Muscle metabolism and activation heterogeneity by combined \(^{31}\)P chemical shift and T\(_2\) imaging, and pulmonary O\(_2\) uptake during incremental knee-extensor exercise

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relaxation time (T2) from 1H magnetic resonance imaging (MRS) (52) interrogate relatively large regions of heterogeneous muscle. During single-leg exercise, unlocalized 31P-MRS data suggest that exercise limitation coincides with a challenge to the muscle [PCr] as it approaches the limit of detection (58). It remains unclear, however, how much whole muscle, or even regional muscle, substrate depletion may occur during exercise with a larger involved muscle mass, where the systemic challenge to homeostasis and the influence of muscle afferent feedback is heightened. The combination of the heterogeneity in muscle metabolism and the technical limitations of available sampling methods greatly confound the in vivo characterization of skeletal muscle fatigue-related changes in relation to exercise limitation in large muscle-mass exercise. A “middle ground” of spatial resolution is offered by 31P chemical shift imaging (CSI), which may shed light on this complexity.

Detecting localized metabolic disturbances during exercise is possible with 31P-CSI (7). CSI can provide a metabolite map, allowing characterization of metabolism between muscles and within single-muscle regions. The method also can balance a need for spatial resolution against the short acquisition times necessary for the temporal resolution required in non-steady-state exercise. CSI has been used to study leg muscles at rest and in recovery from exercise (16, 27, 41), in forearm (21, 44) and dorsi-/plantar flexor (19, 48) muscles during steady-state exercise, and in the quadriceps muscles during constant-power single-leg exercise and recovery (45), although not during a dynamic bilateral leg activity. In addition, muscle metabolic heterogeneity at the limit of tolerance will reflect the diversity of muscle recruitment, as well as the biochemistry of supporting energy conversion. The tissue water spin-spin transverse relaxation time (T2) from 1H magnetic resonance imaging (MRI) provides the opportunity to estimate muscle activation patterns (1) in relation to measures of local (31P-CSI) and whole body [pulmonary oxygen uptake (V̇O2)] metabolism during bilateral leg exercise in the bore of a superconducting magnet (62).

Our aim was, therefore, to characterize the dynamic interrelationships between whole body VO2, whole and regional quadriceps 31P metabolism and intracellular pH (pHi), together with muscle activation by 1H-MRI, across the full range of exercise intensity. To achieve this, we used 31P-CSI of one thigh and T2-weighted 1H MRI of the other thigh during bilateral ramp-incremental knee extension to the limit of tolerance. We hypothesized that localized measurements would reveal potential regional metabolic limitation, which is obscured by traditional global measures of metabolism (V̇O2) and whole quadriceps 31P-MRS.

MATERIALS AND METHODS

Ethical approval. The ethics committee at St. George’s Hospital Trust, London, approved this study, and all procedures complied with the latest revision of the Declaration of Helsinki. Written, informed consent was obtained from all volunteers before their participation in the study.

Participants and exercise protocols. Six healthy, active men were recruited for this study (mean ± SD: 24 ± 3 yr; 184 ± 3 cm; 88 ± 7.6 kg). Participants were screened for known disease and completed an exercise history and a physical activity readiness questionnaire.

All exercise tests were completed on an magnetic resonance (MR)-compatible, computer-controlled electromagnetically braked knee-extension ergometer (MRI Ergometer Up/Down, Lode BV, Groningen, the Netherlands). The participants lay prone with their feet strapped into molded plastic stirrups attached to aluminum arms linking the ergometer cranks. Their hips were secured to the scanner bed with padded nylon and Velcro straps to minimize hip flexion/extension. This setup, similar to an exercise model previously described (62), allowed for ~35° (limited by the scanner bore) of bilateral, alternate knee-flexion/extension to drive an electromagnetically braked flywheel. No resistance was present during knee flexion, other than the work required to lift the mass of the lower leg.

Fig. 1. Composite schematic of the measurement protocols during two ramp incremental exercise tests. The protocol consisted of a resting stage, a 5-W baseline exercise stage, and a ramp incremental exercise phase tailored to bring about intolerance after 3 complete chemical shift imaging (CSI) stages. For CSI, the data-acquisition stages each lasted 256 s. On the y-axis, * denotes an example peak power, but this varied for each participant. T2, transverse relaxation time; MRS, magnetic resonance spectroscopy.

