Thigh muscle activation distribution and pulmonary $\dot{V}o_2$ kinetics during moderate, heavy, and very heavy intensity cycling exercise in humans

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Endo MY, Kobayakawa M, Kinugasa R, Kuno S, Akima H, Rossiter HB, Miura A, Fukuba Y. Thigh muscle activation distribution and pulmonary Vo₂ kinetics during moderate, heavy, and very heavy intensity cycling exercise in humans. Am J Physiol Regul Integr Comp Physiol 293: R812-R820, 2007. First published April 25, 2007; doi:10.1152/ajpregu.00028.2007.-The mechanisms underlying the oxygen uptake (Vo₂) slow component during supra-lactate threshold (supra-LT) exercise are poorly understood. Evidence suggests that the Vo₂ slow component may be caused by progressive muscle recruitment during exercise. We therefore examined whether leg muscle activation patterns [from the transverse relaxation time (T2) of magnetic resonance images] were associated with supra-LT $\dot{V}o_2$ kinetic parameters. Eleven subjects performed 6-min cycle ergometry at moderate (80% LT), heavy (70% between LT and critical power; CP), and very heavy (7% above CP) intensities with breath-by-breath pulmonary Vo₂ measurement. T2 in 10 leg muscles was evaluated at rest and after 3 and 6 min of exercise. During moderate exercise, nine muscles achieved a steady-state T2 by 3 min; only in the vastus medialis did T2 increase further after 6 min. During heavy exercise, T2 in the entire vastus group increased between *minutes 3* and 6, and additional increases in T2 were seen in adductor magnus and gracilis during this period of very heavy exercise. The Vo₂ slow component increased with increasing exercise intensity (being functionally zero during moderate exercise). The distribution of T2 was more diverse as supra-LT exercise progressed: T2 variance (ms) increased from 3.6 \pm 0.2 to 6.5 \pm 1.7 between 3 and 6 min of heavy exercise and from 5.5 ± 0.8 to 12.3 ± 5.4 in very heavy exercise (rest = 3.1 ± 0.6). The T2 distribution was significantly correlated with the magnitude of the $\dot{V}o_2$ slow component (P < 0.05). These data are consistent with the notion that the Vo₂ slow component is an expression of progressive muscle recruitment during supra-LT exercise.

oxygen uptake; slow component; T2 time; muscle use patterns

PULMONARY OXYGEN UPTAKE ($\dot{V}o_2$) during moderate intensity, constant-load cycling exercise below the lactate threshold (LT) approaches a steady state with an exponential time course (phase II) after a short delay (phase I) and attains a steady state within 2–3 min. However, during exercise at supra-LT work rates, the $\dot{V}o_2$ response is more complex, and the fundamental (phase II) kinetics are supplemented by an additional delayed phase that causes a secondary rise in $\dot{V}o_2$, termed the "excess" $\dot{V}o_2$ or the $\dot{V}o_2$ "slow component" (30, 43). The consequence of this delayed $\dot{V}o_2$ slow component is that $\dot{V}o_2$ attains levels

greater than those projected from the sub-LT $\dot{V}o_2$ -work rate relationship (15). Additionally, these supra-LT $\dot{V}o_2$ kinetics (as well as the responses of other physiological variables, such as blood lactate and ventilation) differ depending on whether exercise is undertaken below (heavy intensity exercise) or above (very heavy intensity exercise) the critical power threshold (CP; also termed the fatigue threshold, θ_F) (15, 27, 30, 32), with exercise above CP resulting in a continuous rise in $\dot{V}o_2$ and blood lactate, whereas a delayed steady state can be achieved during heavy intensity work rates (32).

Despite considerable attention, the mechanism determining the Vo₂ slow component during constant-load cycle ergometer exercise remains conjectural (e.g., Refs. 14, 16, 19, 20, 24, 30, 31, 39, 41). Poole et al. (31) have demonstrated that a large majority (~90%) of this additional oxygen consumption originates in the exercising limb (using femoral arterial and venous blood gas and flow sampling during cycle ergometry), which has since been corroborated (37) in the profile of intramuscular phosphocreatine (PCr) breakdown (a proxy for the mediation of oxidative phosphorylation). However, the cause of this additional requirement for oxidative energy provision is still disputed. The most commonly held view, which has yet to be refuted, is that the fiber recruitment pattern of the muscles engaged in exercise is progressively altered during the supra-LT exercise itself, possibly as a result of progressive fatigue in the early recruited motor units, with the fatigue leading to progressive recruitment of less-efficient fibers [i.e., fibers with poor ATP production-to-O₂ consumption ratio (P: O₂) or high energy cost of force production characteristics] or the fatiguing fibers manifesting a reduced efficiency. These hypotheses are unified by their requirement for progressive recruitment as a consequence of ongoing fatigue that leads to increased oxygen consumption during constant power output exercise, resulting in the expression of the Vo2 slow component (5, 11).

