Changes in tissue water content measured with multiple-frequency bioimpedance and metabolism measured with \(^{31}\)P-MRS during progressive forearm exercise

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Raja, Mohan K., Graydon H. Raymer, Gerald R. Moran, Greg Marsh, and R. Terry Thompson. Changes in tissue water content measured with multiple-frequency bioimpedance and metabolism measured with \(^{31}\)P-MRS during progressive forearm exercise. J Appl Physiol 101: 1070–1075, 2006. First published June 22, 2006; doi:10.1152/japplphysiol.01322.2005.—Multiple-frequency bioimpedance analysis (MFBIA) has been used to determine the cellular water composition in the human body. It is noninvasive and has demonstrated good correlations with other invasive measures of tissue water. However, the ability of this method to study transient changes in tissue water in specific muscle groups has not been explored. In this study, MFBIA was used to assess changes in forearm intracellular water (ICW), extracellular water (ECW), and total water (TW) in seven healthy volunteers during and after a progressive wrist flexion exercise protocol. In an identical trial, \(^{31}\)P magnetic resonance spectroscopy (\(^{31}\)P-MRS) was used to assess changes in intracellular pH and phosphocreatine (PCr). At the completion of exercise, forearm ICW increased 12.6% (SD 0.07, \(P = 0.003\)), TW increased 10.1% (SD 0.06, \(P = 0.005\)), and no significant changes were recorded for ECW. A significant correlation was found between the changes in intracellular pH and changes in ICW during exercise (\(r = -0.84, P = 0.018\)). With the use of regression analysis, average changes in PCr, and pH were found to predict changes in ICW (\(R^2 = 0.98, P = 0.005\)). In conclusion, MFBIA was sensitive enough to measure transient changes in the exercising forearm muscle. The changes seen were consistent with the hypothesis that intracellular acidification and PCr hydrolysis are important mediators of cellular osmolality and therefore may be responsible for the increased volume of water in the intracellular space that is often recorded after short-term high-intensity exercise.

intraacellular water; extracellular water; phosphorus-31 magnetic resonance spectroscopy; intracellular pH; phosphocreatine

CONSIDERING SEVERAL DISEASES are associated with altered fluid balance in the body (including human immunodeficiency virus, cancer, infections, septic shock, kidney failure, and multiple sclerosis), a quick and accurate quantification of changes in cellular hydration status may be an invaluable clinical and diagnostic tool. For this reason, bioelectrical impedance analysis (BIA) has become an attractive technique for studying cellular water because of its noninvasive nature, simplicity of use, and relatively low cost. BIA can provide estimates of total tissue water (TW), intracellular water (ICW), and extracellular water (ECW). Past studies have demonstrated good correlations between BIA-measured volumes of TW, ICW, and ECW and dilution-determined volumes (bromide and deuterium oxide dilution) (8, 15, 11). BIA-measured ICW has also been correlated with estimates of ICW made from measures of total body potassium (8, 27).

One application of BIA that has not yet been evaluated is the ability to examine exercise-induced changes in tissue water content in a specific muscle group. Previously, isotopic dilution methods have demonstrated a significant increase in ICW levels after exercise (25, 26). Although an accurate means of assessing ICW levels in the whole body, isotopic dilution techniques are unfortunately invasive and not ideally suited for routine clinical usage. MRI studies have also suggested exercise-induced increases in ICW levels [hypothesized from transverse relaxation time increases (23, 9)]. However, despite being noninvasive, MRI is an expensive procedure with limited availability in most areas.

BIA provides estimates of water content by utilizing the electrical properties of living tissue. When exposed to an alternating electrical current, the impedance of tissues is proportional to their fluid content. The polarized nature of cellular membranes permits cells to be modeled as capacitors (24). Depending on the frequency of the applied current, cell membrane impedances vary. With a low-frequency or direct current, there is no conduction through a capacitor, and thus conductivity through a tissue is governed primarily by ECW. Conversely, with high-frequency or alternating current, impedance decreases and current is allowed to flow throughout both the ECW and ICW compartments. In multiple-frequency BIA (MFBIA), complex impedance measurements (collected from 4 to 1,024 kHz) are fit to a biophysical model (29) providing estimates of TW, ECW, and ICW concentrations. Past studies have demonstrated good correlations between MFBIA estimates of TW and ICW compared with both bromide and deuterium oxide dilution-determined volumes (8, 15, 11). However, MFBIA has typically been used to quantify whole body water at rest (8, 17). To our knowledge there have been no MFBIA studies in the literature examining transient water levels in a specific body segment throughout exercise.

