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Non-invasive Monitoring of Lactate Dynamics in Human Forearm Muscle After Exhaustive Exercise by ^1H -MRS at 7T

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Abstract

Despite its importance in energy metabolism, lactate in human skeletal muscle has been difficult to detect by non-invasive ^1H MRS mainly due to interference from large water and lipid signals. Long echo-time (TE) acquisitions at 7 Tesla effectively attenuates the water and lipid signals in forearm muscle allowing direct observation of both lactate resonances, the methine at 4.09 ppm and the methyl at 1.31 ppm. Using this approach, we are able to monitor lactate dynamics at a temporal resolution of 32 sec. While lactate was not detectable at rest, immediately after an acute period of exercise to fatigue the forearm muscle, lactate rose to a level comparable to that of creatine (~30 mmol/kg wet weight). In a typical ^1H MR spectrum collected using a TE of 140 ms, the lactate methine and methyl resonances both appear as doublets with an unusually large splitting of ~20 Hz due to residual dipolar coupling. During muscle recovery following exercise, the lactate signals decay rapidly with a time constant of $t_{1/2} = 2.0 \pm 0.6$ min ($n = 12$ subjects). This fast and simple lactate detection method may prove valuable for monitoring lactate metabolism in cancer and in sports medicine applications.

Keywords

Skeletal muscle; exercise; lactate; metabolism; MRS

INTRODUCTION

Lactate has long been a focus of research in skeletal muscle metabolism and also a central topic of controversy concerning the relationships among lactate production, acidosis, and

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fatigue (1-6). Once thought to be a waste product, lactate is increasingly considered to be a source of fuel, continuously produced and metabolized in the body to maintain energy homeostasis (6). In skeletal muscle, the lactate concentration can fluctuate dramatically, ranging from ~ 1 mmol/kg ww at resting state to ~ 30 mmol/kg ww at exhaustion (7-13). During exercise, lactate exported into blood from active muscle can be taken up and metabolized by heart, brain, liver and inactive muscle (10,14-15). Persistent high lactate levels, other than from endurance exercise, may be a sign of tissue hypoxia or mitochondrial dysfunction (16-21) while failure to elevate lactate after strenuous exercise could signify a glycolytic defect such as that seen in McArdle's disease (22,23).

The functional importance of lactate, either as a preferred source of energy over glucose or as an end-product of anaerobic metabolism, arises from its strategic location in the overall metabolic network (6). Conversion of pyruvate to lactate or oxidation of pyruvate in the citric acid cycle is controlled by multiple factors including cytosolic and mitochondrial redox state, the phosphorylation state of pyruvate dehydrogenase, and the mitochondrial acetyl-CoA/CoASH ratio. If one could easily monitor dynamic changes in lactate and acetylcarnitine after exercise, ^1H MRS at high field could become a powerful tool to explore the relationships of these complex metabolic processes. Lactate has been difficult to monitor by ^1H MRS *in vivo* (24-33). In addition to the low levels of lactate in normal tissues, this problem is primarily due to the fact that the two resonances of lactate, the methyl resonance at 1.3 ppm and the methine resonance at 4.1 ppm, are typically hidden by the much more intense signals of lipid and water, respectively (34, 35). So far, the vast majority of methods for detection of lactate *in vivo* have been based on spectral editing, various quantum filtering and/or polarization transfer techniques for selective observation of lactate signals while filtering out unwanted water and lipid signals, often together with potentially useful metabolite signals (29). However, these methods are complicated, theoretically and technically, and often involve presumptions of the precise values of the chemical shifts and the H-H coupling for setting sequence parameters. In the case of skeletal muscle, such approach may lack general applicability, since the H-H coupling of lactate depends also on orientation of muscle fibers (32,33,36), which is subject to change among different muscle groups and individuals. Therefore, an alternative approach is needed not only for methodological generality and simplicity, but also for capturing information from all possible metabolites that coexist with lactate, so that a more integrated picture of lactate metabolism can be obtained.

In this study, we monitored the lactate ^1H MR signals from both methine and methyl protons in human forearm muscle *in vivo* using a basic STEAM sequence with a long TE (140 ms) without water or lipid suppression. We demonstrate that it is feasible to monitor in real-time lactate disappearance from muscle following a short duration of strenuous exercise while also observing the signals from creatine, acetyl-carnitine, intramyocellular lipids (IMCL) simultaneously. It is expected that the method may find promising application for diagnosis of performance or metabolic disorders and for understanding the transition from anaerobic to aerobic metabolism.

