which they participated were orienteering and 100- or 200-m sprints, respectively, and they had been specifically training for these events for >12 h/wk for at least 3 yr. In addition to this grouping, based upon event, maximum pulmonary O2 consumption (V\textsubscript{O2max}) measured during treadmill running was used as a selection criterion with V\textsubscript{O2max} being 32 ml\textpermin\textperkg in the ER group and <60 ml\textpermin\textperkg in the SR group. Mean age, height, body weight, and V\textsubscript{O2max} were 32 ± 5 yr, 175 ± 5 cm, 67 ± 6 kg, and 71.6 ± 7.0 ml\textpermin\textperkg in the ER group and 24 ± 5 yr, 180 ± 10 cm, 78 ± 11 kg, and 56.5 ± 4.6 ml\textpermin\textperkg in the SR group.

Experimental Protocol and Setup

The experimental protocol focused on calf muscle functional hyperemia and metabolic recovery at reperfusion after an ischemic plantar flexion bout performed until exhaustion.

An amagnetic pneumatic ergometer interfaced to a PC allowed workload programming and the instant reading of work output. Before the experiment, each subject was familiarized with the ergometer and the plantar flexion movement while lying supine in the magnet. For the actual experiment, an air-cuff was wrapped above the knee of the dominant leg, inflated to 220 Torr. Physiological parameters (heart rate, arterial blood pressure, and blood oxygen saturation) were monitored using an NMR-compatible patient-monitoring device (MAFGLIFE, ODAM-Bruker, Wissembourg, France). The subject then performed plantar flexions with this leg until exhaustion. The phosphocreatine (PCr) depletion was assessed online by standard \textsuperscript{31}P NMR spectroscopy. To help the subjects keep plantar flexions at a constant rate of 0.5 Hz, a gradient pulse generating an audible signal was inserted in the NMR sequence at this rate.

When the subject could no longer maintain the required work rate, the interleaved multiparametric functional (mpf) NMR acquisitions were started while the thigh cuff was still inflated. One minute later, the arterial cuff was released and the mfp NMR acquisitions were continued for the next 14 min, with one complete imaging and spectroscopy dataset being collected every 1.5 s. The importance of remaining still throughout the recovery period was explained to all subjects, and there was good compliance.

The experiments were carried out in a 4-T, 46-cm internal bore, superconducting magnet (Magnex 4/60) interfaced to a Bruker Biospec NMR spectrometer. The calf of the subject’s dominant leg was carefully positioned inside a 17-cm inner diameter transversal electromagnetic \textsuperscript{1}H transmit and receive volume coil, and an 8-cm-diameter custom-built \textsuperscript{31}P surface coil was slid underneath the gastrocnemius (Fig. 1).

The magnetic field (B\textsubscript{0}) homogeneity was optimized with Fastmap, an automatic localized first- and second-order shim procedure (20). The other technical adjustments and the acquisition of reference images and spectra in resting conditions took ~20 min. A \textsuperscript{1}H-NMR muscle water reference spectrum was recorded for the quantification of [Mb] (9) (Eq. 3). One reference \textsuperscript{31}P-NMR spectrum (8 scans) was also acquired, and using our own version saturation inversion recovery (SATIR) of pulsed arterial spin labeling (ASL) (42), we collected a series of 100 perfusion NMR images to evaluate calf muscle perfusion at rest.

Multiparametric Functional NMR

Calf muscle perfusion, intracellular oxygenation, and energy metabolism were studied simultaneously by rapidly interleaved acquisi-

![Fig. 1. Position of the leg on the \textsuperscript{31}P surface coil inside the transversal electromagnetic (TEM) volume coil with the foot placed on the pedal of the ergometer. B\textsubscript{0}, static magnetic field.](image-url)
tions of SATIR ASL perfusion imaging, $^1$H spectroscopy of deoxymyoglobin, and $^{31}$P spectroscopy of the high-energy phosphate metabolites (7, 41). This interleaved acquisition scheme was initially proposed and implemented in our lab and was here driven by the multiscan control (MSC) tool developed and made commercially available by Bruker. A complete dataset was generated every 1.5 s and comprised the following acquisitions.