One likely explanation to account for an increase in ICW that occurs during exercise may be the increased tissue osmo-
lality because of the accumulation of hydrogen ions (H\(^+\)), inorganic phosphate (Pi), and lactate (32, 33). As these species accumulate within the cell, the increasing hyper-tonicity may result in a shift of water molecules to the intracellular space. Phosphorus magnetic resonance spectroscopy (\(^{31}\)P-MRS) can be used to directly or indirectly monitor these metabolites. Thus \(^{31}\)P-MRS can be used in combination with MFBIA to explore whether any relationships exist between the measurements of the two techniques.

The purpose of this study was, therefore, twofold: first, to determine whether MFBIA is sensitive enough to study the changes in ICW levels in human muscle during a progressive exercise protocol to volitional fatigue, and second, to compare any MFBIA-measured changes in ICW to changes in high-energy phosphates as measured by \(^{31}\)P-MRS. Specifically, we hypothesized 1) that MFBIA would be sensitive enough to measure small changes in ICW, 2) that increases in ICW would be correlated with changes in intracellular pH (pH \(= -\log[H^+]\)), and 3) that increases in ICW would also be correlated with changes in Pi and phosphocreatine (PCr) concentrations.

METHODS

Subjects. Seven healthy men [age 27 yr (SD 6), mass 82 kg (SD 9)] volunteered to participate in this study. The \(^{31}\)P-MRS protocol was approved by the University of Western Ontario Review Board for Research Involving Human Subjects and the Hamilton Health Sciences/McMaster University Research Ethics Board.

Experimental protocol. Subjects were studied on two separate occasions by an identical exercise protocol. On one occasion, MFBIA was used to collect ICW and ECW data. On the other occasion, \(^{31}\)P-MRS was used to collect muscle pH and PCr data. The order of MFBIA vs. \(^{31}\)P-MRS was randomized and separated by at least 2 days.

The exercise protocol was identical for both tests and consisted of progressive wrist flexion exercise to volitional fatigue performed on a custom-built wrist ergometer. Previous to the start of exercise, subjects lay supine on a table positioned next to the ergometer. The dominant arm of each subject was positioned inside the ergometer by placing the arm in full extension and then abducting to 90°. The forearm was placed in the pronated position so that subjects could grasp the lever of the ergometer, which was aligned such that the pivot of the lever centered on the axis of the wrist joint. With the arm in this position, the contracting forearm musculature was positioned at heart level, thus ensuring adequate perfusion during the relaxation phase of each contraction-relaxation cycle. The subjects remained supine throughout the protocol.

The exercise consisted of repeatedly depressing the lever at a frequency of 0.5 Hz (1-s contraction/1-s relaxation) through a range of motion of \(\sim 70^\circ\). This action raised and lowered a water reservoir through the use of a cable and pulley system. A metronome set at 0.5 Hz was used to keep subjects on pace.

After a 3-min period during which resting measurements were taken, subjects accommodated to the exercise protocol with 3 min of wrist flexion against low resistance (1.1 kg). After this initial accommodation period, subjects continued to exercise while resistance was increased in a ramplike fashion by pumping water into the reservoir, via a roller pump (Cole-Parmer Instruments, Chicago, IL), at constant rate of 0.25 l/min. Subjects exercised to volitional fatigue. The work done by the subject was calculated by using the known repetition rate (0.5 Hz), the arc distance of the lever (0.10 m), and the weight of the reservoir and water [1.1 kg + (flow rate \times exercise time)] using standard physical relationships. This produced a ramp slope of 0.12 W/min from an initial load of 0.53 W (accommodation period). The actual flow rate of water into the reservoir was calculated as the total volume of water added during the exercise test divided by the time to fatigue. After the completion of exercise, subjects rested supine with the arm positioned in the ergometer while recovery measurements were taken for 20 min.

MFBIA. MFBIA measurements of the forearm were performed using a SEAC model SF3 (Uniquest, St. Lucia, Queensl and, Australia). Electrodes were placed in a tetrapolar arrangement with one current driving electrode (3M Red Dot Ag/Au) placed on the dorsal aspect of the hand and a second current electrode placed on the dorsal aspect of the forearm \(\sim 2\) cm from the elbow. Voltage sensing electrodes were placed between the current electrodes, \(\sim 4\) cm away from the current electrodes to help eliminate surface conduction effects. Measurements were taken at multiple frequencies logarithmically spaced between 4 and 1,024 kHz (~300 data points), and the impedances measured were fit to a Cole-Cole plot (8). The values for the resistance at zero and infinite frequencies were calculated from the fit and used to estimate the ICW, ECW, and TW (8). With this arrangement, MFBIA data were collected every 72 s.

