Energy metabolism and interstitial fluid displacement in human gastrocnemius during short ischemic cycles


Energy metabolism and interstitial fluid displacement in human gastrocnemius during short ischemic cycles. J. Appl. Physiol. 85(4): 1244–1251, 1998.—Energy metabolism and interstitial fluid displacement were studied in the human gastrocnemius during three subsequent 5-min ischemia-reperfusion periods [ischemic preconditioning (IP)]. The muscle energy balance was assessed by using near-infrared spectroscopy (NIRS) and 31P-nuclear magnetic resonance spectroscopy (31P-NMRS). The interstitial fluid displacement was determined by combining NIRS and 23Na-NMRS. No changes in total energy consumption or in the fractional contribution of the underlying energy sources (oxygen consumption, anaerobic glycolysis, and Lohmann reaction) were observed in the muscle during the tested IP protocol. Oxygen consumption in the muscle region of interest, as estimated by NIRS, was –8 µmol·100 g−1·min−1 and did not change during IP. Phosphocreatine and ATP concentrations did not change over the whole experimental period. A slight but significant (P < 0.05) increase in intracellular pH was observed. Compared with the control, a 10% greater interstitial fluid content per muscle unit volume was observed at the end of the IP protocol. It is concluded that, at variance with cardiac muscle, repeated 5-min ischemia-reperfusion cycles do not induce metabolic changes in human gastrocnemius but alter the interstitial fluid readjustment. The techniques developed in the present study may be useful in identifying protocols suitable for skeletal muscle preconditioning and to explain the functional basis of this procedure.

ISCHEMIC PRECONDITIONING (IP) consists of one or more ischemia-reperfusion cycles of a given organ or tissue (23). This procedure has been shown to improve myocardial tolerance to a subsequent ischemic stress under experimental (28) and clinical (33) conditions. The effects of IP were also demonstrated in skeletal muscles of animals, where it appears to reduce the size of experimental infarcts (25, 26) and to enhance postischemic function (18).

The mechanism by which IP operates has not yet been elucidated. Several hypotheses have been put forward. These are mainly based on the release by the tissues of substances such as bradykinin, adenosine, nitric oxide, opioids, and free radicals. However, none of the latter appears to explain the effects of IP. Among the hypothesized mechanisms by which repeated ischemia could operate, changes in energy metabolism (28) and/or localized edema (34), were proposed. With regard to the latter, cellular stretch could trigger the release of active substances, hence the effects of IP (34).

To our knowledge, the effects of repeated ischemia-reperfusion cycles have not been assessed in human skeletal muscle. The purposes of the present study were to investigate possible changes of energy metabolism and interstitial fluid shifts in human gastrocnemius induced by IP. The novelty of the present approach is that the above mechanisms, possibly underlying IP, could be assessed by combining near-infrared spectroscopy (NIRS) (15, 20) with 31P-nuclear magnetic resonance spectroscopy (NMRS) and NIRS with 23Na-NMRS, respectively.

MATERIALS AND METHODS

Subjects

The present investigation was conducted in a total of 50 healthy subjects (20 women) divided into three groups. Each subject participated in only one experimental protocol. All groups of male subjects were age and weight matched. The reason for assessing different subject pools was that the various measurements were carried out in different laboratories (group 1 at L’Aquila, Italy; groups 2 and 3 in Geneva, Switzerland). In addition, the experimental protocols adopted for groups 2 and 3, respectively, were rather demanding, which led us to work with different subject pools. Differences between men and women are only considered within group 1. The subjects were aware of the purpose of the study and the related risks. The protocols were approved by the ethical committee of the Medical School of the Universities of L’Aquila and Geneva.

Group 1. Resting baseline absolute concentration of deoxyhemoglobin ([Hb]d) and oxyhemoglobin ([HbO2]d) were determined in the gastrocnemius of 17 men [age 33.5 ± 8.5 (SD) yr, body weight (BW) 75.2 ± 8.5 kg] and of 20 women [age 29.7 ± 3.6 yr; BW 56.1 ± 7.3 kg] by a phase-modulated NIR photometer. Adipose tissue thickness (ATT; fat plus skin layers) was measured by a skinfold caliper.