A single perfusion image using pulsed ASL. The SATIR imaging protocol applied alternatively a slice-selective inversion pulse (positive tagged image) or a nonselective inversion pulse (negative tagged image), in both cases associated with a slice-selective saturation (42).

Perfusion images were 6-mm-thick axial views of the leg acquired with a half-Fourier single-shot (SS) fast spin echo (or-RARE) sequence, over a field of view of 22 cm × 11 cm, with an acquisition bandwidth of 100 kHz and a reconstruction matrix of 128 × 64 giving a pixel size of 1.7 × 2 mm$^2$. The half-Fourier acquisition consisted of a train of 36 echoes, with a Gaussian radio-frequency (RF) imaging pulse of 1 ms (with half height: 2.740 Hz) and an echo spacing of 4.7 ms, resulting in an effective echo time of 19 ms and an acquisition time of 170 ms. The SATIR module was placed 0.82 s before SS-RARE imaging. Inversion and saturation RF pulses were an 8-ms hyperbolic secant (2,200 Hz) and a 3-ms 7-lobe sinc (1,030 Hz). The optimum evolution time would have been the muscle T1 value (i.e., 1.6 s at 4 T); however, it was shortened to obtain a better temporal resolution and a more adequate sampling of the rapid perfusion changes in this protocol.

A single $^1$H-NMR spectrum of deoxymyoglobin. The n-8 proton of the proximal histidine F8 of Mb in the deoxygenated state was selectively excited by an 0.8-ms Gaussian pulse (64 accumulations, 256 complex points, acquisition time 6 ms).

A single $^{31}$P-NMR spectrum. The high-energy phosphates were excited by a single 0.5-ms square pulse, and the free-induction decay (FID) 2048 complex data points were collected in 128 ms.

NMR Data Processing

The MSC tool automatically distributed the raw interleaved data in three distinct imaging, $^1$H, and $^{31}$P spectroscopy files, which were immediately ready for processing with standard ParaVision and XWIN NMR Bruker software.

SATIR Perfusion Images

A temporary perfusion map was extracted by summing the differences between six successive pairs of images acquired between 12 and 30 s after arterial occlusion release. Regions of interest (ROIs) were traced inside sections of triceps surae activated by exercise, carefully excluding voxels containing lipids or vessels. Identical ROIs were selected in all the images of the series, and perfusion ($\dot{i}$) was calculated according to Eq. 1 (42)

$$\dot{i} = \frac{\lambda}{T} \cdot \ln \left( \frac{M_{SS}(T) - M_{SS}(T)}{M_{SS}(T) + M_{SS}(T)} \right) \cdot \left[ 1 - \exp(\dot{r}1 \cdot T) \right] + 1 \right)$$

where $M$ stands for the image intensity in muscle ROI after slice-selective (SS) and nonselective (NS) inversion; $T$ is the ASL time (0.82 s); $\lambda$ is the tissue/blood partition coefficient (0.9), and $r1$ is the muscle spin-lattice relaxation rate (0.66 s$^{-1}$).

Early postexercise reperfusion ($\dot{i}$12s) was measured in each individual as the mean of three successive measurements between 9 and 15 s after cuff release. For each individual, the whole perfusion dataset of 600 points was fitted with a gamma function, and the maximal postexercise hyperperfusion ($f_{max}$) was taken as the maximum value of the fitted curve.

$^1$H Deoxymyoglobin Spectra

After a 100-Hz line-broadening exponential multiplication and Fourier transformation, zero- and first-order phases of the Mb spectrum were adjusted manually on an end-exercise acquisition. All FIDs of the series were processed using these same parameter settings. After automatic baseline correction (+20/−20 ppm), the Mb peak of each spectrum was quantified by integration over 10 ppm.