MATERIALS AND METHODS

Human Subjects

The protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. Prior to the MRS study, informed written consent was obtained from all participants. All subjects ($n = 12$) were normal healthy males with no history of peripheral vascular, systemic or myopathic diseases. Other details are summarized in Table 1. To avoid possible exercise-associated physiological variations among subjects, all subjects were asked to refrain from any physical activity of moderate or high intensity prior to the study, and subjects sat at rest for 20 minutes prior to the scan. Heart rate and blood oxygen saturation level were monitored during the scan. All subjects were interviewed again 24 hours after the NMR exam. The exercise study, including pre-scan, intermittent hand squeeze until volitional fatigue, and post-scan (total duration of 30 min or less), was tolerated by all subjects, with only reports of slight discomfort in the shoulder of the exercised arm at the end of the scanning session. One subject reported dizziness after exit from the scanner but was stable after 10 min of rest. There were no reports of headaches, vertigo or related neurological complaints 24 hours after the MR exam.

Forearm Exercise Protocol

Forearm exercise was conducted in the scanner by instructing each subject to repeatedly squeeze a rubber ball (ϕ 68mm, moderate hardness) until they experienced forearm muscle fatigue. Each squeeze was 2 sec long in duration, holding tight for 1 sec, followed by relaxation for 1 sec. During the exercise, the subjects were instructed to initiate each squeeze at the “clicking” sound generated by gradient turn-on, which repeated every 2 sec. For this study, no data were used during exercise (dummy scans) because of motion artifacts. Only data acquired during pre- and post-exercise time periods, with the hand and forearm remaining in the same position, were used for analysis.

Spectroscopy

All subjects were positioned head-first and prone in the MRI scanner (7T Achieva, Philips Medical Systems, Cleveland, OH), with the forearm positioned parallel to the magnetic field on top of the detection coil (Philips Medical Systems, Cleveland, OH). The forearm was secured with a Velcro strap (~ 5 cm wide) and a foam pad to minimize motion during the experiment. The coil, used for both transmitting and receiving in quadrature mode, consisted of two tilted, partially overlapping 10 cm loops. The center of the coil was positioned approximately $1/3$ of the distance along the forearm away from the elbow toward the wrist. Axial, coronal, and sagittal T2-weighted turbo spin echo images were acquired for voxel planning. Typical parameters were: field-of-view (FOV) 140×140 mm, time of repetition (TR) 2 s, echo time (TE) 75 ms, turbo factor 16, in-plane spatial resolution 0.6×0.7 mm², slice thickness 4 mm, gap 2 mm, bandwidth 178 Hz, number of acquisitions (NA) one, and acquisition time 1.2 min. The ¹H MR spectra were collected from a voxel centered at flexor digitorum profundus (FDP) muscle, with a typical volume of 5.8 ± 2.1 mL (anterior-posterior, 9 - 18 mm; right-to-left, 10 - 20 mm; foot-to-head, 20 - 30 mm), using a STEAM sequence. The 2nd order iterative volume-of-interest automatic shimming was applied prior to the base-line spectral acquisition, and no new shimming was carried out in the post-

exercise dynamic scans. All spectra collected during the exercise period were not used in this study. The spectral acquisition parameters were TR 2 s, TE 140 ms, spectral bandwidth 8 kHz, 16-step phase cycling, 8192 points, NA = 16. The data acquisition time for each scan was 32 s. After Fourier transformation and phasing, the ^1H MR spectra were fitted using ACD software (Advanced Chemistry Development, Inc., Toronto, Canada) as reported previously (34). The intensity of lactate signals was used to evaluate lactate decay half-time constant $t_{1/2}$, assuming a mono-exponential process. The ^1H chemical shifts of all metabolite resonances in the muscle were referenced to creatine $-\text{CH}_3$ (Cr3) at 3.02 ppm.

Variable-TE spectra were acquired using TE 100, 140, 180, 220 and 260 ms, at a constant TR of 2 s and NA of 16 on three different subjects ($n = 3$ with 2 male and 1 female). The T_2 of the creatine methyl group under resting conditions as evaluated from signal intensity vs TE data assuming mono-exponential decay. Lactate methine T_2 value was evaluated from spectra acquired at TE 100 and 140 ms after exercise, with correction for the concentration decay. T_1 values were measured ($n = 3$ with 2 male and 1 female) using an inversion recovery sequence with TE = 140 ms, TR = 4.0 s, NA = 8, inversion bandwidth of 3 kHz, and varying inversion delay time (TI) from 35 to 2800 ms (7 data points). As reported previously (34), the intensity of the lactate signals was fitted by an exponential recovery process, which took into account of partial saturation of signals and the change of lactate concentration during the post-exercise period using the known disappearance rate of lactate from muscle.