Group 2. Simultaneous 31P-NMRS and concentration changes in Hb (∆[Hb]) and HbO2 (∆[HbO2]) were obtained throughout the adopted IP experimental protocol (see below) in a group of eight male subjects [age 38.0 ± 9.4 yr; BW 78.5 ± 16.7 kg] by a 1.5-T magnetic resonance spectrometer and by a continuous-wave NIR photometer, respectively.

Group 3. 23Na-NMRS data were obtained in five different male subjects [age 37.4 ± 12.0 yr, BW 73.4 ± 15.9 kg] during an IP protocol the same as was adopted for group 2. In group 3, a control 23Na-NMRS experiment was also carried out.

NIRS Measurements

Absolute [Hb]d and [HbO2]d values, required for the calculation of the oxygen consumption rate (O2), were measured by a
The procedure is applicable only if the total hemoglobin (Hb) concentration is constant and if, in the investigated muscle region of interest, [HbO₂]b and [Hb]b appearing in Eq. 1, as indicated above, cannot be measured by the continuous-wave NIRS-500 photometer. Therefore, the following values (obtained separately by the multisource, phase-modulated OM NIA spectrometer in group 1 subjects) were adopted: [HbO₂]b = 100 µM and [Hb]b = 21.1 µM.

Thus Eq. 1 turns out to be

\[
\dot{O}_2 = \frac{4 \cdot 121.1}{1.13} \frac{\Delta[HbO₂] + 100}{\Delta[HbO₂] + \Delta[Hb] + 121.1} \quad (4)
\]

Mean \( \dot{O}_2(\bar{O}_2) \) was calculated over 4-min intervals

\[
\dot{O}_2(\bar{O}_2) = \frac{1}{4} \int_0^4 \dot{O}_2 \, dt \quad (5)
\]

The maximum error in \( \dot{O}_2(\bar{O}_2) \) calculation has been assessed for the whole range of experimental values obtained in the present study by means of a mathematical simulation (unpublished data) and found to be <10%.

**31P-NMR Measurements**

31P-NMR spectra were obtained with a hybrid system comprising a Picker (Picker International, Highland Heights, OH) whole body imaging system (1.5-T superconducting magnet, 90-cm bore) and a NMR console (Surrey Medical Imaging Systems, Guildford, UK). A double-tuned \(^{(1}H-^{31}P\), 5-cm-diameter radio-frequency surface coil was used. Shimming was performed manually on the proton signal by using first-order correction. The transmitter-pulse duration and amplitude were chosen so as to maximize the in vivo \(^{31}P\) signal-to-noise ratio from the target sample. Acquisition parameters were 100 µs for the radio-frequency pulse duration, 1,024 points per free-induction decay (FID), a spectral width of 1,000 Hz, and a 3-s repetition time. A single spectrum consisted of the sum of 32 FIDs, corresponding to an acquisition time of 96 s per spectrum. The FIDs were filtered with an exponential function (line broadening 3 Hz) to improve the signal-to-noise ratio. Evaluation of the relative concentrations of phosphocreatine (PCr) and ATP (IATP), and the measurement of the chemical shift of \( P_i \), Cr, α-ATP, and β-ATP was performed by using a nonlinear least square fitting method in the time domain (31a, 32). Prior knowledge was incorporated for the β-ATP triplet, imposing the same frequency splitting between the three peaks. The α-, β-, and γ-ATP peak areas within each multiplet were linked together by using the following constants of proportionality: 1:1, 0.5:1:0.5, and 1:1. Chemical shifts were used to compute intracellular pH (27) and Mg\(^{2+}\) concentration (17).