The end-ischemic exercise Mb area was taken as the 100% desaturation value. [Mb] was calculated by referencing fully desaturated Mb to muscle water resonance (9)

$$[Mb] = \frac{A_{Mb} \cdot RG_{water} \cdot ns_{water} \cdot N_{water} \cdot M_{water}}{nswater \cdot RG_{Mb} \cdot ns_{Mb} \cdot M_{Mb}}$$

where $A_{Mb}$ is the resonance area, $RG_{Mb}$ is the signal receiver gain, $ns_{Mb}$ is the number of scans programmed for each spectrum, $N_{water}$ is the number of hydrogen atoms contributing to the resonance per molecule, and $M_{water}$ is the molar concentration of water.

To determine the Mb resaturation time constant ($r_{Mb}$), a monoeponential decay was fitted to the Mb data points collected immediately after cuff release (Fig. 2). Based on [Mb] and $r_{Mb}$, the muscle reoxygenation rate constant ($M_{reoxy}$) was calculated as

$$M_{reoxy} = \pi \cdot [Mb] / r_{Mb}$$

where $\pi$ is the oxyphoric capacity of Mb (1.36 ml O$_2$/g of Mb) and [Mb] is expressed here in grams per liter (Mb molecular weight = 17,000).

$^{31}$P Spectra of High-Energy Phosphates

The $^{31}$P FIDs were summed four by four and were processed in a similar fashion to the $^1$H spectra, except for an 8-Hz line-broadening exponential multiplication. $P_i$ and PCr integration limits were set to

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Fig. 2. Typical curves of perfusion (A), myoglobin (Mb) resaturation (B), and phosphocreatine (PCr) resynthesis (C) during recovery, obtained from processing NMR interleaved data of 1 subject.
5.6/3.5 ppm and 1.5/1.5 ppm, respectively. Muscle intracellular pH was calculated from the chemical shift (δ) between the P, and PCr peaks (51):

$$\text{pH} = 6.75 + \log \left[ \frac{-3.27 + \delta}{5.69 - \delta} \right]$$ (4)

Cr rephosphorylation data points were fitted monoexponentially to determine the actual Cr rephosphorylation time constant (τPCr). Also, the pH measured during early recovery was used to correct for the effect of acidosis on the maximum Cr rephosphorylation time constant (τPCrcor) (24).

Mitochondrial oxidative production of ATP (VATP) was calculated as

$$\text{VATP} = \frac{Q_{\text{max}}}{1 + \frac{K_m \cdot Z}{P/P\text{Cr}}}$$ (5)

with $K_m \cdot Z$ estimated to be 0.8 in humans (10). $Q_{\text{max}}$ is the maximum oxidative ATP production rate and depends on τPCr and on an estimation of PCr concentration at rest ([PCr]rest ~15 mM wet tissue) (34).

$$Q_{\text{max}} = \frac{[\text{PCr}]_{\text{rest}}}{\tau\text{PCr}}$$ (6)

Statistical Analyses

Data are presented as means ± SD, except when stated otherwise. After the normality of distribution had been tested for each parameter, results between and within groups were compared using one-way or two-way ANOVA (NCSS and Vassarstat softwares).

RESULTS

[Mb]

[Mb] was significantly higher in the calf muscles of the endurance athletes than in the sprinters (ER 0.28 ± 0.06 mM vs. SR 0.20 ± 0.03 mM; P < 0.05; Fig. 3). Additionally, [Mb] was found to correlate negatively with the time to peak hyperemia ($r = 0.58$, $P = 0.03$) and to correlate positively with mitochondrial oxidative production of ATP ($r = 0.70$, $P = 0.008$) (Fig. 4).

Ischemic Exercise

Total work developed during the ischemic exercise bout was higher in the SR group than in the ER group (677 ± 193 J vs. 476 ± 90 J; $P = 0.03$), due to both a larger number of plantar flexions (102 ± 28 vs. 80 ± 12; $P = 0.08$, corresponding to exercise durations of 235 ± 66 and 198 ± 31 s, respectively) and a higher maximal power output (3.3 ± 0.3 W vs. 2.9 ± 0.2 W; $P = 0.01$). Calf muscle cross section was larger in the SR athletes (70 ± 12 cm² vs. 48 ± 6 cm²; $P = 0.03$). As a consequence, the work normalized to calf section was not different between groups (10.2 ± 2.7 J/cm² in ER vs. 11.4 ± 3.9 J/cm² in SR; $P = 0.5$).