\(^{31}\)P-MRS. \(^{31}\)P-MRS data were acquired on a 1.89-T custom-built magnetic resonance system with a 4-cm-diameter surface coil situated on the ventral aspect of the forearm, 10 cm distal to the medial epicondyle of the humerus. A 3-ms adiabatic 90° pulse was used in acquiring spectra, with a 12-μs delay time, spectral width of 4,000 Hz and 2,048 complex data points. Spectra consisted of six averages over 36 s (TR = 6 s), collected sequentially throughout rest, exercise, and recovery.

Data analysis. MFBIA measurements were normalized by expressing muscle water content as a percentage of resting values. ICW changes during exercise were compared with changes in pH, PCr, and Pi by use of Pearson correlation coefficients. Stepwise multiple linear regression was used to determine the extent to which Pi, pH, and PCr changes could predict the percent changes in ICW.

\(^{31}\)P-MRS data were fit in the time domain by the Levenberg-Marquardt algorithm (18) with a priori knowledge (4) to determine the relative concentrations of phosphorus containing metabolites (ATP, PCr, and Pi). Changes in PCr and Pi were normalized to β-ATP. Intracellular pH was determined from the chemical shift of Pi with respect to PCr (28).

The phosphorylation potential, which can be estimated in vivo as the ratio P/PCr, was examined in this study as the logarithm (log P/PCr) to facilitate detection of a threshold in P/PCr (19). Piecewise linear regression analysis was applied to plots of log P/PCr, pH, and ICW vs. power output for determination of this threshold. During recovery, Pi was normalized by the sum of Pi + PCr (28) and compared with changes in water content.

To test the hypothesis that changes in TW, ICW, and ECW were occurring throughout exercise and recovery, these data were analyzed by a one-way ANOVA with repeated measures. All data are presented as means (SD). Significance was set at P < 0.05.

RESULTS

Comparison of power outputs. Peak power outputs from each subject during both exercise bouts did not differ significantly from one another [average peak power output MFBIA = 1.75 W (SD 0.08), average peak power output \(^{31}\)P-MRS 1.69 W (SD 0.28) (P = 0.32)]. Additionally, individual subject power outputs were found to be similar in each of the exercise bouts (MFBIA vs. \(^{31}\)P-MRS, r = 0.86, P = 0.01). For MFBIA, exercise lasted 9.94 min (SD 1.50). For \(^{31}\)P-MRS, exercise lasted 10.29 min (SD 2.39). Exercise times did not differ significantly between the two bouts (P = 0.457). As well, individual subject exercise times from the two exercise bouts were found to be significantly correlated to one another (MFBIA vs. \(^{31}\)P-MRS, r = 0.81, P = 0.03).
Changes in ICW and ECW in the forearm during exercise are shown in Fig. 1. For ICW, results of a one-way ANOVA revealed a main effect for percentage of peak power output ($P = 0.003$). Likewise, a main effect for TW was also observed during exercise ($P = 0.01$). In both cases, this indicated that water content increased as exercise progressed. In contrast, analysis of ECW during exercise revealed no significant changes as power output increased ($P = 0.252$). At end exercise, forearm ICW was found to have increased by 12.6% (SD 0.07, $P = 0.003$). TW increased by 10.1% (SD 0.06, $P = 0.005$) whereas no significant change was found in ECW.

During recovery, ICW was found to decrease toward baseline values. A significant effect was found for time ($P = 0.00001$). Mean changes in ICW during recovery [compared with pH and P/(P1+PCr)] are shown in Fig. 4. Also, a significant time effect was found for measurement of TW during recovery ($P = 0.03$). As was the case during exercise, no significant changes were detected in ECW during recovery ($P = 0.251$). At the end of exercise, the fraction of total forearm muscle water attributed to ICW was found to be 0.85 (SD 0.03), whereas the ECW fraction was 0.15 (SD 0.03).

Comparison of MFBIA and $^{31}$P-MRS measurements during exercise. A comparison of changes in ICW and changes in pH throughout exercise is shown for a representative subject and group averages in Fig. 2. Pearson correlation coefficients were used to examine the relationships between changes in P/β-ATP ($\Delta P$), PCr/β-ATP ($\Delta PCr$), log(Pi/PCr) [$\Delta log(Pi/PCr)$], pH ($\Delta pH$), and percent changes in ICW ($\% \Delta ICW$). The $\Delta$ represents end-exercise values minus end-warm-up values. The only significant correlation found was between $\Delta PH$ and $\% \Delta ICW$ ($r = -0.84, P = 0.018$; Fig. 3). The correlations of $\Delta P$, $\Delta PCr$, and $\Delta log(Pi/PCr)$ with $\% \Delta ICW$ were $r = 0.52 (P = 0.225), r = -0.62 (P = 0.138),$ and $r = 0.59 (P = 0.166),$ respectively.