Fig. 2. Profile of O2 uptake (V̇O2; ○) and whole-quadriceps unlocalized phosphocreatine concentration ([PCr]; ○) during the ramp incremental exercise phase in a representative participant. Arrows represent the beginning/end of 5-W and ramp incremental phases. [PCr] is given as percentage of resting, with the y-axis inverted to show more clearly the relationship to V̇O2.
Once familiarized, the physiological responses to this exercise mode are reproducible between visits in the same subjects (62). Therefore, each participant underwent a comprehensive series of familiarization visits to account for learning effects and also to ensure consistent muscle activation patterns among multiple MR measurements. Participants completed up to six ramp incremental tests until exercise responses could be reproduced on three successive occasions.

For the MR measurements, participants completed two ramp incremental exercise tests in a random order on separate days for each participant, to produce task failure in the average of the density map within each quadriceps muscle. Scan planning relied on 31P-CSI with 8-cm slice thickness, 16 × 16 phase encoding steps, and a 48-cm field of view. The volume of each voxel was 3 × 3 × 8 cm, or 72 cm³, and voxel selection for specific muscles was done with reference to the multislice MRI. A TR of 1 s was used, and a two-dimensional 31P metabolite map was generated every 256 s. This acquisition time was determined from pilot measurements to ensure good spectral data from the quadriceps and represents an optimal compromise between signal-to-noise, temporal resolution, and spatial resolution.

Data analyses for 31P measures. Signal intensities, frequencies, and line widths of Pi, PCr, and ATP were quantified using Java-based version of the magnetic resonance user interface (43). Uncalibrated concentrations for PCr and Pi were determined from assuming β-ATP concentration of 8.2 mM (28, 54). pH was estimated from the chemical shift of Pi (40):

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

where \( \delta \) is the chemical shift of the Pi peak relative to PCr. In the case of Pi peak splitting (51, 57), a Pi signal intensity-weighted mean of the two pH values was used for analyses (53).

31P-CSI postprocessing was done using SAGEIDL software (GE, Milwaukee, WI, version dev2000.3) to allow for voxel shifting to the proper region of tissue to characterize the knee-extensor muscles. Signal intensities, frequencies, and line widths were transformed into a time series for each region of interest on the 31P metabolite map.

1H-MRI. At rest and immediately following the limit of tolerance, T₂-weighted 1H images were obtained with the whole body imaging coil and used to estimate muscle activation. Changes in the spin-spin relaxation time, T₂ (ΔT₂) from rest to peak exercise were used to estimate relative muscle activation during ramp incremental exercise. The 31P surface coil under the right quadriceps caused artifact in the T₂ image, and, therefore, ΔT₂ was determined in the muscles of the left leg. Quantitative apparent-T₂ maps were constructed pixel by pixel from dual-fast-spin echo 1H images (TR of 4,000 ms; echo times of 30 and 63 ms), assuming exponential decay, and quantified from the average of the density map within each quadriceps muscle. Scan time for T₂ imaging was 176 s.

### Table 1. T₂ at rest and immediately after the limit of tolerance in the muscles of the quadriceps

| Participant No. | RF Preexercise | RF Postexercise | VM Preexercise | VM Postexercise | VI Preexercise | VI Postexercise | VL Preexercise | VL Postexercise |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 1               | 45             | 49             | 52             | 51             | 63             | 64             | 63             | 52             |
| 2               | 48             | 63             | 51             | 55             | 59             | 62             | 52             | 75             |
| 3               | 46             | 62             | 49             | 52             | 63             | 66             | 54             | 56             |
| 4               | 46             | 70             | 51             | 57             | 61             | 60             | 56             | 63             |
| 5               | 49             | 55             | 46             | 49             | 47             | 57             | 35             | 51             |
| 6               | 47             | 50             | 48             | 45             | 49             | 49             | 49             | 51             |
| Mean (SD)       | 47 (2)         | 58 (8)*        | 49 (2)         | 52 (4)         | 57 (7)         | 60 (6)         | 51 (9)         | 58 (10)        |

Values are in ms. T₂, spin-spin relaxation time; RF, rectus femoris; VM, vastus medialis; VI, vastus intermedias; VL, vastus lateralis. Different from the preexercise value (*P = 0.02).