The effect of forearm orientation on the lactate signal was studied in one subject at two different forearm angles relative to the static B_0 magnetic field: 0° (parallel to B_0) and 20° . Intrasubject reproducibility study was conducted on two subjects in two different sessions, separated by at least two weeks, by comparing the variance in signal intensity, splitting, and decay half-time constant of lactate methine resonance using the same exercise routine. Intra-session reproducibility test was performed on lactate signals splitting pattern at TE 140 and 100 ms. Four scans were repeated with alternated orders (TE 140 ms $\rightarrow$ TE 100 ms, and then TE 100 ms $\rightarrow$ TE 140 ms) under otherwise the same acquisition and shimming condition, from a small voxel located within flexor digitorum profundus (FDP) muscle.

RESULTS

Detection of Lactate Signals in Forearm Muscle

Lactate was not detectable with this approach in resting forearm muscle. After acute fatiguing exercise, lactate appears as two doublets (Fig. 1). The downfield doublet, assigned to the lactate methine proton ($-\text{CH}-$), resonates at 4.09 ppm. With a flat spectral baseline at TE 140 ms, the methine doublet is clearly resolved from the neighboring water signal at 4.67 ppm (~200-fold larger) and creatine methylene $-\text{CH}_2-$ signal at 3.93 ppm (Fig. 1b and 1e). The other doublet, assigned to lactate methyl ($-\text{CH}_3$) protons, appears at 1.31 ppm ($\delta = 3.78$ ppm between methine and methyl resonance). This latter signal falls in the narrow band between the signals of intra- and extramyocellular lipids (IMCL and EMCL). Though partially overlapped by IMCL signal at 1.27 ppm, the lactate methyl doublet is fully-resolved in the difference spectrum (subtracting pre- from post-exercise spectra) as shown in Fig. 1f.

The lactate methine and methyl doublets showed an unusual splitting, $\nu = 20$ Hz, in comparison to the expected $^3J_{\text{HH}}$ coupling constant (7 Hz), indicating a possible contribution from residual dipolar coupling (RDC) (36). The methyl doublet at 1.31 ppm is somewhat broader than the methine doublet at 4.09 ppm (methine 10.1 ± 0.6 Hz vs methyl 12.4 ± 0.2 Hz) while the ratio of the two signal intensities measured $\sim 1 : 3.4$, close to that expected.

In addition to the appearance of the lactate signals, the post-exercise ^1H MR spectrum also reveals concomitant change in the intensity of water and acetyl-carnitine signals (Fig. 1). Specifically, the intensity of water signal at 4.67 ppm and acetyl-carnitine acetyl signal at 2.12 ppm is increased by 2.7-fold and 3.1-fold, respectively, while their linewidths remained nearly the same as observed in pre-exercise spectra.

Effect of Exercise and Recovery on the Lactate Signal

As summarized in Fig. 2a, the peak intensity of the lactate methine signal in each post-exercise ^1H MR spectrum (relative to the creatine methyl signal at 3.02 ppm in the pre-exercise spectrum) ranged from 0.12 to 0.56 among individuals and averaged 0.25 ± 0.15 ($n = 12$). Correcting this ratio by a factor of 3 to convert this number to methyl equivalents, the lactate content in post-exercise forearm muscle can be estimated at roughly 22 mmol/kg wet weight (assuming total creatine, the sum of Cr and PCr, = 30 mmol/kg wet weight (37)). However, this value likely underestimates the actual lactate concentration because of differences in the relaxation times between lactate and the methyl creatine resonance (measurements discussed in more detail below).

After correction for relaxation time differences, for this group of subjects ($n = 12$, male 6 and female 6), the peak lactate measured in male subjects (42.2 ± 22.7 mmol/kg ww) tended to be higher than that measured in female subjects (22.9 ± 5.0 mmol/kg ww; $p = 0.07$). For the exercise protocol employed, it took longer for the male subjects to become fatigued (2.6 ± 0.9 min) in comparison to the female subjects (1.7 ± 0.4 min). This difference was of borderline statistical significance ($p = 0.06$). However, there was no difference in rate of lactate decay from muscle between the males and females (2.0 ± 0.7 min vs 1.9 ± 0.4 min, $p = 0.71$). Furthermore, neither age nor BMI was a significant determinant of the lactate concentration or the rate of lactate clearance.

Following exercise, the activated muscle begins to relax and the lactate ^1H MR signals decay rather rapidly, with an average half-time constant $t_{1/2}$ of 2.0 ± 0.6 min ($n = 12$), as evaluated from the methine signal using mono-exponential model. Among different individuals, the $t_{1/2}$ values ranges from 1.0 to 2.8 min (Fig. 2b), indicating the variation in muscle response to the exercise.