Stepwise linear regression was used to determine which exercise-induced changes in metabolites could predict the observed changes in ICW. This analysis indicated that $\Delta PH$, $\Delta P$, and $\Delta PCr$ all significantly predicted $\% \Delta ICW$ changes ($P = 0.005$) according to the equation:

$$\% \Delta ICW = 100 \times \left[ (-0.53 \pm 0.09) \times \Delta PH \right] - \left( 5.53 \times 10^{-2} \pm 0.01 \right) \times \Delta PCr - \left( (5.21 \times 10^{-2} \pm 0.01) \times \Delta Pi \right) - (0.17 \pm 0.03), \ R^2 = 0.98$$

Piecewise linear regression analysis revealed significant breakpoints for individual plots of pH and log(Pi/PCr) vs. power output. The average pH threshold occurred at 1.13 W (SD

Fig. 1. Percent changes in forearm water during exercise. Average changes in intracellular (ICW) and extracellular water (ECW) in the forearm during wrist flexion exercise. Although ICW significantly increased throughout exercise, ECW was found to remain close to baseline values. Multiple-frequency bioimpedance analysis (MFBIA) measurements are normalized as a percentage of resting values. Data are plotted as a percentage of maximum power output. Error bars indicate $\pm$ SD.

MFBIA measurement of tissue water during exercise and recovery. Changes in ICW and ECW in the forearm during exercise are shown in Fig. 1. For ICW, results of a one-way ANOVA revealed a main effect for percentage of peak power output ($P = 0.003$). Likewise, a main effect for TW was also observed during exercise ($P = 0.01$). In both cases, this indicated that water content increased as exercise progressed. In contrast, analysis of ECW during exercise revealed no significant changes as power output increased ($P = 0.252$). At end exercise, forearm ICW was found to have increased by 12.6% (SD 0.07, $P = 0.003$). TW increased by 10.1% (SD 0.06, $P = 0.005$) whereas no significant change was found in ECW.

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Piecewise linear regression analysis revealed significant breakpoints for individual plots of pH and log(Pi/PCr) vs. power output. The average pH threshold occurred at 1.13 W (SD
whereas the average log(Pi/PCr) threshold occurred at 1.09 W (SD 0.44). No thresholds were detected for individual plots of ICW vs. power output.

**Comparison of MFBIA and 31P-MRS measurements during recovery.** Figure 4A illustrates the changes in pH and ICW during recovery. After the cessation of exercise, mean ICW began to decrease toward baseline values whereas pH continued to decrease for ~2 min before recovering toward baseline values. In contrast, Fig. 4B shows that mean changes in P/(P_i+PCr) more closely approximated mean changes in ICW, as recovery toward baseline occurred immediately after exercise ended.

**DISCUSSION**

In this study we examined the exercise-induced increases in tissue water in the forearm and the metabolic factors that may contribute to these changes. We found that, using MFBIA, it is possible to observe changes in tissue electrical resistance, consistent with alterations in tissue hydration state, throughout a ramped wrist flexion exercise. Furthermore, by comparing MFBIA and 31P-MRS measurements made during exercise, it appears that increases in ICW content are driven to a great extent by decreases in pH, increases in P_i, and the depletion of PCr.

Ideally, the two measurement techniques utilized in this study would have been performed simultaneously during a single bout of exercise. However, because of the technical difficulties of performing MFBIA inside the bore of a magnet, subjects had to perform the identical exercise on two separate occasions. To reduce the likelihood of a training effect, subjects had performed the exercise protocol used in this study at least two times before actual data collection. This was reflected by the significant correlations in subject peak power outputs between both exercise bouts. Furthermore, no significant differences were found between peak power outputs among the two bouts. Thus subjects exerted themselves to a similar extent regardless of the measurements being made. The interindividual metabolic response to exercise was also found to be comparable between repeated trials as evidenced by similar end exercise pH values. In contrast, ICW volumes are dynamic and change from day to day [dependent on factors such as hydration, ion status, posture, and fluid distribution (29)]; thus absolute values were not used for MFBIA. Data were normalized as a percentage of resting values, thereby accounting for subject variation and making comparisons possible.

Because of motion-related errors with MFBIA, electrodes could only be placed dorsally on the forearm. Thus this was in contrast to 31P-MRS, whereby the surface coil was positioned under the ventral aspect of the forearm. However, MFBIA measures impedance of the entire volume between electrodes. Therefore, even though electrodes were placed over inactive dorsal forearm muscles during exercise, active ventral muscles predominantly contributed to the differences in measured impedances.