Consistent with this notion, Barstow et al. (6) demonstrated that the amplitude of the $\dot{V}o_2$ slow component (during cycle ergometry in humans) was positively correlated with the percentage of type II fibers in quadriceps biopsy samples, fibers that are thought to manifest a high energy cost of force production (5, 11). Furthermore, Krustrup and colleagues (21, 22) have shown in muscle biopsy samples of the quadriceps that PCr and glycogen depletion became increasingly more

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widespread during work rates engendering a Vo2 slow component. Investigations using surface electromyography (EMG), however, have not consistently corroborated this progressive recruitment hypothesis (40, 41). More recently, Saunders et al. (39) have used the ¹H transverse relaxation time (T2) during magnetic resonance imaging (MRI) of skeletal muscle as an index of muscle activation (1, 12, 25, 33, 36) to investigate this suggestion. They showed a linear correlation between the magnitude of the $\dot{V}o_2$ slow component (between 3 and 15 min of cycle ergometry) and T2 increases in muscles of the leg [which were both reduced by endurance training; Saunders et al. (38)], consistent with the progressive recruitment hypothesis for the Vo₂ slow component mechanism. However, these authors did not clarify the distribution of T2 values within each muscle of the upper leg nor did they distinguish between the differing systemic responses of exercise in the heavy and very heavy intensity domains.

Because skeletal muscle T2 is sensitive to muscle water, which is in turn greatly influenced by lactate concentration and its associated proton (as well as other osmotically active ions that accumulate above LT; e.g., Refs. 1, 3, 25, 33), we hypothesized that muscle T2 increases would be more pronounced during very heavy intensity exercise than during exercise at either heavy or moderate intensity. Furthermore, because MRI allows all the muscles of interest to be investigated (rather than more regional superficially weighted measurements from surface EMG), we aimed to investigate T2 in cross-sectional images of each of the 10 muscles of the upper leg (including the gluteus maximus; GM) during exercise to resolve spatially oriented skeletal muscle "activation maps." Thus we further hypothesized that T2 increases in the 10 muscles investigated would manifest an increasing diversity as intensity increased, and that the extent of this diversity would be reflected in the magnitude of the $\dot{V}o_2$ slow component.

We therefore aimed to determine the relationship between muscle T2 changes and the $\dot{V}o_2$ slow component magnitude (between 3 and 6 min of cycle ergometry) during moderate (sub-LT), heavy (between LT and CP), and very heavy (supra-CP) exercise.

METHODS

Subjects. Eleven healthy Japanese subjects (8 females and 3 males, none being habitually active) volunteered to participate in this study and gave written informed consent as approved by the ethics committees of the institutions involved (in accordance with the Declaration of Helsinki). Subjects averaged (mean \pm SD) 28 \pm 9 yr, 162 \pm 7 cm, and 56 \pm 9.7 kg.

Exercise protocols. The subjects initially performed a ramp incremental exercise test to the limit of tolerance, at a rate of 12 W/min (for the female subjects) or 20 W/min (for the male subjects), on an electromagnetically braked cycle ergometer (232c-XL, Combi, To-kyo, Japan) at 60 rpm. During the incremental exercise, ventilatory and gas exchange parameters were measured breath by breath, and capillary blood was sampled every 2 min for blood lactate determination (see below). The V-slope method (9) was used to determine the LT, with concomitant corroboration from the onset of systematic increases in the ventilatory equivalent for $\dot{V}o_2$ ($\dot{V}_E/\dot{V}o_2$) and end-tidal Po₂, without a concomitant rise in the ventilatory equivalent for CO₂ output ($\dot{V}_E/\dot{V}co_2$) or fall in end-tidal Pco₂ (43).