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Pulmonary gas exchange. Participants breathed through a low-resistance (<0.1 kPa l⁻¹ s⁻¹ at 15 l/s), low-dead-space (90 ml) mouthpiece for the measurement of respired gases, as previously described (62). Flow rates and volumes were measured with a custom-designed nonmagnetic turbine flow sensor (VMM, Interface Associates, Laguna Niguel, CA), while a quadrupole mass spectrometer designed nonmagnetic turbine flow sensor (VMM, Interface Associates, Laguna Niguel, CA), while a quadrupole mass spectrometer measured gas concentrations from respired gas sampled from the mouthpiece (QP9000; CaSE, Gillingham, Kent, UK). During the 31P-MRS measurements from respired gas sampled from the mouthpiece, Laguna Niguel, CA), while a quadrupole mass spectrometer designed nonmagnetic turbine flow sensor (VMM, Interface Associates, Laguna Niguel, CA), while a quadrupole mass spectrometer measured gas concentrations from respired gas sampled from the mouthpiece (QP9000; CaSE, Gillingham, Kent, UK). During the 31P-MRS experiment, the mass spectrometer and computer were housed in the scanner control room, outside the Faraday cage. The volume transducer signal was filtered to prevent conduction of radio frequency noise into the scanner room. Due to the length of the respired gas capillary tubing (13.7 m), the measured time delay between the near-instantaneous turbine flow signal and the gas concentration signals was used to align the signals for breath-by-breath analysis (6). This increase in transit delay and sample line length did not influence the 5–95% rise time of the quadrupole mass spectrometer or the signal fidelity of respired gases (62).

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The flow sensor and gas analyzers were calibrated before each experiment. The turbine volume transducer was calibrated in the bore of the scanner with a 3-liter syringe (Hans Rudolph, Shawnee, KS). The calibration was completed with flow rates ranging from 0.2 to 6 l/s, mimicking flow rates expected for humans at rest and during exercise. After the completion of the flow sensor calibration, the flow volumes were verified over 25 syringe strokes of varying flow rates and accepted when the means were within ±0.01 l, with a SD and coefficient of variation (CV) of 0.02 l and 1%, respectively. Additionally, the mass spectrometer was calibrated with two high-precision-certified gases (BOC Group, Guildford, UK) with concentrations of O₂, CO₂, and N₂ spanning the physiological range (O₂, 21 and 10%; CO₂, 0 and 8%; N₂, 79 and 82%), and verified using room air. Following each experiment, the mass spectrometer calibrations were checked by resampling the calibration gases.

Data analyses for pulmonary gas exchange. To improve signal to noise, VO₂ breath-by-breath data were filtered by removing values residing more than 4 SD from the local mean. Data were time aligned, interpolated, averaged into 10-s bins, and ensemble averaged across like transitions. Peak values were calculated from 20-s means immediately before the limit of tolerance.

Statistical analyses. Changes in VO₂, muscle activity, and metabolites with exercise were assessed with repeated-measures ANOVA or paired t-test, where appropriate. Heterogeneity was characterized using a normalized dispersion, or CV (expressed as %). Statistics were completed using the Statistical Package for the Social Sciences (SPSS v17.0, SPSS, Chicago, IL).

RESULTS

During ramp incremental exercise, participants achieved a peak power of 42 ± 10 W, which was reproducible within 2 ± 2 W. Following an initial kinetic phase, VO₂ rose progressively to achieve a peak VO₂ of 1.98 ± 0.30 l/min, which equated to ~55% of cycling maximum VO₂ in these participants. Consequently, the dynamic VO₂-[PCr] relationship from the unlocalized 31P-MRS measurements was approximately linear in all participants (Figs. 2 and 3). The mean slope and intercept of this relationship were −0.13 ± 0.02 l·min⁻¹·mM⁻¹ and 4.34 ± 0.53 l/min, respectively. The CV for the slope and intercept of the VO₂-[PCr] were ~15% for each of the partic-
ipants, with the regression function for five of the participants showing a slope and intercept CV of ~7%.

Resting [PCr], [Pi], and pH, from unlocalized MRS were 29 ± 1 mM, 7 ± 1 mM, and 7.08 ± 0.03, respectively. At peak exercise, [PCr], [Pi], and pH, were 20 ± 4 mM, 19 ± 5 mM, and 6.91 ± 0.08. Therefore, from rest to peak exercise, [PCr] decreased by 9 ± 4 mM (P = 0.002), [Pi] increased by 12 ± 4 mM (P = 0.001), and pH, decreased (P = 0.001) by 0.18 ± 0.07.

