Interrelationship of oxidative metabolism and local perfusion demonstrated by NMR in human skeletal muscle

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Commissariat à l'Energie Atomique-Service Hospitalier Frédéric Joliot, Groupe de Résonance Magnétique Nucléaire, 91401 Orsay Cedex, France, and Cardiac Unit and Nuclear Magnetic Resonance Center, Massachusetts General Hospital, Boston, Massachusetts 02114

Toussaint, J. Jean-François, Kenneth K. Kwong, Fidelis M’Kparu, Robert M. Weisskoff, Paul J. LaRaia, and Howard L. Kantor. Interrelationship of oxidative metabolism and local perfusion demonstrated by NMR in human skeletal muscle. J. Appl. Physiol. 81(5): 2221–2228, 1996.—Using nuclear magnetic resonance (NMR), we have examined the relationship of high-energy phosphate metabolism and perfusion in human soleus and gastrocnemius muscles. With 31P-NMR spectroscopy, we monitored phosphocreatine (PCr) decay and recovery in eight normal volunteers and four heart failure patients performing isometric plantar flexion. By using echo-planar imaging, perfusion was independently measured by a local [inversion-recovery (T1-flow)] and a regional technique (NMR-plethysmography). After correction for its pH dependence, PCr recovery time constant is 27.5 ± 8.0 s in normal volunteers, with mean flow 118 ± 75 (soleus and gastrocnemius T1-flow) and 30.2 ± 9.7 ml·100 ml−1·min−1·mm−1 (NMR-plethysmography-flow). We demonstrate a positive correlation between PCr time constant and local perfusion given by y = 50 – 0.25x (r² = 0.68, P = 0.01) for the 8 normal subjects, and y = 64 – 0.24x (r² = 0.83, P = 0.0001) for the 12 subjects recruited in the study. Regional perfusion techniques also show a significant but weaker correlation. Using this totally noninvasive method, we conclude that aerobic ATP resynthesis is related to the magnitude of perfusion, i.e., O2 availability, and demonstrate that magnetic resonance imaging and magnetic resonance spectroscopy together can accurately assess muscle functional status.

MUSCLE FUNCTION is dependent, in part, on the capacity of myocyte mitochondria to synthesize ATP from the electron transport chain. Energy stores can thereby be easily maintained at rest and replenished at an increased rate during strenuous exercise. By using 31P-nuclear magnetic resonance (NMR) spectroscopy (MRS), phosphocreatine (PCr), ATP, and Pi, can be noninvasively and dynamically measured in humans. An improved rate of ATP synthesis has been demonstrated in athletes and associated with improved performance (28). In contradistinction, in heart failure patients slowed ATP synthesis is associated with compromised performance (24). In both of these conditions muscle performance is also affected by tissue perfusion (17, 33), which is another major determinant of functional status.

Using a newly developed NMR approach to measure perfusion, we have examined the relationship between muscle metabolism and microcirculation in humans. Techniques for assessing perfusion have been developed in vivo in animal models (10) and applied in human brain (20) and human muscle studies (37). We have used a combination of these methodologies to calculate perfusion of the soleus (Sol) and gastrocnemius (Gc) muscles during reactive hyperemia and compared local with global perfusion measurements. We then examined the correlation between local muscle perfusion and metabolism. We have elected to study the recovery phase of metabolism after ischemic exercise, as recently reported in the investigations of Blei et al. (6), to examine the PCr recovery time constant (tPCr), which is a marker of aerobic ATP synthesis rate and is independent of workload.

METHODS

Experimental Subjects

Eight nonsmoking healthy volunteers (30 ± 7 yr) participated in the study. These normal volunteers had no history of medical problems and had not been physically trained on a regular basis in the last 3 mo; three of them were occasional runners (2–4 h/mo). To obtain a broader range of flow and metabolic data, we included heart failure patients: heart failure is associated with abnormalities of muscle metabolism (24, 26) and perfusion (17). Four patients were studied under the same conditions as the healthy volunteers: two were New York Heart Association (NYHA) class II, and two were NYHA class III (8). Patients had not been engaged in a program of physical rehabilitation at the time of examination. This investigation was approved by the Massachusetts General Hospital Committee on Research of Human Studies, and informed consent was obtained from each subject.