**23Na-NMR Measurements**

23Na-NMR spectra were obtained with a double-tuned \(^{(1}H-^{23}Na\), 5-cm-diameter radio-frequency surface coil. The shimming procedure was the same as for \(^{31}P\)-NMR spectra. Relative concentration of total Na (Na\(^{-}\); intracellular and extracellular) was detected with a standard one-pulse NMR sequence. The transmitter-pulse duration and amplitude were chosen so as to maximize the in vivo \(^{23}Na\) signal-to-noise ratio from the target sample. Acquisition parameters were 120 µs for the radio-frequency pulse duration, 512 points per FID, a spectral width of 5,000 Hz, and a 164-ms repetition time. A single spectrum consisted of the sum of 80 FIDs, resulting in an acquisition time of 13.12 s per spectrum. The FIDs were first filtered with an exponential function (exponential coefficient 3 Hz) to improve the signal-to-noise ratio.
Analysis of the spectra ($^{23}$Na-NMR spectra are characterized by only one peak) was performed by using the same software as for $^{31}$P-NMRS.

Experimental Protocol

Group 1 ($n = 37$). Absolute $[\text{HbO}_2]$ and $[\text{Hb}]$ measurements were performed in the right gastrocnemius muscle while the subject was seated with the lower legs down. The probe was placed on the medial line of the upper third of the gastrocnemius medialis. The data were collected over a time interval of 5 min after an initial 5-min readjustment period. The tracings were averaged to improve the signal-to-noise ratio. To make the measured values comparable among subjects, the photometer was tested and calibrated in a phantom before each measurement. An additional tracing, in the same phantom, was obtained at the end of each measurement to check the stability of the equipment. ATT was measured at the same location.

Group 2 ($n = 8$). After the control values at rest (5 min) were recorded, a sequence of three cycles, each consisting of 5-min ischemia followed by 5-min reperfusion, was performed in the right leg of the subject. The subject was lying supine inside the NMR magnet, with the calf placed over the $^{31}$P-NMR radio-frequency coil. The right leg was tightly fixed to the sliding board to avoid motion artifacts. The transmitting and receiving optodes of the NIRS photometer were placed on the gastrocnemius medialis 2 cm proximal to the radio-frequency coil (10). Their relative distance (3 cm) was ensured by a rigid 3-mm-thick custom-made holder (29). Measurements were performed by a pair of optical fibers (length 6 m). Ischemia was induced by inflating a nonmagnetic cuff (width 15 cm) up to 350 mmHg around the leg, 1 cm distal to the groin. Continuous acquisition of $^{31}$P-NMRS and NIRS data was carried out simultaneously.

Group 3 ($n = 5$). The same protocol as for group 2 was repeated for $^{23}$Na-NMRS. In addition, control $^{23}$Na-NMR measurements were carried out in separate sessions before the IP protocol. In both cases, the subjects were asked not to lie supine within 2 h before the experiment. The initial $^{23}$Na-NMR spectra were acquired 10 min after the lying position was assumed.

Statistics

Data are reported as means ± SD. Significance of differences in all measurements within each group was analyzed by means of the Student’s paired t-test. Significance level was set at $P < 0.05$.

RESULTS

NIRS: Aerobic Metabolism

$[\text{Hb}]$ and $[\text{HbO}_2]$ in the region of interest in the resting gastrocnemius muscle are shown in Fig. 1 as a function of the ATT. Solid and open circles refer to women and men, respectively. As appears in Fig. 1A, $[\text{Hb}]$ does not vary as a function of ATT both in women and men. Mean $[\text{Hb}]$ values were $13.6 ± 4.5$ and $21.1 ± 7.5$ µM for women and men, respectively. By contrast, $[\text{HbO}_2]$ is definitely higher in muscle than in fat, as shown by the data at low ATT values (Fig. 1B), the trend being similar in both women and men. From Fig. 1 it also appears that most $[\text{Hb}]$ and $[\text{HbO}_2]$ values for men are to be found in the intervals 5–30 and 70–200 µM, respectively. These data are adopted (on the basis of the simulation) to define the constants appearing in Eq. 4.