T2 imaging revealed activation variability in the four quadriceps muscles contributing to the exercise. In the RF muscle, T2 increased from rest to exercise (47 ± 1.6 vs. 58 ± 8.2 ms; P = 0.02), while no change was detected in the VM muscle (P = 0.15), VI (P = 0.22), or VL (P = 0.22) (Table 1). However, this approach obscured individual differences in T2 changes among muscles, as there was a considerable variability in activation pattern of the knee extensors among participants (24). Therefore, the postexercise muscle activation was ranked from largest to smallest T2 change for each participant. A significant main effect was present in ranked muscle activation by ∆T2 (F = 5.9; P = 0.007; η² = 0.54), with two of four rank orderings different from one another (Table 2), indicating large variability in activation among muscles. Additionally, three of four muscles showed a ∆T2 that was different from zero, indicating that at least three of the four knee-extensor muscles were active (to varying degrees) in each participant (Table 2). At the limit of tolerance, T2 heterogeneity in each participant was ~21% to +32% of the knee-extensor mean T2.

[PCr], [Pi], and pH, changes from rest to end-exercise varied considerably across the knee-extensor muscles (Figs. 4, 5, and 6). The largest changes in each variable occurred in the RF muscle, with a significant interaction (power × muscle) present (F = 10.0; P = 0.02; η² = 0.67). No single voxel showed “complete” depletion of PCr, the lowest CV (in RF) reaching ~8 mM in three participants (Fig. 7). The CV for 31P metabolites at each power across the knee-extensor muscles increased from rest to peak exercise (IPCR, 11 ± 5 vs. 30 ± 12%, P = 0.01; [Pi], 35 ± 9 vs. 63 ± 22%, P = 0.03). The relatively small CV for pH, (~1–4%) reflects its logarithmic scale; thus, for [H+], CV increased from rest to peak exercise (6 ± 3 vs. 34 ± 26%; P = 0.04). As the CSI metabolite maps are a mean of the entire ~4-min acquisition, the trajectories of metabolite changes were extrapolated to exercise cessation using linear regression between the final two CSI acquisitions in the ramp incremental protocol (Figs. 5 and 6). At the point of intolerance [PCr], [Pi], and pH, in the RF were extrapolated to 11 ± 9 mM (or 36 ± 27% resting), 23 ± 11 mM, and 6.64 ± 0.29, respectively. Extrapolated [PCr] in two participants was reduced to 3.5 mM, or ~10% of resting [PCr] (e.g., Fig. 5).

At peak exercise, Pi peak splitting was evident in the RF voxel of all six participants. Additionally, all participants showed at least three of four knee extensors with Pi peak splitting during exercise. When pH was calculated from the chemical shift of Pi, the difference at peak exercise for the split Pi voxels in the RF muscle was 6.70 ± 0.23 vs. 6.92 ± 0.18 (P = 0.0006).

The changes in [PCr], [Pi], and pH, from rest to end exercise were used to rank the four muscles of the quadriceps in order of metabolic disturbance, as described above for T2. A significant effect was present for ranked ∆[PCr] (F = 13.8; P = 0.01; η² = 0.74), ∆[Pi] (F = 19.5; P = 0.004; η² = 0.80), and ∆pH, (F = 9.9; P = 0.02; η² = 0.66) (Tables 3, 4, and 5). This analysis indicated that the metabolic disturbance in the four muscles (reduction in [PCr], and increase in [Pi]) was significantly different from one another (Tables 3 and 4). This was also the case for ∆pH, except that the two muscles showing the greatest change (mostly RF and VL) were not different from each other (Table 5).

**DISCUSSION**

**Activation of the knee extensors and regional metabolism.** The lagged profiles of whole body VO2 and unlocalized [PCr] were consistent with an approximately exponential response to the linear power forcing (Fig. 2). The resulting slopes of the linear phase of the dynamic VO2-[PCr] relationship were similar among participants, reflecting that the whole-muscle oxidative capacity, total creatine concentration, and ATP rephosphorylated per ½ O2 reduced (P/O) varied only slightly among participants, reflecting that the whole-muscle oxidative capacity, total creatine concentration, and ATP rephosphorylated per ½ O2 reduced (P/O) varied only slightly among participants, reflecting that the whole-muscle oxidative capacity, total creatine concentration, and ATP rephosphorylated per ½ O2 reduced (P/O) varied only slightly among participants.
distribution of $^{31}$P metabolite changes across the knee-extensor muscles during ramp incremental exercise, which was revealed by localized $^{31}$P-CSI (Figs. 6 and 7). The heterogeneity of $[\text{PCr}]$ and $[\text{Pi}]$ between individual muscles approximately tripled from rest to the limit of tolerance, and the heterogeneity of $[\text{H}^-/\text{H}^+]$ increased approximately fivefold. This “metabolic strain” is related to muscle activation and the metabolic properties of the muscle region. Muscle activation was assessed in the present study by the change in $T_2$ (38). $T_2$ reflects mainly the accumulation of osmotically active ions and water associated with muscle activation (1). During our knee-extensor exercise protocol, over the range of extension from $\sim$145 to $180^\circ$, the greatest disturbance to $[\text{PCr}]$, $[\text{Pi}]$, and $p\text{H}_i$ (Figs. 5 and 6) and $\Delta T_2$ (Table 2) was most commonly seen in the RF muscle. However, muscle activation patterns were highly variable among individuals for this exercise mode, prompting comparisons using the rank order of muscle activity. Using this approach, at the limit of tolerance, three of four knee-extensor muscles showed at least some activation ($\Delta T_2$ different from zero; Table 2), consistent with being biomechanically “available” to contribute to power production during prone knee extension. Importantly, and contrary to our hypothesis, no single muscle region showed complete depletion of $[\text{PCr}]$, even when responses were extrapolated to end-exercise from the time of the CSI acquisitions. Thus, our principal finding is that the limit of tolerance was reached with an apparent energy reserve of $\sim$10% of $[\text{PCr}]$ in the most metabolically active muscle region, with heterogeneous metabolic responses across the active muscle group.