1H-Magnetic Resonance Imaging (MRI) of Muscle Perfusion

Methods for plethysmographic and longitudinal relaxation constant (T1)-flow measurements have been previously described (37). In this study, we calculated perfusion from two successive series of T1-flow and two NMR-plethysmographic (P) series after 5 min of ischemia. We performed perfusion measurements before 31P-MRS studies because it was not technically possible to perform both perfusion and metabolic measurements at the same time (although new sequences are presently developed that may soon allow such interleaved determinations) (6). Also, exercise performance modifies interstitial water content (21), alters the T1 and transverse relaxation constant (T2) (13), and may change the conditions and assumptions for perfusion calculation in the model we used in this study. Subjects came for a single 2-h MRI/MRS session, whereas they performed four ischemic tests (perfusion-imaging) and three ischemic exercises (metabolism-spectroscopy).

Images were performed on a General Electric Signa 1.5-tesla whole body magnet equipped with resonant gradients (Advanced NMR Systems, Wilmington, MA) by using a saddle
transmit/receive coil with a 20-cm diameter placed around the calf. Echo-planar imaging sagittal scout images were obtained, and an axial single-slice image was chosen at the largest calf diameter. Image parameters for inversion-recovery and spin-echo sequences were field of view = 200 \times 100 \text{ mm}, resolution = 3 \times 1.6 \text{ mm}, and slice thickness = 10 \text{ mm}. Perfusion was calculated by two independent methods (37).

T1-flow technique. The first calculation was obtained from the difference in 1/T1. T1 was measured from an echo-planar inversion-recovery sequence [initial inversion time (TI), 50 ms; 15 TI increments of 150 ms; echo time (TE) 18 ms; repetition time (TR) 4,500 ms; and total acquisition time 1 min 3 s]. Images were taken at rest and during and after a 5-min ischemic period produced by the inflation of a thigh cuff to 230 mmHg. Images were centered at the same level where the spectoscopic measurements are performed. Perfusion flow changes (ΔQ) were calculated with use of the equations

\[
\Delta Q = \frac{1}{T1} \cdot K \cdot R1 - K \cdot R1
\]

where ΔR1 is the change in the longitudinal relaxation rate and K is the water partition coefficient (K(37)). And ΔR1 = (1/T1 reperfusion - 1/T1 rest) (Ref. 10; see APPENDIX).

This technique allows both local and global assessments of flow from T1 maps with regions of interest (ROI) excluding the visible vessels. We performed a local measurement for soleus and gastrocnemius (Sol and Gc T1-flow) and a global perfusion measurement by using a ROI summing all the muscular regions in the slice (leg T1-flow). Excluding the major vessels resulted in a measurement area covering 60–80% of the muscle group (Fig. 2). The results of two successive series were averaged for each subject.

NMR-P. The first calculation was obtained from NMR-P, which was adapted from conventional strain-gauge plethysmography and performed with a 5-min ischemic period followed by the deflation of the thigh cuff from 230 to 60 mmHg (37). As with the conventional method, we imaged a single slice at the largest muscle diameter, assuming a constant slice thickness (this parameter is directly determined from the gradient strength in the slice direction and does not change during reperfusion). It has been previously demonstrated that monoslice results were linearly correlated with multislice measurements (7). We monitored volume increase due to venous outflow blockage at a time of a large arterial inflow. NMR-P-flow is obtained from the slope of the volume-time curve. This measurement is obtained in 6–12 s, a time necessary for allowing leg volume change (see Fig. 2 in Ref. 37). NMR-P-flow values are averaged from two successive series.