Typical time courses of $\Delta[\text{Hb}]$, $\Delta[\text{HbO}_2]$, and $\Delta[\text{Hb}]_{\text{tot}}$ during the IP protocol are shown in Fig. 2 for a typical subject. The horizontal solid bars represent the ischemic periods (ISPE). As expected, a constant decrease in $\Delta[\text{HbO}_2]$ is accompanied by an increase in $\Delta[\text{Hb}]$. Individual tracings have similar patterns, but $[\text{Hb}]_{\text{tot}}$ during ISPE (i.e., the intervals during which O$_2$mean was calculated) varies considerably among subjects. The largest observed $\Delta[\text{Hb}]_{\text{tot}}$ was on the order of 10 µM, being within the concentration range (−5 to 10 µM). The values are well within the limits imposed for the simulation. The $\Delta[\text{Hb}]_{\text{tot}}$ values recorded at baseline level and at the end (after 35 min) of the IP protocol are essentially the same. These results, combined with the
23Na-NMRS data, are required to assess interstitial fluid shifts (see DISCUSSION).

\(\dot{O}_2\)_{mean} in the muscle region of interest, as a function of ATT, calculated by Eq. 5, is shown in Fig. 3. Circles, stars, and crosses correspond to the first, second, and third ISPE, respectively. No \(\dot{O}_2\)_{mean} differences were found among the three ischemic cycles. The data on the left of the curve reflect \(\dot{O}_2\)_{mean} of mainly muscle, which could be estimated at \(-8\ \mu\text{mol}\cdot100\ \text{g}^{-1}\cdot\text{min}^{-1}\).

**DISCUSSION**

As indicated at the beginning of this study, one purpose of the study was to investigate possible total energy changes, both aerobic and anaerobic (lactic and alactic), in the human gastrocnemius during the IP protocol.
NIRS Measurements: $\tilde{O}_2$

Among the investigated subjects, ATT varies in the range 0.2–1.2 cm (see Fig. 1). Because it was estimated (21) that the NIR light penetrates into the tissue (adipose layer plus muscle) about one-half the distance between the light source and the detector, different fractions of the two main tissue components are monitored at each ATT by the two optodes. Skeletal muscle appears to contain more hemoglobin than does adipose tissue, and its largest fraction is oxygenated (HbO2). Because of the “banana” shape of the observed region of interest (21), the highest (mainly muscle) and the lowest (mainly fat) $O_2$mean experimental values appearing in Fig. 3 are compatible with the results obtained by Elia (13), who found $O_2$mean values of 7.99 and 2.81 µmol·100 g$^{-1}$·min$^{-1}$ for muscle and adipose tissue, respectively.

Muscle myoglobin (Mbtot) has an absorption spectrum similar to that of hemoglobin. Therefore, Mbtot does interfere with the NIRS measurement of muscle. On the basis of values in human gastrocnemius, i.e., on average, 4 mg/g (24) or 41.9 µM, one can estimate its weight as a confounding factor at ∼5–10% of the whole hemoglobin signal, an amount that may be considered negligible for the present calculation. However, the presence of myoglobin, whatever its concentration, does not influence $O_2$ calculation because the transformation of four molecules of oxygenated myoglobin to deoxygenated myoglobin is equivalent, from the optical standpoint, to the transformation of one molecule of HbO2 to Hb. This leads in any case to the utilization of four molecules of $O_2$, and Eqs. 1 and 5 remain valid.

The use of the phase-modulated OMNIA photometer for the determination of $[\text{Hb}]_b$ and $[\text{HbO}_2]_b$ in group 1 would require that the tissue within the region of interest be homogenous. As appears in Fig. 1A, $[\text{Hb}]_b$ values in two homogeneous regions of interest (mainly fat or mainly muscle, respectively) are practically identical. A possible optical artifact due to the heterogeneity of the investigated tissue layers would necessarily lead.
to average [Hb]b levels different from the values found for mainly fat vs. mainly muscle. This rules out tissue heterogeneity as a possible confounding factor for [Hb]b measurements. It necessarily follows that, if [Hb]b values are reliable, then, on the basis of the OMNIA algorithm, [HbO2]b values must also be correct. This leads us to conclude that the measured [HbO2]b and [Hb]b values reflect actual physiological conditions.

Absolute [Hb]b and [HbO2]b values are required to calculate "true" O2mean during IP by Eqs. 1 and 5 (see MATERIALS AND METHODS).