As anticipated, the dynamic spectra show that lactate methine and methyl signals decay synchronously (Figs. 3 and 4). For example, in Figure 3 the dynamics of both methine and methyl doublets are clearly illustrated since the small IMCL signal at 1.27 ppm has minimal interference with the lactate methyl signal. Generally, however, the IMCL signal is large and often overlaps the upfield lactate methyl doublet (Fig. 4b). In those cases, a difference spectrum is necessary to differentiate the lactate methyl and IMCL resonances (Fig. 4c), but essentially the same information is obtained. In contrast to the methyl resonance, the

methine was always well-resolved (Figs. 3 and 4) and therefore more suitable for quantitative analysis. The appearance of the lactate methine signal is a marker for the existence of lactate methyl resonance in the lipid region.

Effect of Forearm Orientation on Splitting of the Lactate Signal

Fig. 5a summarizes the variation in lactate methine doublet splitting (ν) among the different subjects. The ν values ranged from a low of 16 Hz to a high of 23 Hz, averaging 19.6 ± 2.3 ($n = 12$). This inter-subject variation may reflect minor difference in muscle fiber orientation relative to B_0 field. A linear correlation was observed between the lactate methine splitting ν and the creatine methylene doublet at 3.93 ppm (Fig. 5b), suggesting a common factor in the splitting of both resonances. Interestingly, the ν value is consistently larger for lactate than for creatine (Fig. 5b). Indeed, when the lactate splitting was less than 18 Hz (4 of 12 cases), the creatine methylene signal appeared as a singlet.

To confirm the relationship between ν and fiber orientation, ^1H MR spectra were collected at two different forearm orientations, 0° (parallel to B_0) and 20° , respectively, from the same subject, using the same coil, and under identical acquisition conditions. As illustrated in Figs. 5c and 5d, the lactate splitting was larger with the muscle positioned parallel to B_0 (0°) compared to when the muscle was 20° off axis. In comparison, the splitting in the creatine methylene resonance was too small to be observed at 20° , but well-resolved at 0° (parallel to B_0).

T_1 and T_2 Measurements

Despite the rapid loss of lactate from muscle during rest after exercise, the T_1 values of both the methine and methyl resonances were measured using an inversion recovery sequence with a TR of 4 s, TE of 140 ms, NA = 8 for a total experimental time of 3.8 min (Fig. 6). The intensity of lactate detected at each delay time was reduced by the known rate loss in lactate concentration due to either metabolism or release from the tissue (see Fig. 3b), and this effect was taken into account for T_1 evaluation (Fig. 6b). A small difference in the rate of signal recovery between the two lactate signals was observed, with recovery of the methine resonance lagging slightly behind recovery of the methyl resonance (Fig. 6a). An analysis of data such as that shown in Fig. 6a yielded a $T_1 = 1.7 \pm 0.2$ s for methine signal (Fig. 6b) and a $T_1 = 1.2 \pm 0.1$ s for the methyl signal (data not shown). Using the same inversion recovery approach, a $T_1 = 1.9 \pm 0.2$ s was measured for the creatine methyl resonance in resting forearm muscle ($n = 3$).

In an attempt to measure T_2 for the lactate resonances as well, several spectra were collected using variable echo delay times (TE from 100 to 260 ms, without water suppression). Spectra collected using either TE = 100 or 140 ms show reasonably good S/N for lactate (Fig. 7) while those collected with longer TE times resulted in poor signal to noise. Therefore, the intensity data obtained at TE 100 and 140 ms were employed to estimate a $T_2 = 109 \pm 10$ ms ($n = 3$) for lactate methine resonance (with correction for the decrease in lactate due to post-exercise decay). Unfortunately, the lactate methyl resonance was not well enough resolved from the much larger IMCL signal at these shorter TE values to allow a reliable estimate of its T_2 value. However, a $T_2 = 167 \pm 12$ ms was obtained for creatine

methyl resonance at 3.02 ppm in resting muscle, which is comparable to the T_2 value of 162.5 ± 4.3 ms reported for the creatine methyl resonance in resting gastrocnemius muscle at 3T (38).

In reference to the total creatine methyl signal at 3.02 ppm (30 mmol/kg ww), the lactate concentration in post-exercise forearm muscle, after correction for the differences in T_1 and T_2 between lactate methine and creatine methyl resonances, was 32.5 ± 18.6 mmol/kg ww ($n = 12$), as compared to 22.5 ± 13.5 mmol/kg ww ($n = 12$) without relaxation time correction.