Exercise Protocol

Subjects were examined in the supine position with the knees fully extended and were instructed to perform submaximal ischemic plantar flexion with the foot on a pedal-shaped lever. Two Velcro straps were placed at the knee and hip levels to secure thigh, pelvis, and abdomen to the table to isolate exercise to the Sol and Gc muscles. In comparison with aerobic exercise, ischemic exercise provokes a quicker and greater decrease of PCr. Ischemia was produced by inflation of a thigh cuff to 230 mmHg. This procedure is well tolerated, provided the frequency of exercise is <1 Hz. The ischemic exercise protocol was divided into five periods (A-E). After the acquisition of a fully relaxed spectrum, three 30-s spectra were acquired during a 1.5-min resting period (period A). The thigh cuff was then inflated, and three spectra were acquired before exercise began (period B). The ischemic exercise followed (period C); plantar flexion was performed at 0.5 Hz with a 3-kg load. PCr resonance was continuously monitored on-screen during rest, exercise, and recovery. We set a threshold of 70% of the resting value (i.e., a reduction [ΔPCr] of at least 30%) to respect the criteria for first-order kinetic of PCr recovery (23). When this threshold was reached (usually after 1–2 min of exercise), we asked subjects to continue to perform at the same frequency until fatigued. The total exercise time ranged from 3 to 6 min for healthy volunteers and 2 to 5 min for heart failure patients with a similar time to fatigue for each subject during the three successive protocols. At the end of exercise, three spectra were acquired with the cuff remaining inflated (period D). The cuff was then deflated, and recovery was monitored for 10 min (period E). After cuff release, PCr recovery reflects ATP synthase activity in the setting of constant ATP content (5). The advantage of studying PCr recovery rate relates to the fact that this parameter only depends on mitochondrial respiratory function (18), is independent of work level, and does not need any correction for active mass (27). The rapidity of PCr depletion during ischemic exercise allowed three successive protocols of ischemic exercise, which were separated by a 10-min period of absolute rest.

31P-MRS

MRS was performed in the same 1.5-tesla General Electric system by using a transmit/receive surface coil (transmitter diameter 21 cm; receiver diameter 7 cm) centered at the maximal calf diameter. Shimming was performed on the proton water signal with a commonly achieved water line width of 6–8 Hz. Spectra were acquired with a flip angle of 60° and a TR of 3 s and corrected for T1 saturation by using a fully relaxed acquisition at rest (TR = 15 s). Spectral width was 2,000 Hz, with 1K data points. Spectra were averaged every 30 s at rest and during exercise and every 15 s during 10 min of recovery. Spectra were Fourier transformed with a 3-Hz line broadening and were integrated peak by peak in the frequency domain. PCr recovery curves were fit with an exponential by using nonlinear regression. We calculated the recovery constant (τPCR) as the average measurement from three successive exercise series. Muscle pH variation was calculated from the chemical shift difference (Δ) of the P, peak compared with PCr according to the equation (Ref. 34)

\[
\text{pH} = 6.75 + \log [(\sigma - 3.27)/(5.69 - \sigma)]
\]

Because PCr recovery depends on the pH reached at the end of exercise (5, 18), we corrected τPCr values for all the experiments during which pH decreased <6.95 according to the relationship (16)

\[
\tau_{\text{PCr}} = 342 - 46 \text{ (minimum pH)}
\]

Reproducibility of τPCr for each individual was calculated as the SD after three measurements. It is expressed as the average of the 12 SD of all the experiments.

Analysis

Data are expressed as means ± SD. Significant difference is assumed for a P < 0.05.

RESULTS

MRS

For the eight normal volunteers, Pi/PCr is 0.13 ± 0.05 at rest. At the end of exercise, this ratio is 0.64 ± 0.27, whereas PCr decreases to 59 ± 10% of its resting value. pH is 7.02 ± 0.04 at rest and significantly decreased to 6.89 ± 0.12 at the end of exercise (mean


\[ \Delta \text{pH} = 0.13 \pm 0.12 \]. ATP resonances do not change significantly during exercise.

A typical experiment during an ischemic plantar flexion is shown in Fig. 1A. This stack of spectra displays the simultaneous variations of PCr (the largest peak) and P\text{i}.

For the eight normal volunteers, \( \tau_{PCr} \) before correction is 31.8 ± 7.6 s. We corrected \( \tau_{PCr} \) values for pH decrease in 12 exercise recoveries of 5 subjects (3 volunteers and 2 patients). The corrected \( \tau_{PCr} \) for the eight normal volunteers is 27.5 ± 8.0 s. Reproducibility is 2.8 s, or 10.2%.