[Hb]b and [HbO2]b cannot be determined by continuous-wave photometers of the type of NIRO-500 used for the present joint NIRS-NMRS protocols. However, even a device such as the phase-modulated OMNIA photometer, by which absolute [HbO2]b and [Hb]b can be assessed, cannot monitor the fractional Δ[Hb] and Δ[HbO2] due to blood volume shifts (Δ[Hb]tot) and due to variations in the Hb-to-HbO2 ratio for any given Δ[Hb]tot in the region of interest. These changes could introduce an error in the O2mean calculation. This is the reason a simulation was made. By the simulation it was proven that the use of Eqs. 4 and 5 yields reliable O2mean data even in the presence of sizeable Δ[Hb]tot and Hb-to-HbO2 ratio changes. Moreover, it was proven that Eqs. 4 and 5 are not highly dependent on [HbO2]b and [Hb]b, and that, in any case, the error in estimating O2mean is <10% for the whole range of experimental values.

It is noteworthy that, apart from extreme ATT values (i.e., nearly mainly muscle or mainly fat), O2mean takes into account different fractions of muscle and adipose tissue. However, in the present case, the fact that during IP O2mean is the same at each ATT value proves that O2mean also does not change for each of the two tissues under consideration. In fact, it would be highly improbable that muscle O2 could totally compensate for adipose tissue O2 at each ATT.

NMRS Measurements: Energy Metabolism

As is well known, the energy necessary for muscle metabolism is released at the onset by the Lohmann reaction and, subsequently, by aerobic and anaerobic glycolysis. Thus, at constant [ATP], the muscle energy balance can be described (4) by the following equation

\[ \dot{E} = \alpha[P\text{Cr}] + \beta[La] + \gamma[O_2] \]

where \( \dot{E} \) is the energy per unit time consumed by the tissue, and \( \alpha, \beta, \) and \( \gamma \) represent the energy equivalent of 1 mol of PCR, lactate (La), and O2, respectively. [PCR] did not change during the IP protocol, which means that no energy was contributed by the Lohmann reaction, which in Eq. 6 is [PCR] = 0. This finding is compatible with previous results (8, 19). The observed small increase in pH (see Fig. 4) may be the consequence of the small (~2°C) muscle temperature drop (5) found during ischemia at room temperature. Such temperature change is too small to affect the recorded PCR level through changes in relaxation times, magnetization, and so on (5). The constancy of [PCR], accompanied by the slight increase in pH, suggests that no anaerobic metabolism takes place during IP. The occurrence of La accumulation not detected by the pH measurements, due to tissue buffering or redistribution of lactic acid outside the cell, is not compatible with a constant [PCR] and must therefore be ruled out. It follows also that, during the IP protocol, [La] = 0. Thus all energy is supplied by the oxidative pathway (O2). Because O2mean is constant during the three ISPE, E also appears to be unchanged.

NMRS Measurements: Fluid Shifts

The second purpose of the present experiment was to identify, by means of 23Na-NMRS (natural abundance, 100%), tissue fluid shifts during IP. This is possible if [Na] in each of the three muscle compartments [i.e., intracellular (~85%), interstitial, and vascular] remains constant throughout the experiment. In this respect, it is noteworthy that, considering the size of the compartments and their [Na], ~20%, i.e., a nonnegligible fraction of the signal, would be of intracellular origin. Indeed, the intracellular Na content does not change in the present IP experimental protocol because muscle metabolism is aerobic. This is confirmed by personal observations (unpublished observations), whereby intracellular Na content by flip-angle independent 23Na triple-quantum-filter NMRS (9, 30) was found to be the same in IP and in control conditions. If this is the case and the volume of intracellular fluids remains constant, possible Na changes detected by means of 23Na-NMRS should be linearly related to the volume changes in extracellular fluids. The assessment by 23Na-NMRS of the fluid shift in the interstitial space of the muscle could therefore be altered by size changes in the vascular bed. For example, it may be seen that transient changes in vascular fluid content accompanying the hyperemic postischemic responses (see Fig. 2) are detected as peaks in the Na tracing (Fig. 5A). However, the vascular bed fluid content can be considered unchanged. In fact, \( \Delta[Hb]_{tot} \) at the beginning and at the end of the experiment appears to be the same (see RESULTS).