Reproducibility

The signal splitting for both lactate and creatine was less at TE 100 ms than at TE 140 ms (Fig. 7). To confirm the observation and exclude the possibility of random artifact from motion and heterogeneity, an intra-session reproducibility study was conducted, with data acquired using alternated orders (TE 140 ms $\rightarrow$ TE 100 ms, and then TE 100 ms $\rightarrow$ TE 140 ms) from a small voxel within FDP muscle. As shown in Fig. 8a, regardless of the timing of the acquisition, the spectra at TE 100 ms, as compared to those at TE 140 ms, consistently produced a narrower splitting for both lactate and creatine resonances.

Inter-session lactate reproducibility was also tested under the condition TE 140 ms with the same exercise routine. As demonstrated in Fig. 8b, highly reproducible lactate signals were obtained from the forearm muscle of the same subject, immediately following exercise, at two different sessions 9-week apart. Quantitative data analysis indicates that the inter-session variance is within 11 % for the key parameter evaluated, including the magnitude and splitting of lactate methine signal, and the half-time constant of lactate decay after exercise.

DISCUSSION

Compared to the various spectral editing approaches to measure lactate in tissues reported over the last two decades (24-31), the long-echo approach described here is simple and the observed spectra are easy to analyze. The two new signals detected in skeletal muscle after a short period of exercise were assigned to lactate based on the following evidence.

First, the chemical shifts of the two resonances were identical to the widely accepted shifts of the lactate methine (4.09 ppm) and methyl (1.31 ppm) resonances, respectively. *Second*, the ratio of the two signal intensities (1:3.4) was within that expectation for a methine-to-methyl proton ratio. *Third*, the splitting pattern of the two resonances signals were similar to each other (a doublet with an inter-peak splitting ν of 19.6 ± 2.3 Hz) and consistent with earlier observations in isolated frog muscle (32,33) and in postmortem mouse leg muscle (18). *Fourth*, both signals decayed post-exercise with an identical $t_{1/2}$ of 2.0 ± 0.6 min, similar to the rate of decay measured in double-quantum studies of human gastrocnemius muscle (25) and in biopsy studies of human quadriceps femoris muscle (7,39). *Fifth*, the lactate signal intensity (after corrections for T_1 and T_2 differences) reflect a lactate concentration of 32.5 ± 18.6 mmol/kg ww ($n = 12$) in fatigued forearm muscle in agreement with needle biopsy results for vastus lateralis muscle (immediately after fatigue 36.2

mmol/kg ww, after 3 min of recovery 18.8 mmol/kg ww) (7), and for quadriceps femoris muscle at fatigue (100.0 ± 5.5 mmol/kg dry weight or 25.0 ± 1.4 mmol/kg wet weight, at 100% VO_2 max) (12). It also agrees with the lactate concentration of 25.3 - 27.2 mM measured for fatigued forearm muscle using lactate-selective spectral editing method (40, 41). Finally, the rate of lactate clearance from forearm muscle was 16.3 ± 13.3 mmol/min/kg ww as estimated from lactate decay half-time constant $t_{1/2}$ of 2.0 ± 0.6 min and initial concentration of 32.5 ± 18.6 mmol/kg ww ($n = 12$). In comparison, a peak lactate efflux rate of 20 mmol/min/L and 4 mmol/min/L was reported by Saltin et al (7) and Jorfeldt et al (42), respectively, based upon muscle biopsy results.

Measurement of the lactate concentration requires knowledge of the T_1 and T_2 relaxation times which is difficult to assess for fast-decaying lactate signals ($t_{1/2} = 2.0 \pm 0.6$ min). In this study, the reason we chose TE 140 ms for T_1 measurements, instead of a shorter TE, is that well-resolved and well-defined signals could be obtained for both lactate and creatine, thus permitting easy and reliable spectral fitting. On the other hand, a longer TE was not favored because of the low sensitivity. It should be noted that the use of limited data points and the presence of residual dipolar coupling may affect the accuracy of relaxation time measurements.

Unanswered questions remain regarding the large lactate splitting ($\nu \sim 20$ Hz) and the splitting pattern of methine resonance (doublet, rather than 1:3:3:1 quartet, as expected from conventional $^3J_{\text{HH}}$ coupling ~ 7 Hz). In a highly-oriented environment such as skeletal muscle, the residual dipolar coupling (RDC) is known to play a major role in the signal splitting of metabolites such as creatine and lactate (32,33,36,38,43), likely due to the restricted tumbling motion of metabolite in the narrow space of oriented myofibrils (41). In a simplified form, the magnitude of RDC is determined by