The curve-fitting PCr recovery for the same volunteer as in Fig. 1A is displayed in Fig. 1B. In this experiment, \( \tau_{PCr} \) is 30 s; \( \tau_{PCr} \) after three successive exercise series for this subject is 30.1 ± 1.1 s.

**Perfusion**

Sol and Gc T1-flow from the eight normal subjects is 149.8 ± 47.1 ml·100 ml⁻¹·min⁻¹. Leg T1-flow is 128.3 ± 43.0 and NMR-P is 30.2 ± 9.7 ml·100 ml⁻¹·min⁻¹. Figure 2A shows an echo-planar imaging spin-echo image of the resting leg of a normal volunteer, with the ROI determined for perfusion measurement, which excludes regions corresponding to the major vessels and fibula; Fig. 2B shows the perfusion map of this leg at the time of reactive hyperemia (RH).

**Relationships Between Flow and Oxidative Metabolism**

The relationship between \( \tau_{PCr} \) and postischemic perfusion rate measured locally by Sol and Gc T1-flow for the eight normal subjects is shown in Fig. 3. A linear relationship is given by \( y = 50 - 0.15x \), \( r^2 = 0.68 \), \( P = 0.01 \).

For the two NYHA class II heart failure patients, the results are the following: \( \tau_{PCr} = 29 \) and 30 s; RH Sol and Gc T1-flow = 160 and 117, total leg T1-flow = 170 and 95, and NMR-P-flow = 44 and 23 ml·100 ml⁻¹·min⁻¹, respectively.

For the two NYHA class III patients, the results are the following: \( \tau_{PCr} = 65 \) and 62 s. The RH Sol and Gc T1-flow is 22 and 33, the total leg T1-flow is 10 and 28, and NMR-P-flow is 5 and 15 ml·100 ml⁻¹·min⁻¹, respectively.

Global perfusion techniques have less powerful correlations with \( \tau_{PCr} \) for the eight normal subjects (Figs. 3-5). These relationships are \( y = 44 - 0.12x \), \( r^2 = 0.38 \), \( P = 0.10 \) for leg T1-flow and \( y = 44 - 0.90x \), \( r^2 = 0.34 \), \( P = 0.17 \) for NMR-P. They do not reach statistical significance. However, when we extend the range of flows and include the four heart failure patients, the correlation between \( \tau_{PCr} \) and RH perfusion rates is significant, with the strongest being calculated with Sol and Gc T1-flow, as demonstrated in Fig. 3. Linear relationships are given by the following: \( y = 64 - 0.24x \), \( r^2 = 0.63 \), \( P = 0.001 \) for Sol and Gc T1-flow; \( y = 60 - 0.23x \), \( r^2 = 0.63 \), \( P = 0.002 \) for leg T1-flow; and \( y = 59 - 0.90x \), \( r^2 = 0.84 \), \( P = 0.001 \) for NMR-P-flow.

**DISCUSSION**

In this investigation, we demonstrate a positive correlation between oxidative metabolism characterized by the rate of PCr recovery measured after ischemic exercise with \(^{31}\)P-MRS and the perfusion rate obtained after RH from \(^{1}\)H-MRI in human skeletal muscle from healthy volunteers and heart failure patients. This result shows that aerobic ATP synthesis after exercise is related to the magnitude of perfusion, i.e., \( \text{O}_2 \) availability, in both normal and diseased muscle.

**PCr Recovery**

PCr recovery provides an excellent estimation of ATP synthesis because of 1) the equilibration of ATP and...
PCr in the creatine kinase reaction and 2) the immediate cessation of ATP production through anaerobic glycolysis at the end of exercise (34). Our \( \tau_{PCr} \) values in the group of healthy volunteers are in excellent agreement with previous publications on adults of the same age by McCully et al. (27) and Marsh et al. (25). These differ substantially from the work of Blei et al. (5) in which a longer \( \tau_{PCr} \) was measured in exercising forearm. However, the population in this study was 10 years older than ours and 15 years older than the group studied by Marsh et al. (25), and it has been previously demonstrated that PCr recovery slows with age (27) and parallels a decrease of maximal rate of \( \text{O}_2 \) consumption (\( \text{VO}_{2\text{max}} \)) (19; 27). Recent data from our group also suggest such a relationship among age, flow, and metabolism (35).