MFBIA measurements indicate a 12.6% increase in ICW at end exercise; an amount in accord with recent work by Damon et al. (7), who reported an 11.9% increase in intracellular volume of excised frog muscle after exercise. These values are similar but slightly higher than previous studies using radiotracers that reported ICW increases of ~10% (14, 25, 26). However, these studies examined ICW changes in leg muscles that are generally comprised of more oxidative muscle fibers than what is found in the forearm, which is comprised of ~47% type I fibers (12). Metabolic accumulation of osmolites would presumably be greater within the exercising forearm muscles, thus eliciting a greater uptake of water into the intracellular compartment.

A possible source of error in MFBIA measurements is the implicit assumption that resistivity of the conductor remains constant. In tissue, the resistivity is largely determined by ion concentration and generally decreases with increasing ion concentration (22). Thus it has been theorized that the increases in ion concentration in the intracellular space during exercise may account for a slight underestimation of ICW. Although it has not been studied in detail, the magnitude of this effect may not be significant (13). Our results support this speculation, because MFBIA measure of ICW fraction at end exercise (ICW fraction of total forearm water = 0.842) was in close agreement to past studies that employed other means of measurement (7, 6, 14, 26).

Although not considered a “gold standard” for measuring tissue water, past studies have characterized the accuracy of bioimpedance analysis. Good correlations have been found between bioimpedance-measured volumes of TW, ICW, and ECW compared with dilution-determined volumes (as measured by both bromide and deuterium oxide dilution) (8, 15, 11). In addition, bioimpedance-measured ICW has also been correlated with estimates of ICW made from measures of total body potassium (8, 27). One criticism of bioimpedance has
been that most of the studies that examine whole body water have employed a wrist-to-ankle electrode placement to gain a measure of whole-body impedance (29). Because biopendence estimates water content by modeling the body as a cylinder, it has been argued that a segmental approach is more valid. By separately measuring arm, trunk, and leg impedances, estimation of body water would theoretically be improved (29, 20). An advantage of the present study is that we examined changes in the forearm, a body segment that closely resembles a cylinder, thus presumably improving estimations of water content. Given the significant effects found during both exercise and recovery, our results indicate that MFBI A is sensitive enough to document changes in tissue resistivity that are consistent with alterations in ICW throughout an exercise protocol. Furthermore, because of its ease of use, noninvasive nature, and portability, it provides a suitable alternative to more invasive methods.

During exercise and recovery, ECW content of the forearm was not found to significantly change. Considering that the ECW volume is much less than the ICW volume, small changes in ECW are therefore more difficult to detect. It is possible that MFBI A is not sensitive enough to detect the individual changes in ECW within the forearm during exercise. However, this raises another question: if significant increases in ICW are found with no corresponding decrease in ECW during exercise, where does the water come from? It is likely that there was an uptake of fluid into the intracellular compartment from the vasculature. It has been shown that fluid transfer does not occur from inactive to active muscle (26) and metabolic production of water from muscle is negligible with short-term exercise (16). Despite the fact that significance was not reached, it was interesting to note that our results indicated that ECW appeared to reach a maximum volume (~8–9% increase from resting) ~8–12 min after the cessation of exercise. This is suggestive of exercise hyperemia, where local blood vessels were no longer compressed by contracting muscles. Thus fluid was slowly drawn into the extracellular space.

The effects of exercise-induced changes in blood flow on MFBI A measurements are not well known. Theoretically, exercise may affect MFBI A measurements by increased blood flow and vascular perfusion in skeletal muscle, possibly resulting in a decrease in impedance and muscle resistivity. However, because the intravascular volume itself accounts for only a small proportion of total tissue fluid volume, it may not contribute much to MFBI A measurements. A study by Cieslar et al. (6) showed that plasma volume accounts for only 1.8% of in vivo rat muscle tissue. Thus changes occurring in plasma volume and flow during exercise would likely only have a minimal affect on gross changes in ICW and ECW compartments. Although beyond the scope of this study, the effects of blood flow on MFBI A are uncertain and thus warrant further study.

The second major finding of this study was the relationship among intracellular acidification, PCr breakdown, and ICW increases during exercise. The accumulation of exercise by-products acts to draw water into the intracellular space, effectively diluting intracellular concentrations to maintain resting conditions. It has recently been demonstrated that treatment of amphibian muscle with NaN (to promote lactate and proton production) caused significantly larger exercise-induced percentage increases in intracellular volume than amphibian mus-
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