$$RDC = sD_o \langle 3\cos^2\vartheta - 1 \rangle / r^3 \quad (1)$$

where r is the distance between the proton pairs interacting *via* through-space dipolar interactions; ϑ is the angle between r and the external magnetic field B_o ; D_o represents a measure of the strength of interaction between molecule and lattice, and s represents the order of the lattice. All the geometric parameters are time-averaged. This RDC model offers a reasonable explanation for the orientation dependence observed for the splitting of lactate and creatine (Fig. 5) but it is not clear whether and how much the conventional $^3J_{\text{HH}}$ coupling might contribute to the signal splitting observed here. If the lactate signal splitting is solely dipolar in nature, then the larger RDC effect observed for the lactate dipolar pair – CH-CH_3 ($r = 2.7$ Å) than for the creatine methylene dipolar pair – CH_2 ($r = 1.2$ Å) would suggest that the combined contribution from D_o and ϑ terms is larger in lactate than in creatine. On the other hand, if the $^3J_{\text{HH}}$ coupling does contribute to the lactate signal splitting, then the appearance of a doublet rather than a quartet for lactate methine resonance would mean that two of the four transitions may have an unfavorable T_2 or distorted phase to be clearly observed. Further simulation work will be necessary to delineate these contributions to the signal, as well as the varying splitting in lactate and creatine signals at different TEs (Figs. 7 and 8).

In contrast, a similar observation of two lactate peaks near 1.3 ppm (15-20 Hz apart) was previously reported for isolated rat and frog muscles (44, 45) and assigned to two lactate pools located in two distinct types of fibers, oxidative and glycolytic. According to the fiber type model, those two peaks are induced by differences in magnetic susceptibility between the two muscle fiber types, which means that they represent two resonance signals with separate resonance frequencies. If this is the case, then one would expect that the frequencies of those two peaks would be independent of TE, in disagreement with our observation that the inter-peak distance is actually modulated by TE in human forearm muscle *in vivo* (Fig. 7). Furthermore, if the difference in magnetic susceptibility between oxidative and glycolytic fibers were responsible for the appearance of two lactate peaks observed here, then one would also expect the similar two peaks with the same inter-peak distance for other co-localized molecules such as creatine. This, again, appears to contradict our observations reported in Fig. 1.

Despite the high lactate concentration immediately after exercise, no pyruvate signal was evident in the post-exercise ^1H MR spectra at the chemical shift expected for pyruvate (2.37-2.40 ppm (46,47)). This is probably due to the high lactate/pyruvate ratio of ~25-40-fold in the exercised muscle, and relatively poor detection limit of the current method.

The accumulation of large quantity of lactate in exercised muscle implies that glycolysis pathway may be highly active during exercise, and therefore the breakdown of carbohydrates (glucose and glycogen) could be the major energy source for the muscle fiber contraction. Indeed, subtraction spectra (Figs. 1f and 4d) in the IMCL region at ~1.3 ppm produced a doublet resonance pattern for lactate methyl that matches to that of lactate methine at 4.1 ppm, thus supporting the notion that IMCL is not significantly involved in the energy production during the acute high-intensity exercise.

Two mechanisms may be responsible for the decay of lactate signal intensity following exercise, including lactate release to the blood (7) and reuse as fuel in mitochondria (1,2,6). Differences in relaxation times between resting state and after exercise could also contribute to decay of the lactate signal intensity following exercise. Though it is not feasible to measure lactate relaxation time in resting muscle due to the low detection limit (Fig. 1), for small metabolites such as carnitine and creatine, a 10% decrease in T_2 induced by exercise (without change in T_1) has been reported, as measured from carnitine trimethylamine (TMA) and creatine methyl in soleus muscle (34). If this is the case for lactate in forearm muscle, then the rate of lactate clearance from muscle could be slightly longer than $t_{1/2}$ 2.0 ± 0.6 min. Nevertheless, the dominance of metabolic pathways in the decay of lactate signal intensity following exercise agrees with the biopsy result ($t_{1/2} \sim 3$ min) reported for vastus lateralis by Saltin et al (7).

Finally, in comparison to spectral editing methods for lactate detection, our long TE approach is much simpler. It is an advantage to be able to simultaneously detect the resonance signals from multiple different metabolites, especially the signal at 2.12 ppm from the acetyl protons of acetylcarnitine, a metabolite indicative of metabolic processes occurring in mitochondria. Therefore, this method could potentially allow one to study both glycolysis and mitochondrial functions simultaneously to obtain a more comprehensive picture of

multiple biological processes in muscle. This simple approach may prove useful because much of our current knowledge regarding muscle lactate metabolism has been drawn from the study of isolated systems or by using invasive methods (48).

In summary, a long echo-time ^1H MRS acquisition strategy at 7T provides a powerful tool for non-invasive, real-time monitoring of lactate accumulation and disposal in human skeletal muscle following strenuous exercise. Given the importance of lactate in energy production and cancer metabolism, the approach presented here may find promising applications in sports medicine and cancer research to help answer questions concerning the role of lactate in cancer cell proliferation and in muscle fatigue and acidosis.