PCr resynthesis rate after ischemic exercise is related to \( \text{VO}_{2\text{max}} \) (23; 29) and depends both on mitochondrial phosphorylative activity and \( \text{O}_2 \) delivery. \( \text{O}_2 \) delivery relies on red blood cell transit time, capillary permeability, and diffusion to the intracellular space (15). Magnetic resonance has the unique capability of assessing these successive steps in human muscle by quantifying blood flow, \( \text{O}_2 \) diffusion through myoglobin saturation curves (6), and mitochondrial function.

In this study, we satisfy the conditions for first-order recovery set forth by Mahler (23), indicating that PCr at the end of exercise should decrease between 20 and 70% of its initial value, ATP should not change, and the pH drop should be minor. According to this model, our results suggest that the higher flow value in our population is associated with better mitochondrial func-
tion and better muscle performance. We did not directly test this hypothesis, for we did not perform any muscle biopsy or measure muscle performance, but other investigators have suggested this relationship (14). Our results are consistent with this hypothesis as illustrated on Fig. 5, in which the most limited patients in NYHA class III are in the initial part of the curve (low flow and high $t_{\text{PCr}}$).

These results and hypothesis are also consistent with the literature in which related changes have been demonstrated during training and detraining. Training increases oxidative enzymes and mitochondrial content (9) as well as capillary density and blood flow (22). Conversely, detraining or deconditioning decreases performance, $V_{O_2\text{max}}$, oxidative enzymes, as well as capillary density and flow parameters. We notice here that the lowest flow and the slowest ATP resynthesis correspond to the most severe patients, those in NYHA class III, with marked exertional intolerance and early fatigue (8), where an adaptive mechanism probably linked to muscle deconditioning is thought to produce a simultaneous alteration of flow and oxidative metabolism (11). These results may suggest that physical activity in normal subjects or congestive heart failure patients contributes to parallel changes of both metabolism and perfusion. Further experiments will be required to demonstrate the dependence of these two important determinants of muscle function and the time course of their alterations.

**Ischemic Exercise**

In our experiment, the cuff is inflated for 1.5 min before and 1.5 min after the ischemic exercise. During these periods, no change of $P_i$ or PCr occurs. Our voluntary exercise protocol yields a result similar to the electrical stimulation protocol used by Blei et al. (6): it confirms that a short ischemic period does not grossly perturb resting mitochondrial metabolism and that no recovery can occur after ischemia until flow and $O_2$ availability are restored (12).

**Flow Measurements**

Ischemia is one of the most powerful stimuli for vasodilation. RH has been extensively studied as an index of maximal flow capacity in normal human subjects, athletes, or heart failure patients. The amplitude of RH in human muscle seems to be reached even after a brief period of ischemia. Patterson and Whelan (30) showed that peak flows during early RH are similar after 3 or 10 min of ischemia; Barcroft (3)
demonstrated that scarcely any further dilation occurred between 5 and 15 min of occlusion, and Sinoway et al. (32) recently showed that RH in the brachial artery plateaus after 3 min of occlusion.

The T1 technique has now been applied for measuring tissue perfusion in numerous organs in both animals and humans. It has recently been compared with validated methods: these works report excellent correlation between the T1 technique by using inversion of arterial water spins and flow probe measurements in perfused heart (41) or radioactive microspheres in brain in vivo (38). Calculating the apparent T1 variations induced by flow changes, we recently reported excellent correlation of this magnetic resonance technique with an independent plethysmographic method in human skeletal muscle (37). In this investigation, we showed that the changes in signal intensity were lasting for ~2 min, with a plateau during the first minute (37). Signal intensities used for T1 measurement are collected over that first minute. However, the determinant points in measuring T1 correspond to the points when the T1 is close to T1. In our experiment, these critical points occur at T14 = 650 ms, T15 = 800 ms, and T16 = 950 ms, i.e., 18, 22.5, and 27 s after cuff release; these time points were chosen to surround the time-to-peak of MRI signal intensity (22 ± 7 s) (37) and are close to the time-to-peak of conventional plethysmography (15 s) (42). Although it does not completely prevent it, the selected scheme may limit a possible underestimation of absolute peak flow by the T1 technique, which may appear at high flow values (38). To reduce total acquisition time, some authors have suggested that a three-point T1 calculation may be sufficient for accurate measurement of flow (38); in any case, increased speed for data collection may further reduce this limitation in the future.