Postexercise Hyperemia

The time course of postischemic exercise muscle reperfusion was markedly different ($P < 0.001$) in the two groups (Fig. 5). Early postexercise hyperperfusion, measured 12 s after occlusion release (t12s), was significantly higher in the ER group.

Fig. 3. Calf muscle concentrations of Mb (4), Mb resaturation time constant (B), muscle perfusion measured 12 s after the release of occlusion (C), and rephosphorylation time constant of creatine (Cr) corrected for pH (D) in endurance-trained (ER) and sprint-trained (SR) groups. Values are means ± SD. *$P < 0.05$, **$P < 0.01$. 
The time to reach the maximal perfusion (T\text{max}) was significantly shorter in the ER group than in the SR group (306 ± 74 vs. 560 ± 240 s, respectively; \( P < 0.05 \)). The maximal values of perfusion (f\text{max}) were comparable in the two groups (f\text{max ER: 56.0 ± 13.1 vs. fmax SR: 57.0 ± 10.4 ml·100 g\(^{-1}\)·min\(^{-1}\)}).

At the end of the 15-min followup period, calf muscle perfusion had not returned to baseline values in the SR group (19.6 ± 3.3 vs. 7.8 ± 1.0 ml·100 g\(^{-1}\)·min\(^{-1}\) before exercise; \( P < 0.05 \)) whereas it had returned to normal preexercise values in the ER group (9.8 ± 4.8 vs. 6.9 ± 3.4 ml·100 g\(^{-1}\)·min\(^{-1}\) before exercise; \( P > 0.05 \); Fig. 5).

Mb Resaturation and Muscle Reoxygenation

\( \tau \text{Mb} \) tended to be shorter in the ER group than in the SR group (13.5 ± 4.1 vs. 19.2 ± 7.0 s; \( P = 0.09 \)), but owing to the differences in [Mb], muscle reoxygenation rate was significantly higher in the ER athletes than in the SR athletes (0.53 ± 0.19 vs. 0.30 ± 0.12 ml O\(_2\)·kg\(^{-1}\)·s\(^{-1}\); \( P = 0.02 \)).

Mitochondrial Oxidative Phosphorylation

\( \tau \text{PCr} \) was very much shorter in the ER compared with the SR group (34 ± 6 s vs. 64 ± 20 s, respectively; \( P < 0.01 \); Fig. 6). This difference remained significant after \( \tau \text{PCr} \) was corrected by 10.220.33.4 on April 3, 2017 http://ajpregu.physiology.org/ Downloaded from

Fig. 4. Relationship between Mb concentration and mitochondrial oxidative production of ATP (VATP) (A), and time to reach maximal level of perfusion (B) during the recovery period in the 2 groups.

(23.6 ± 5.6 ml·100 g\(^{-1}\)·min\(^{-1}\)) than in the SR group (16.6 ± 4.7 ml·100 g\(^{-1}\)·min\(^{-1}\); \( P < 0.05 \); Fig. 3).

The time to reach the maximal perfusion (T\text{max}) was significantly shorter in the ER group than in the SR group (306 ± 74 vs. 560 ± 240 s, respectively; \( P < 0.05 \)). The maximal values of perfusion (f\text{max}) were comparable in the two groups
for pH (\(r_{\text{PCr}_{\text{cor}}} = 20 \pm 6\) and \(33 \pm 13\) s, respectively; \(P < 0.01\)).

For all subjects, end-exercise pH (ER \(6.69 \pm 0.20\) and SR \(6.69 \pm 0.06\)) was significantly lower than resting pH (ER \(7.05 \pm 0.03\) and SR \(7.09 \pm 0.05\), \(P < 0.0001\) and <0.05, respectively). End-exercise pH and minimum pH values at the beginning of the recovery period (ER \(6.51 \pm 0.18\) and SR \(6.44 \pm 0.18\)) were not different between the two groups.