It necessarily follows that the observed decrease in Na at the end of the experiment (Fig. 5) is attributable only to a loss of interstitial fluid. This shift is probably due to the fluid redistribution to the upper part of the body when the subject is tilted from standing to supine (3). The behavior of the fluid content as a function of time in control conditions (Fig. 5B) is similar to that found by Berg et al. (3), even though the time constant is somewhat shorter: 26.4 vs. up to 37 min. This difference might be methodological. In fact, Berg et al. 1 A similar estimate may be made by a different approach. This is based on the knowledge of 1) muscle blood volume [8.54 ml/100 ml (see Ref. 31)]; 2) the amount of fluid in the interstitial space [10 g/100 ml (see Ref. 1)]; and 3) muscle water content [74.1 g/100 g (see Ref. 34)]. The calculated intracellular water turns out to be ~25% if allowance is made for the presence of the so-called "broad component" of the 23Na-NMRS signal described by Kushnir et al. (22) that could reduce the NMR-"visible" Na.
measured muscle volume changes by plethysmography and bioelectrical impedance analysis. In the present case, Na values represent only the fluid compartments, whereas the surrounding tissues are neglected.

Should the water shift in the gastrocnemius be only due to gravity, then Na level at the end of the IP protocol (35 min; Fig 5A) would be equal to the level found 20 min after the onset of the control measurement (Fig 5B). In fact, during the ISPE the water cannot leave the legs because of the cuff, and the time available for the fluid shift would be 35 – 15 min = 20 min. The actual Na level after 20 min (Fig 5B) was 86.35 ± 0.71%. This is significantly lower (P < 0.05) than the final level in the IP protocol recorded in Fig. 5A. This difference becomes significant only after the third ISPE. Thus, after IP, less water is lost than expected. A mechanism other than gravity must therefore affect muscle fluid redistribution, which leads to a relative increase, or more appropriately, to a lesser decrease, in the interstitial water content. This is at variance with a previous hypothetical interpretation by our group (6), whereby Na disappearance was attributable, in part, to the Na “trapping” (i.e., NMR invisible) in the interstitial space.

In conclusion, it was shown for the first time, to our knowledge, that 1) three 5-min ISPE followed by 5-min reperfusion intervals do not induce significant changes in the total energy consumption (E) in the human gastrocnemius; and 2) the same protocol is associated with a reduction in the postural fluid shift observed in the muscle when the body is tilted from the vertical to the supine position. The latter change obtained by combined 23Na-NMRS and NIRS may be a precursor of edema, which is a possible determinant of IP. In addition to the above-mentioned main findings, the following results were obtained: 1) [HbO2] values in resting muscle and adipose tissue are the same; 2) the O2mean of the resting gastrocnemius has been confirmed to be on the order of 8 µmol · 100 g−1 · min−1, whereas that of adipose tissue is 2 µmol · 100 g−1 · min−1; 3) during repeated 5-min ischemia-reperfusion cycles, anaerobic metabolism does not contribute energy to the muscle; and 4) on assumption of the lying position, the decrease in the interstitial fluid in the gastrocnemius is characterized by a half-time of 24.6 min.

We thank Dr. C. Robino of Hamamatsu, Italy, for the loan of the NIKOS00. The project was supported by the Centro Interuniversitario Grandi Apparecchiature Biomediche nelle Neuroscienze (Italy), the Foundation Ernst and Lucie Schmidheiny (Switzerland), the Swiss National Science Foundation (no. 31–47075.96), and European Union Contract BMH4-CT96.1658.

Address for reprint requests: T. Binzoni, Centre Medical Universitaire, Département de Physiologie, 1211 Genève 4, Switzerland (E-mail: Tiziano.Binzoni@medecine.unige.ch).

Received 16 October 1997; accepted in final form 1 June 1998.

REFERENCES