**Considerations for spatial resolution.** The interacting constraints on spatial and temporal resolution for CSI are a potential limitation of our study. The voxel sizes (72 cm$^3$) equate to $\sim$80 g of tissue (assuming a muscle tissue density of $\sim$1.1 g/cm$^3$), rendering CSI a “blunt” tool compared with biopsy. The single-voxel mass ($\sim$5% of the active muscle) greatly exceeds that of muscle biopsy ($\sim$0.005% of active muscle) where single fibers have been reported with near-complete PCr depletion (31). To this point, numerous voxels in the present study exhibited Pi peak splitting, consistent with previous measurements (57), suggesting that intravoxel compartmentalization of metabolites was present in our...
data. Nevertheless, our data show that only very small, localized muscle regions are likely to demonstrate metabolically limiting conditions at the limit of tolerance during bilateral leg exercise. It also provides an important additional frame of reference for the distribution of the metabolic disturbance within the muscle mass available for exercise. This is because the data suggest that the true proportion of muscle mass reaching metabolically limiting conditions resides in between the resolutions of the CSI used here and the muscle biopsy technique.

From available biopsy data, ~10% of muscle fibers in the sample exhibit [PCr] approaching zero (operationally, <5 mmol/kg dry mass) when sampled after submaximal heavy exercise (31); these fibers were part of a distribution with a mean [PCr] of ~30 mmol/kg dry mass with a CV of ~15% (31). Our data show a mean extrapolated [PCr] in the RF muscle at the limit of tolerance of ~10 mM (wet mass), with a CV of ~30%. Therefore, given the values from biopsy studies, it seems reasonable to suggest that [PCr] will approach zero in at least 10% of the muscle fibers within a highly metabolically active voxel. However, not all voxels characterizing the active muscle showed this magnitude of metabolic challenge. Across the entire mass engaged in exercise, the true regions with intracellular conditions limiting force production may be far smaller than local biopsy results suggest, and probably of the order of <5%, dispersed widely through the muscle i.e., PCr is depleted in ~10% of the fibers within only one of the four active quadriceps muscles measured by CSI. In other words, intramuscular metabolic conditions that would severely impair muscle power production during voluntary large muscle-mass exercise are observed only in a very limited fraction of the recruited muscle. Whether these conditions, in very small muscle volumes, contribute to bringing about task failure remains unclear.

Heterogeneity in skeletal muscle perfusion and metabolism during exercise. Within a limb muscle region during exercise, or even within a single muscle (13), Q is unevenly distributed, which has the potential to disrupt oxygen transport and mitochondrial oxygen utilization (47). During isometric knee-extension in humans, the quadriceps muscle exhibits a wide heterogeneity of Q (25). Our data also show wide variation in metabolic changes, with the RF muscle exhibiting the largest disturbance. Whether these two dispersions are well matched, or even related, remains uncertain.