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Figure 1. Anatomy of human forearm muscle and population-averaged ^1H MR spectra of forearm muscle collected before and after exercise

(a) Cross-sectional T2W image of human forearm muscle acquired with a partial volume coil at 7T. Abbreviations: P, flexor digitorum profundus (FDP); S, flexor digitorum superficialis (FDS); R, flexor carpi radialis (FCR); U, flexor carpi ulnaris (FCU). Population-averaged 7T ^1H MR spectra collected from a single voxel centered at FDP muscle, before (c) and immediately after (b) exercise, with enlarged region shown in (d) and (e) for clear view of lactate and other coexistent metabolites. The spectra represent average of spectra collected from 12 individuals, normalized to the signal of creatine at 3.02 ppm (pre-exercise). The subtraction (post-exercise – pre-exercise) spectrum (f) illustrates the doublet pattern of lactate methine and methyl signals, with spectral fitting of lactate resonances shown in (g) (blue trace: individual peak fitting; orange trace: sum of the fitted peaks; black trace: experimental data; red trace: residual of the fitting). The upfield signal of the doublet assigned to lactate methyl resonance at 1.3 ppm may contain contribution from co-resonating IMCL bulk $-(\text{CH}_2)_n$ -resonance. The ^1H MRS was acquired with STEAM sequence using TR = 2 s, TE = 140 ms, NA = 16 (scan time 32 s). Abbreviations: Lac, lactate; acetyl, acetyl signal of acetylcarnitine (AcCtn); Cr3, methyl signal of creatine; Cr2, methylene signal of creatine; EMCL, extramyocellular lipid; IMCL, intramyocellular lipid.

Figure 2. Comparison of lactate production and clearance among individuals

Signal intensity (a) and decay rate (b) of lactate in forearm muscle after exercise, measured from methine resonance of lactate, for the group of subjects studied ($n = 12$). The intensity of lactate was scaled to that of creatine methylene resonance prior to exercise.

Figure 3. Dynamic 7T ^1H MR spectra collected from forearm muscle before and after exercise (a) T2w images showing the localization of the voxel in the forearm muscle. (b) Dynamic ^1H MR spectra, collected from the forearm muscle of a 23-year old man with BMI 21 kg/m², showing the synchronous change of lactate methyl and methine signals following hand-grip exercise. Note that, due to the nearly negligible amount of intramyocellular lipid (IMCL) in this subject, it is possible to unambiguously assign the doublet at 1.31 ppm to lactate methyl resonance. The ^1H MRS was acquired from a voxel of dimension $20 \times 25 \times 34 \text{ mm}^3$ with STEAM sequence using $\text{TR} = 2 \text{ s}$, $\text{TE} = 140 \text{ ms}$, $\text{NA} = 16$. The post-exercise spectra represent consecutive scans with a temporal resolution of 32 s. Abbreviations: Lac, lactate; Cr2, methylene signal of creatine; EMCL, extramyocellular lipid.

Figure 4. Dynamic 7T ^1H MR spectra collected from forearm muscle before and after exercise (a) T2w images showing the localization of the voxel in the forearm muscle. Lactate methine resonance at 4.09 ppm is fully resolved as doublet (b), while due to IMCL interference, the lactate methyl resonance at 1.31 ppm is partially resolved (c). The lactate methyl resonance is fully resolved as doublet in the subtraction spectra (d). Note the match in resonance pattern and time-course between lactate methine and methyl signals. These dynamic spectra were acquired from a voxel of size 8.1 mL ($16 \times 19 \times 26 \text{ mm}^3$) with STEAM sequence using TR = 2 s, TE = 140 ms, NA = 16. The post-exercise spectra represent consecutive scans with a temporal resolution of 32 s. Abbreviations: Lac, lactate; Cr2, methylene signal of creatine; EMCL, extramyocellular lipid; IMCL, intramyocellular lipid.

Figure 5. Lactate signal splitting

(a) Comparison of the magnitude of lactate signal splitting among subjects studied ($n = 12$). (b) Linear correlation of signal splitting between lactate methine and creatine methylene resonances ($n = 8 / 12$; for the rest 4 subjects, lactate splitting is smaller than 18 Hz while creatine splitting is too small to be resolved). ^1H MR spectra at forearm orientation parallel to B_0 (c) and 20° off B_0 (d), as indicated by the central white arrow in T2w images. Note that, for both lactate and creatine, the splitting is smaller at 20° than at 0° (forearm parallel to B_0). ^1H MR spectra were acquired with STEAM sequence using $\text{TR} = 2$ s, $\text{TE} = 140$ ms and $\text{NA} = 16$. Abbreviations: Lac, lactate; Cr2, methylene signal of creatine; Cr3, methyl signal of creatine; acetyl, acetyl signal of acetyl-carnitine.