The calculated mitochondrial VATP was higher in the ER than in the SR group (0.28 ± 0.05 vs. 0.16 ± 0.04 mM/s, \(P < 0.001\)).

Creatine rephosphorylation time constant was negatively correlated with calf muscle reperfusion at 12 s (\(r = 0.56; P = 0.05\)) (Fig. 6) and positively correlated with \(\tau_{\text{Mb}} (r = 0.75; P = 0.003)\) (Fig. 6). Identical correlations were found between the pH-corrected creatine rephosphorylation time constant and muscle perfusion (\(r_{\text{PCr}_{\text{cor}}} = 48.7–1.0 \times f_{12s}; r = 0.55, P = 0.05\)) and with Mb resaturation time constant (\(r_{\text{PCr}_{\text{cor}}} = 3.6 + 1.6\tau_{\text{Mb}}; r = 0.75, P = 0.004\)) (Fig. 6).

**DISCUSSION**

One fundamental finding of this study was the significant elevation of skeletal muscle [Mb] (by \(\approx 40\%\)) in a group of endurance-trained runners when compared with their sprint-trained counterparts. This is a unique observation that has not been previously documented, possibly in part due to the typically short-term interventional approaches that have been employed in the past (25, 33, 50). In contrast, the current investigation studied subjects who were either long-term sprint or endurance trained. In parallel, three interleaved NMR acquisitions revealed a greater metabolic and perfusional capacity in the endurance-trained subjects and a strong correlation between intracellular reoxygenation and metabolic recovery. Unfortunately, the cross-sectional design of this investigation does not answer the obvious question of “nature over nurture?”

However, in light of the differing [Mb], this study affords the opportunity to examine the metabolic and vascular capacity of these two groups and shed some light on the role of Mb in human skeletal muscle.

**Role of Mb in Human Skeletal Muscle**

The general heterogeneity of fiber type within human muscle has meant that simple associations between aerobic potential of differing muscle groups and the abundance of Mb within each has not been possible, as it has in nature (28). Previously, several investigators have utilized endurance training to promote aerobic adaptations and evaluated the effect on [Mb]. The majority of such interventional studies have failed to augment [Mb] in humans (25, 33, 50). Terrados et al. (52) however, have demonstrated an increased Mb mRNA and protein level after endurance training, but only under hypobaric conditions.

Although the training periods have been relatively short (5–8 wk), similar training time frames have successfully changed a host of other O2 transport-related variables (48).

A single study, focused on detraining (14), tracked, among other things, [Mb] and found that the cessation of training in long-term endurance-trained subjects for 12 wk did change [Mb], which was not different from sedentary controls. As studies in rats have demonstrated an increase in [Mb] with endurance training (37), this was interpreted as a potential species difference in terms of the adaptive response to exercise. However, there are several significant differences between the cross-sectional human study performed by Coyle et al. (14) and the current research. First, subjects in the previous study were heterogeneous in terms of both training modality (cyclists, runners, and swimmers), gender, and the length of time involved in regular exercise training (1–20 yr), whereas in the current study all subjects were involved in highly specific regimented military training for endurance or sprint athletes for at least 3 yr. Second, the current \(^1\)H NMR spectroscopy method assessed a much larger volume (several hundred cm\(^3\)) of calf muscle for [Mb] rather than the \(\approx 40\)-mg biopsy samples that Coyle et al. (14) collected from either the vastus lateralis or the gastrocnemius, avoiding sampling site variability.

At the molecular level, Mb mRNA expression in humans has been documented to increase as a consequence of hypoxic endurance training (54). Of interest, the Mb mRNA response was similar to that of vascular endothelial growth factor (VEGF) (54), which is supportive of a link between Mb and O2 transport, as VEGF regulation has been associated with not only increased capillarity but with functional indexes of O2 transport in humans (48).