The matching of Q to V̇O₂ is an important determinant of exercise tolerance (46, 61), so there is considerable interest in measuring both variables with high temporal and spatial resolution. Due to the technical constraints in achieving the required resolutions, this has proven to be a difficult task, even while at rest (39). Unlike in the myocardium (35) or brain (34), there appears to be little or no relationship between local Q and metabolism (i.e., as judged by glucose uptake or PCr breakdown) in exercising skeletal muscle (20, 26, 36, 48). This is in contrast to measures made during rest and exercise-recovery in which Q-V̇O₂ matching was relatively homogenous (39), or in the case of estimating metabolic activity from free fatty acid uptake, which was correlated with Q (36). Measuring spatially resolved Q with arterial spin labeling (ASL) in concert with PCr measurement in humans (48) showed a poor relationship of perfusion to metabolism, although differences in oxidative capacity between muscle regions complicate interpretation of these comparisons. Also, volume changes, movement artifact, and fluid redistribution each present a substantial

### Table 3. Δ[PCr] in the muscles of the quadriceps

| Participant No. | Largest Δ[PCr] | Muscle | 2nd Largest Δ[PCr] | Muscle | 2nd Smallest Δ[PCr] | Muscle | Smallest Δ[PCr] | Muscle |
|-----------------|----------------|--------|-------------------|--------|---------------------|--------|-----------------|--------|
| 1               | 9.6            | VI     | 6.5               | RF     | 3.5                 | VM     | 2.2             | VL     |
| 2               | 15.1           | RF     | 7.9               | VI     | 6.7                 | VL     | 5.4             | VM     |
| 3               | 16.5           | RF     | 8.9               | VI     | 5.3                 | VM     | 3.5             | VL     |
| 4               | 27.2           | RF     | 10.3              | VI     | 2.9                 | VM     | ~4.0            | VL     |
| 5               | 10.6           | RF     | 9.9               | VI     | 5.7                 | VL     | 3.2             | VM     |
| 6               | 20.3           | RF     | 13.7              | VI     | 10.3                | VM     | 5.7             | VL     |
| Mean (SD)       | 16.5 (6.5)     |        | 9.5 (2.5)*        |        | 5.7 (2.7)†          |        | 2.7 (3.6)‡      |        |

*Difference in phosphocreatine concentration between rest and end-exercise (Δ[PCr]) values are presented in mM and ordered from largest to smallest Δ[PCr]. Different from the next largest rank-ordering of Δ[PCr] (P = 0.03, †P = 0.01, ‡P = 0.02).

### Table 4. Δ[Pi] in the muscles of the quadriceps

| Participant No. | Largest Δ[Pi] | Muscle | 2nd Largest Δ[Pi] | Muscle | 2nd Smallest Δ[Pi] | Muscle | Smallest Δ[Pi] | Muscle |
|-----------------|--------------|--------|-------------------|--------|-------------------|--------|-----------------|--------|
| 1               | 7.3          | RF     | 6.1               | VM     | 2.1               | VI     | ~0.3            | VL     |
| 2               | 16.5         | RF     | 8.8               | VI     | 4.9               | VM     | 4.5             | VL     |
| 3               | 19.7         | RF     | 4.6               | VL     | 4.1               | VI     | 3.4             | VM     |
| 4               | 30.3         | RF     | 8.5               | VI     | 3.0               | VM     | 0.5             | VL     |
| 5               | 10.1         | RF     | 3.6               | VI     | 2.1               | VM     | ~2.5            | VL     |
| 6               | 20.1         | RF     | 6.2               | VL     | 5.5               | VI     | 3.3             | VM     |
| Mean (SD)       | 17.3 (8.2)   |        | 6.3 (2.1)*        |        | 3.6 (1.4)†        |        | 1.5 (2.7)‡      |        |

*Difference in Pi concentration between rest and end-exercise (Δ[Pi]) values are presented in mM and ordered from largest to smallest Δ[Pi]. Different from the next largest rank-ordering of Δ[Pi] (P = 0.01, †P = 0.02).
technical challenge for ASL in contracting skeletal muscle, as opposed to its application to measuring brain Q where these complications are mostly absent (56). Recent reports do show sufficient signal-to-noise with ASL in exercise recovery, where movement artifact and muscle volume change are smaller and slower compared with those during rhythmic contraction (64, 65). However, the estimation of local Q/V\(\dot{O}_2\) during exercise through ASL and CSI has yet to be revisited since the initial methodological report (48). From the limited data available, it appears as though local Q is poorly matched to local metabolism during exercise.

Additional evidence for the regional disruption of the balance between muscle O\(\dot{2}\) delivery and consumption is available using multichannel near-infrared spectroscopy. Near-infrared spectroscopy also indicates a poor relationship between local Q/V\(\dot{O}_2\) with wide distributions in deoxygenation among quadriceps muscle regions during constant-power exercise (deoxygenation amplitude CVs \(\sim\)10–50\%) (29). This is similar to the variation in metabolism for the large locomotor knee-extensor muscles during dynamic exercise observed in our study. However, without simultaneous high-resolution measurements of perfusion, the dynamic Q-V\(\dot{O}_2\) relationships in skeletal muscle remain elusive.