Figure 6. Measuring T_1 relaxation time in post-exercise forearm muscle

The subject was a 42 year old man with BMI 25 kg/m². A total of seven inversion recovery spectra were acquired after exercise (a). Each of the seven spectra represents 8 acquisitions, with TR 4 s, TE 140 ms and voxel size $16 \times 21 \times 29$ mm³ at varying inversion delay time (TI, from bottom to top: 35, 262, 529, 854, 1270, 1848 and 2800 ms). (b) The inversion recovery curve of lactate methine doublet at 4.1 ppm for T_1 analysis (solid line), taking into account of signal partial saturation and lactate decrease occurring during post-exercise recovery. The dotted curve in (b) represents the trend of inversion recovery assuming invariable lactate level. The localization of the voxel was indicated in T2w coronal image (c). Abbreviations: Lac, lactate; AcCtn, acetylcarnitine; Cr2, methylene signal of creatine; Cr3, methyl signal of creatine.

Figure 7. Effect of echo-time (TE) on lactate signals

7T ^1H MR spectra acquired from forearm muscle of a 26 year old female subject with BMI 22 kg/m^2 at TE 100 ms (a) and 140 ms (b). Note, for both lactate methine and creatine methylene resonances, a smaller splitting was observed at TE 100 ms than at TE 140 ms (inset). These spectra were acquired with STEAM sequence using $\text{TR} = 2 \text{ s}$ and 16 acquisitions (32 s) from a voxel of size 6.1 mL ($14 \times 17 \times 22 \text{ mm}^3$) as indicated by the T2w coronal image. Abbreviations: Lac, lactate; AcCtn, acetylcarnitine; Cr3, methyl signal of creatine; Cr2, methylene signal of creatine; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid.

Figure 8. Reproducibility of lactate signals

(a1-a4) Representative 7T ^1H MR spectra demonstrating the reproducibility of the effect of TE on lactate splitting patterns. The subject was a 28 year old man with BMI 27 kg/m^2 . The spectra were acquired within the same session at TE 140 ms (a1 and a4) and TE 100 ms (a2 and a3), in the time order a1 $\rightarrow$ a2 $\rightarrow$ a3 $\rightarrow$ a4 following exercise, from the same voxel (3.2 ml , dimension $11.3 \times 14.4 \times 19.5 \text{ mm}^3$), as indicated by the inset T2w image, under the same shimming condition. Note the smaller splitting at TE 100 ms than at TE 140 ms. (b1 and b2) Representative 7T ^1H MR spectra demonstrating the inter-session reproducibility of lactate methine and methyl signals under the same acquisition condition and exercise protocol. The data were acquired from forearm muscle of a 23 year old female subject with BMI 21 kg/m^2 at two different sessions 9-week apart (b1 $\rightarrow$ b2), using STEAM sequence with TR = 2 s, TE = 140 ms, NA = 16, acquisition time 32 s, voxel size 6.0 mL ($14 \times 17 \times 25 \text{ mm}^3$). Abbreviations: Lac, lactate; AcCtn, acetylcarnitine; Cr3, methyl signal of creatine; Cr2, methylene signal of creatine; Acetyl: acetyl signal of acetyl-carnitine; IMCL, intramyocellular lipid; EMCL, extramyocellular lipid.

Table 1

Lactate Concentration, Decay Rate, Demographic and Exercise Measurements for the Human Subjects Studied ($n = 12$)

	All subjects	Male	Female	<i>p</i> -value
Subject No.	12	6	6	
Age (year)	33 ± 8	35 ± 7	31 ± 9	0.41
Body weight (kg)	73 ± 16	82 ± 16	65 ± 13	0.07
BMI (kg/m ²)	25 ± 4	26 ± 3	24 ± 6	0.45
Heart Rate at Rest (bpm)	70 ± 11	74 ± 12	65 ± 10	0.19
Maximal Heart Rate during Exercise (bpm)	92 ± 12	92 ± 12	92 ± 12	0.96
Exercise Duration (min)	2.1 ± 0.8	2.6 ± 0.9	1.7 ± 0.4	0.06
Lactate Concentration (mmol/kg ww)	32.5 ± 18.6	42.2 ± 22.7	22.9 ± 5.0	0.07
Lactate Disposal Rate Constant $t_{1/2}$ (min)	2.0 ± 0.6	2.0 ± 0.7	2.0 ± 0.5	0.71
Lactate Signal Splitting (Hz)	19.6 ± 2.3	18.8 ± 2.4	20.3 ± 2.1	0.27