In addition to the current observation of an increased [Mb] in the muscle of endurance-trained runners when contrasted with their sprint-trained counterparts, the following observations, afforded by the interleaving of metabolic and vascular NMR acquisitions, support the role of Mb as an important factor in both O2 transport and oxidative capacity. 1) \(r_{\text{PCr}}\), an accepted indicator of skeletal muscle oxidative capacity, was both shorter in the endurance-trained group and negatively correlated with [Mb] across all subjects (\(r = 0.58\)). It should be noted that, although there was a significant difference in pulmonary \(V_{\text{O}_2}\) (measured while running on a treadmill) between the two groups, with the endurance-trained group having the highest values, there was no significant relationship between \(V_{\text{O}_2}\) and [Mb]. This is in contrast to the correlation with between [Mb] and \(r_{\text{PCr}}\) and highlights the potentially confounding results that can accompany the combination of central and peripheral assessments of metabolic capacity. 2) The time to reach maximal perfusion after cuff release was also both shorter in the endurance-trained group and negatively correlated with [Mb] across all subjects (\(r = 0.56\)). 3) Finally, \(r_{\text{Mb}}\), although not significantly reduced, tended to be shorter in the endurance trained group and was positively correlated with \(r_{\text{PCr}} (r = 0.75)\). In summary, these NMR data reveal that [Mb] is increased in human muscle with a high oxidative capacity and a highly responsive vasculature, and the rate at which Mb resaturates is well correlated with the rephosphorylation rate of Cr, each of which implies a teleological role for Mb in O2 transport with highly oxidative human skeletal muscle.

**Postischemic Exercise Reperfusion Patterns**

The noninvasiveness of the method, the possibility to repeat measurements at a high rate, the spatial localization, and the ability to quantify perfusion in absolute terms (42) contribute to make the ASL NMR the technique of choice when a thorough evaluation of tissue perfusion is sought. Here, this methodology provided novel information: very different post-exercise perfusion patterns in calf muscles of endurance and sprint athletes. Early reperfusion was markedly more elevated
in the endurance athletes than in the sprinters (Figs. 5 and 6). To a large extent, this may reflect the increased vasodilatory responsiveness of the muscle arteriolar bed associated with endurance training compared with force training (22). The increased capillary density in endurance-trained muscles (8, 33) may also somewhat contribute to the high perfusion observed in this group. Muscle fiber type distribution and capillarization are significantly modified by endurance training (1, 13, 30). A relationship was found between the high perfusion levels in muscles, the increase in type I fiber fractional volume, and the increase in capillarization (capillary density and/or capillary/fiber ratio) induced by endurance training (2, 23). Indeed, potentially contributing to the diverging group results, Bond et al. (5) reported that high-intensity resistance training decreased maximal blood flow and attributed this increase in vascular resistance to the hypertrophy of type II fibers associated with the form of exercise training employed by the SR group.

It is, however, noteworthy that the maximum level of perfusion achieved in the activated muscles was not statistically different between the two groups, suggesting the absence of structural differences of the arteriolar beds at maximum vasodilation between the two groups.

Postischemic exercise muscle hyperperfusion lasted for a relatively long time period compared with the exercise duration. More importantly, the hyperperfusion amplitude and duration was out of proportion to the metabolic debt generated by the exercise. Although this mismatch was recognized long ago (4), it has generally been overlooked but was exemplified in this protocol. As clearly demonstrated by the interleaved spectroscopic acquisitions, muscle cells reoxygenated and high-energy phosphates stores replenished within a fraction of a minute while the hyperperfusion state was maintained in some cases for >15 min. The exact mechanisms responsible for the persistence of this high level of perfusion in the recovering muscle are not known at the present time. A role of the endothelium in the regulation of perfusion in the recovering muscle is likely but remains to be clearly identified in the later phases of this process (15, 26). Recent microdialysis data show that among the metabolites released in the interstitial space by the exercising muscle, some, such as adenosine and bradykinin, disappear soon after exercise while high concentrations of other molecules and ions such as lactate, hydrogen, and phosphate ions are present in the interstitium for at least several minutes (29, 31, 49). Interstitial hyperosmolarity has been shown to induce dilation of rat skeletal muscle arterioles (32). One may reasonably hypothesize that the postexercise hyperperfusion facilitates the clearance of these metabolites and ions.