We previously evaluated the T1 technique in human muscles by comparing its measurements with NMR-P (37); the correlation was excellent (r^2 = 0.84), although results between the two techniques differed by a factor of 3.8. The principal explanation for this discrepancy may be related to the well-known underestimation of muscle perfusion by plethysmography (31). The T1 method provides results that are close to the more accurate measurements obtained with thermodilution. Furthermore, the use of inflowing water protons as a magnetically labeled tracer is directly related to the amount of blood flow entering the studied slice and does not depend on factors unrelated to perfusion, such as skin and connective tissue compliance, or distention of capacitive vessels, which may reduce plethysmography accuracy. However, in a novel approach to study the relationship between noninvasive evaluation of muscle perfusion and metabolism, it seemed important to us to compare a technique that has been employed for more than 50 years in muscle investigations and a recently developed method which has shown excellent agreement with gold-standard techniques of perfusion (38, 41).

Flow and Metabolism

In both RH and functional hyperemia, a high perfusion rate results in increased O_2 availability and allows a quick recovery of high-energy phosphate metabolites after exercise. Our study demonstrates a correlation between the RH Sol and Gc T1-flow and the $t_{PCr}$, showing that a strong relationship exists in skeletal muscle between oxidative metabolism and tissue perfusion. A similar relationship was shown between O_2 consumption and flow by Andersen and Saltin (2) with more invasive techniques.

This relationship is better assessed when metabolism and perfusion are isolated to the same group of muscles (local perfusion measurement). The lower correlation coefficient found with the global measurement in healthy volunteers may be due to the contamination of anterior muscles (tibialis anterior), which have a different microvasculature than antigravity muscles and have a lower flow value (37). It seems likely from the good relationship obtained in the whole study group of volunteers and patients that this result may be due to a difference of sensitivity; statistical significance threshold for global perfusion techniques may have been reached with a population somewhat larger. However, the internal consistency of our results is evidenced by the similar origins of the different curves: for the 12 subjects, at x = 0, $t_{PCr} = 64, 60, and 59 s$ for Sol and Gc T1-flow, leg T1-flow, and NMR-P-flow, respectively.

Two groups studied the relationship between flow and high-energy phosphate metabolism with $^{31}$P-MRS. Wiener et al. (39) investigated the relationship between acutely reduced flow measured from Doppler ultrasound and oxidative metabolism in normal forearm muscle. They demonstrated an increase of P_i/PCr and a decrease of pH for each workload studied under a 50% reduction of forearm blood flow. Moreover, they also showed a significantly slowed recovery for P_i/PCr after cessation of exercise, which may be interpreted as a reduced phosphorylation activity. On another hand, Williams et al. (40) studied muscle metabolism in a situation with limited flow in a group of patients suffering from peripheral arterial occlusive disease. They also demonstrated a good correlation between P_i recovery rate and an angiographic index of arterial resistance, which is probably related to flow. These studies and the results presented here support the hypothesis that local perfusion is an important determinant of ATP synthesis in human skeletal muscle.

Heart Failure Patients

To further characterize the flow-metabolism relationship, we extended the investigation to a population known to have abnormalities of muscle metabolism and perfusion: four heart failure patients were studied by using the same protocol as with the healthy volunteers.

In a heart failure population, flow alterations depend on the muscle studied (17) and on the timing of compensatory mechanisms (43): flow as well as metabolism abnormalities may resolve with training (1). Two of the four patients were NYHA class II, with slight
limitation of physical activity: they had a PCr recovery and blood flow values similar to those of the normal volunteers. The two other patients in NYHA class III were clinically limited, as expected from more severely ill subjects (8). Their \( r_{PCr} \) were the longest of all, and their perfusion rates were minimal. Including the results of these four patients with the data from the normal volunteers slightly changed the relationship between \( r_{PCr} \) and flow. This suggests that a mechanism takes place during the evolution of the disease that maintains the link between flow and metabolism. The heart failure patients were included to have a broad range of flow and metabolism, but a larger group should certainly be studied before any conclusive statement can be made about this disease state (36). NMR may be the preferred method to further study the relationship between flow and metabolism in these patients.