**Potential mechanisms for exercise limitation.** The last measurement in the CSI series represents the regional averages over the final \(~\)4 min of the \(^{31}\)P dynamics during ramp incremental exercise (Fig. 1). Therefore, the final CSI metabolite map best represents metabolite concentrations at \(~\)2 min before the point of intolerance. When the trajectories of metabolite changes are extrapolated to exercise cessation, assuming linear dynamics (60), an energy reserve still remains within even the most metabolically active muscle (Fig. 6, 11 ± 9 mM, or 36 ± 27\% resting, 23 ± 11 mM, and 6.64 ± 0.29 for [PCr], [Pi], and pH\(_i\), respectively).

The presence of a remaining energy “reserve” at the limit of tolerance raises the question of what causes the exercise to be terminated. This finding is consistent with previous demonstrations and suggests that peripheral muscle “energetic failure” may not be responsible for exercise limitation (22, 49, 58). However, the regions of tissue that manifest the greatest disturbances in pH\(_i\), [Pi], and [PCr] may also be responsible for the generation of neural feedback signals that contribute to a sense of effort and therefore exercise limitation (4). Additionally, while the reduction in [PCr] fails to explain interference with power production per se (55), it is indicative of increases in [ADP], [Pi], and \(\text{H}^+\) that cause muscle fatigue during high-intensity exercise through disruptions in cross-bridge cycling and Ca\(^{2+}\) handling (2). High [ADP] can disrupt power output (9), and Pi is inversely correlated with force output (63), likely due to the disruption in myosin detachment by competitive binding of ADP and Pi to the myosin head (15, 66). Further effects on power output with high [Pi] are expected from a reduction in Ca\(^{2+}\) sensitivity at troponin C and Ca\(^{2+}\) availability by precipitate formation in the sarcoplasmic reticulum (2, 59).

While the proposed role of pH\(_i\) in mammalian skeletal muscle fatigue has been questioned (59), some reports show that it may be responsible for fatigue at, or near, physiological temperatures via alterations in Ca\(^{2+}\) sensitivity (11, 15, 50). Additionally, changes in [Pi] and pH\(_i\) both contribute to increases in the diprotonated Pi species (H\(_2\)PO\(_4^-\)) (63), which is implicated in fatigue. Whether or not the relationship between [H\(_2\)PO\(_4^-\)] and decline in force production is causal, there is a strong correlation between muscle fatigue and [H\(_2\)PO\(_4^-\)] (8), which can, therefore, be used as an index of the potential disruption in force production. Following this logic, the distribution of [H\(_2\)PO\(_4^-\)] in the present data shows muscle regions that are expected to show a substantial reduction in peak force output at the limit of tolerance (Fig. 8) (8). This analysis suggests that peak force production in whole voxels

### Table 5. \(\Delta pH_i\) in the muscles of the quadriceps

| Participant No. | Largest \(\Delta pH_i\) | 2nd Largest \(\Delta pH_i\) | 2nd Smallest \(\Delta pH_i\) | Smallest \(\Delta pH_i\) |
|-----------------|------------------------|-----------------------------|-----------------------------|------------------------|
|                 | Muscle                 | Muscle                      | Muscle                      | Muscle                 |
| 1               | VM 0.14                | VL 0.12                     | VI 0.06                     | RF 0.02                |
| 2               | RF 0.22                | VL 0.19                     | VI 0.05                     | VM −0.04               |
| 3               | RF 0.24                | VL 0.06                     | VI 0.01                     | VM −0.01               |
| 4               | RF 0.61                | VL 0.06                     | VI 0.06                     | VM 0.02                |
| 5               | RF 0.10                | VL 0.08                     | VL −0.01                    | VL −0.04               |
| 6               | RF 0.34                | VL 0.19                     | VI 0.07                     | VM 0.06                |

Mean (SD) 0.28 (0.18) 0.12 (0.06) 0.04 (0.03)* 0.002 (0.04)†

*Difference in intramuscular pH between rest and end-exercise (\(\Delta pH_i\)) values are ordered from largest to smallest \(\Delta pH_i\). Different from the next largest rank-ordering of \(\Delta pH_i\) (\(\ast P = 0.01, \dagger P = 0.02\)).