Exercise is associated with an increase in the T2 relaxation time of the participating muscles. These T2 changes have been unequivocally related to global and local fluid displacements during exercise (36, 40). It is of interest to note that the time frame of the postmuscle exercise T2 changes approximately follows the time course of postexercise muscle hyperperfusion, and T2 tends to normalize faster in red muscle compared with white muscle (40). A more comprehensive understanding of the relationship between postexercise muscle T2 changes and perfusion will require further investigation. At this point, a confounding effect of the T2 changes on the calculation of perfusion with our ASL method can be excluded since the T2 weighting of the NMR images cancels out in our procedure (42).

An original contribution of this work is the identification of specific muscle reperfusion patterns in the long-distance vs. short-distance runners. Perfusion vs. time is shifted to the right in the sprinters (Fig. 5). Along the same line of observations as ours, it has been reported using Doppler ultrasound that the time for femoral blood flow to return to normal after exercise was inversely related to $V_{O_2}$ max in high-level sportsmen (38). Again, the mechanisms responsible for these specific responses are unknown. Interestingly, the perfusion integrals, or the volume repaid to the muscles, are identical in the two groups. A simple explanation might be that the higher and faster early reperfusion in endurance athletes facilitates the clearance of metabolites responsible for the prolonged muscle vasodilatation and hence accelerates the return to baseline perfusion level.

**Implications for $O_2$ Supply and Demand in Human Skeletal Muscle**

The study employed a unique high time resolution and multiparameter examination of human skeletal muscle’s attempt to restore homeostasis after an extreme perturbation (cuffed exercise). This affords the opportunity to examine the interactions between $O_2$ supply and demand, not only in skeletal muscle as a whole, but also allows us to contrast endurance- and sprint-trained muscle. Exercise combined with complete cuff occlusion resulted in the complete deoxygenation of Mb and a large depletion of PCr. The rate at which the rephosphorylation of PCr takes place is commonly accepted to represent metabolic capacity, with the acceptance that this is an entirely oxidative process and occurs as rapidly as possible and that this process occurs in an environment where $O_2$ supply is not limited (21, 43, 46). Mb deoxygenation and subsequent resaturation can act as an endogenous probe of intracellular $P_{O_2}$, indicating $O_2$ availability (47).

En mass, Mb reoxygenated within 15–20 s after the cuff release; this may be interpreted as revealing a minimal need for either the facilitation of $O_2$ diffusion or the buffering of $O_2$ concentration by Mb at this point. As rPCr was significantly longer than this in both groups, it suggests that only the initial PCr replenishment is accomplished with the aid of Mb facilitated $O_2$ transport, but as the rate of PCr recovery declines (exponential recovery, Fig. 2) the flux of $O_2$ from blood to mitochondria is adequate to meet this requirement without Mb desaturation.

The perfusion profile after the cuff release for the endurance-trained subjects was quite different from that of the sprint-trained subjects, such that f12, the time to maximal flow, and the return to resting perfusion levels were all faster. However, despite this apparently enhanced bulk $O_2$ delivery, Mb resaturation rates although revealing a tendency to be faster in the endurance-trained group were not statistically shorter in this group. In light of the marked difference in rPCr (whether corrected or uncorrected for pH), these similar Mb resaturation values may be solely the consequence of differing metabolic capacities: a faster metabolic capacity (rPCr) in the endurance-trained subjects will tend to delay the resaturation of Mb.

In conclusion, the current interleaved assessment of muscle perfusion, tissue reoxygenation, and PCr recovery facilitates a
better understanding of how O2 supply and demand interact within skeletal muscle and paves the way for similar studies with varied levels of inspired O2 in healthy subjects and in diseases thought to have a peripheral muscle component such as chronic heart failure and chronic obstructive pulmonary disease (44).

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