Conclusion

In human Sol and Gc, PCr recovery after ischemic exercise is correlated with the perfusion rate measured in these two muscles during RH over a broad range of perfusion rates. This result demonstrates that ATP availability in human skeletal muscle. This highlights the necessity to develop a noninvasive technique capable of quantifying local flow in vivo. The association of MRI and MRS in interleaved sequences may be the instrument of choice to assess muscle function in many physiological and pathological states.

APPENDIX

T1-Model

In Detre et al. (10) and Kwong et al. (20), the Bloch equation including tissue flow effect is

\[
dM_b / dt = [(M_b^* - M_b)/T1] + \dot{Q} M_a - (\dot{Q}/K) M_b
\]

where \( M_b^* \) is fully relaxed proton density of the tissue, \( M_b \) is tissue longitudinal magnetization, \( M_a \) is blood longitudinal magnetization, \( T1 \) is longitudinal relaxation constant of the tissue in the absence of flow, \( \dot{Q} \) is blood flow (in ml·100 g\(^{-1}\)·min\(^{-1}\)), and \( K \) is the partition coefficient; it is the ratio (quantity of nonmagnetized water spins/g muscle)/(quantity of nonmagnetized water spins/ml blood).

The apparent proton density \( M_{app} \) can then be deduced as

\[
M_{app} = [(M_b^*/T1) + \dot{Q} M_a]/(1/T1 + \dot{Q}/K)
\]

and the apparent \( T1 \) of the tissue including flow effect \( (T1_{app}) \) is given by \( T1_{app} = 1/T1 + \dot{Q}/K \).

Thus a flow variation can be measured from a change of \( T1_{app} \). If we assume tissue \( T1 \) remains constant, a change in blood flow \( \Delta Q \) will lead to a change in the observed \( T1_{app} \):

\[
\Delta Q/K = \Delta (1/T1) = \Delta R1 \cdot K.
\]

It is the difference of \( 1/T1_{app} \) measured under two distinct flow conditions that allows the calculation of a flow difference. In this case, we chose the reference value during complete ischemia (no-flow condition) and therefore calculated the absolute flow value \( Q \) during reperfusion as \( Q = \Delta R1 \cdot K \).

NMR-P

We adapted the conventional plethysmographic technique and studied the effect of flow changes on the leg volume during reperfusion while an increase in venous pressure is simultaneously applied. For that purpose, the 5-min ischemic period is followed by a deflation of the thigh manometer from 230 to 60 mmHg to block venous outflow, but not arterial inflow, which is maximal at that time. High arterial inflow results in a rapid leg volume increase until venous pressure equals cuff pressure. With a constant slice thickness (STk), we measure the volume changes \( \Delta V \) from the change of cross-sectional area \( (\Delta CS\text{A}) \) determined with an automated segmentation analyzing program as \( \Delta V = \Delta CS\text{A} \cdot STk \) (in ml).

Because the volume increase is solely due to inflowing arterial blood, flow is determined from the slope of the volume-time \( (t) \) curve: \( \dot{Q} = \Delta V/\Delta t \) (see Fig. 2 in Ref. 37). With the assumption of a constant density of muscle tissue, flow is expressed in ml·100 g\(^{-1}\)·min\(^{-1}\).

We thank David A. Chessler and Jerry L. Boxerman for very helpful discussions, Terry A. Campbell for outstanding technical assistance, and Cecile Brillaut-Salvat for participation in all steps. We would like to thank the staff and members of the Massachusetts General Hospital-Nuclear Magnetic Resonance Center for general support, assistance in operating the NMR systems, and helpful discussions.

We gratefully acknowledge the support of the Association Française contre les Myopathies and the Harold M. English Fund (to J.-F. Toussaint); National Heart, Lung, and Blood Institute Grant RO1-HL-3971; the Eugene H. and Patricia C. Remmer Fund; and the Ruth and Frank Stanton Fund (to H. L. Kantor).

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Received 11 July 1995; accepted in final form 25 March 1996.

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