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Fig. 8. Calculated concentration of diprotonated (monanionic) Pi (H\(_2\)PO\(_4^-\)) (63) plotted for each participant at rest and at the limit of tolerance of incremental exercise in the 4 knee-extensor muscles: RF, VI, VL, VM. Symbols are as defined in Fig. 5 legend. The resting value (★) is the mean of all 4 knee extensors. Shaded panels show expected peak force as a percentage of the baseline muscle value, calculated as a function of H\(_2\)PO\(_4^-\) concentration on the basis of the relationship observed in a study of human first dorsal interosseus muscle (8).

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in four of the six participants would be reduced by >10%. Whether this influence contributes to exercise limitation directly though failure of power production, or indirectly via feedback from muscle afferents, or some other process, remains to be determined. Nevertheless, while single voxels may demonstrate evidence of energetic fatigue in the present study, it appears that these regions reflect only a small proportion (~<5%) of the active muscle mass.

It is currently unclear whether neural afferent feedback from stimuli arising in these regions is necessary for limiting exercise tolerance in healthy subjects. Studies using intrathecal fentanyl demonstrate that afferent feedback is vital for proper “calibration” of central motor output in healthy individuals (3–5), and that by blocking these signals it is possible to improve exercise tolerance, at least in chronic obstructive pulmonary disease (17). While the improvement in chronic obstructive pulmonary disease patient exercise tolerance was more likely from reduced ventilatory drive and reduced dyspnea, the signals generated in the periphery were presumably a driving force for a complex feedback cascade contributing to exercise limitation.

**Implications for sampling techniques.** Metabolic distributions within skeletal muscle are largely attributed to the differing enzymatic and energetic profiles of type I and II muscle fibers, and the distribution of metabolites and substrate depletions in these fibers have traditionally been investigated through biopsy. Naturally, this technique provides the advantage of direct measurement of specific cellular metabolites. Biopsy studies suggest large reductions in PCr during high-intensity cycling exercise (and accumulation of Pi, ADP, and H+) (18, 31). However, these data are of limited use in determining the responses over an entire muscle or muscle group. Biopsy volume (~50–300 mg) is typically assumed to be representative of the entire volume of muscle contributing to the exercise task (~1–3 kg per leg, based on thigh mass of ~5–7 kg in adult men (12)). It is clear from the large variability in phosphorus metabolite concentrations reported here and elsewhere that the biopsy technique may well miss fibers that approach limiting levels or even show depletion of PCr. The biopsy technique also offers no spatial and temporal information, unless multiple sampling sites per muscle are used (raising obvious ethical and practical concerns).

Data in the literature and from our study therefore indicate that single-site sampling techniques, such as biopsy, are insufficient to characterize whole-muscle metabolism during voluntary exercise (24, 33). Based on the heterogeneity in skeletal muscle reported here and elsewhere (10, 14, 19, 24, 48), a greater spatial and temporal fidelity is needed to assess the changes in metabolic activity and fatigue-related metabolites in relation to limitation during large muscle-mass exercise.

**Conclusions.** While whole body \( \dot{V}O_2 \) was linearly related to whole quadriceps [PCr] during ramp incremental exercise, the limit of tolerance was attained with a remaining intramuscular energy store and heterogeneous dynamics in all \(^{31}P\) variables examined. The CSI technique suggests that ~<5% of the quadriceps muscle volume was metabolically challenged at the limit of bilateral knee extension, despite activity in the majority of the quadriceps (based on \( T_2 \) change) indicating the ability of these muscles to contribute power production. These data suggest that single-site sampling techniques are inadequate to characterize the heterogeneous nature of muscle metabolism during dynamic exercise in humans. While we have used a spatially resolved technique, which substantially improves interpretation for muscle metabolite changes over unlocalized \(^{31}P\)-MRS, Pi peak splitting was apparent in most muscle regions, indicating that further compartmentalization may mask regions of limitation. How these signals are integrated to bring about exercise intolerance in large muscle-mass exercise remains to be determined.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: D.T.C., F.A.H., C.L., and H.B.R. analyzed data; D.T.C., F.A.H., J.R.G., and H.B.R. interpreted results of experiments; D.T.C. prepared figures; D.T.C. and H.B.R. drafted manuscript; D.T.C., F.A.H., S.A.W., D.J.M., C.L., J.R.G., G.J.K., and H.B.R. edited and revised manuscript; D.T.C., F.A.H., S.A.W., D.J.M., C.L., J.R.G., G.J.K., and H.B.R. approved final version of manuscript; F.A.H., B.J.W., S.A.W., G.J.K., and H.B.R. conception and design of research; F.A.H., B.J.W., D.J.M., and H.B.R. performed experiments.

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