The subjects subsequently performed a series of exercise tests on the same cycle ergometer to determine the power-duration curve and estimate CP, according to methods previously described (26). Briefly, each subject performed at least four different high intensity, constantload exercise tests to the limit of tolerance, the aim being to bring the subject to the limit of tolerance within $\sim 2-10$ min. Each trial was randomized and undertaken on a separate day. Exhaustion in each test was defined as the point at which the subject could no longer maintain a pedaling rate of 50 rpm despite verbal encouragement. CP was estimated using the equation $P = W' \cdot (1/t) + CP$, where CP and W' are the intercept and slope, respectively, of the linear regression between power (P) and 1/time to exhaustion (1/t).

Each subject then performed a series of constant work rate cycle ergometer exercise tests at moderate intensity (80% of LT; 59 \pm 15 W), heavy intensity (at 70% of the difference between LT and CP; 117 \pm 29 W), and very heavy intensity (a work rate >CP expected to elicit the limit of tolerance in 12 min; 146 \pm 35 W); each was followed by 4 min of rest. The protocols were repeated three to six times, in a randomized sequence and on separate days ($\dot{V}o_2$ series), to provide appropriate confidence for precise $\dot{V}o_2$ kinetic characterizations (23). Subsequently, the subjects performed cycle ergometry (on the same ergometer) close to the bore of an MRI magnet (MR series; see *MRI*, below) at the same work rates used in the $\dot{V}o_2$ series. Here, each of the three work rates was performed for either 3 or 6 min to obtain T2 images at the same time points used to measure the $\dot{V}o_2$ slow component. All of these exercise tests were performed at the same time of day, each on different days.

Cardiopulmonary measurements. Ventilatory and gas exchange responses were determined breath by breath using a computerized metabolic measuring system (RM-300, Minato Medical, Osaka, Japan). Before each exercise test, a hot-wire flow sensor and gas analyzers were calibrated using a known volume of air (at several mean flow rates) and gas mixtures of known concentration, respectively. Heart rate (HR) was monitored continuously via a three-lead electrocardiotachogram (ECG) (BP-306, Colin, Komaki, Japan). A second-by-second time course was calculated for each variable by interpolation, and the data were stored on disk for further analysis.

MRI. For the MR series, subjects initially lay in the bore of 0.2-T superconducting magnet (AIRIS II, Hitachi Medical, Tokyo, Japan) to obtain functional (T2) MR images of the right thigh. Subjects then came out of the magnet and performed the required (randomized) cycle ergometer test (described in Exercise protocols, above) immediately after which they moved quietly but quickly back into the bore of the MR magnet (~5-m distance) for further thigh imaging (the second scan commencing \sim 45 s after exercise cessation). Resting and postexercise enhanced T2 cross-sectional images of the thigh were acquired according to the method previously described by Kinugasa et al. (18). Briefly, standard spin-echo MR cross-sectional images were taken at two points on the leg: 1) 60% between the trochanter and patella in the right thigh (to estimate T2 in the 9 thigh muscles), and 2) an additional 4 cm superior to this (for T2 estimation in the GM). Ten-millimeter-thick transaxial images (repetition time, 1,500 ms; echo times, 30 and 60 ms) were collected using a whole body coil. A 256 \times 180 matrix was acquired with one excitation and a 32-cm field of view. Scan time was 318 s.

Calculations of T2 were made separately on each of the nine muscles of the thigh and GM using product-specific imaging software (AIRIS II). The mean T2 value at rest and immediately after the 3rd and 6th min of exercise at each of the three different intensities was calculated from two to three ~2- to 3-cm² regions of interest for each of 10 muscle groups: rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI), sartorius (Sar), gracilis (Gr), adductor magnus (AM), adductor longus (AL), semitendinosus (Semi), and GM. Anatomic MR images were analyzed using a modified version of the National Institutes of Health (NIH) image software package (written by Wayne Rasband at the NIH, Bethesda, MD, and available by anonymous ftp: **ftp://zippy.nimh.nih.gov/**). Anatomic cross-sectional areas of each image were obtained by tracing the outline of each thigh muscle. Muscle activation at each

intensity and time point was then evaluated from the average T2 value for each muscle.

Analysis. Vo_2 responses were time interpolated second by second and averaged across each like-intensity transition for each subject. The response kinetics were estimated using nonlinear regression (i.e., Marquardt-Levenberg Algorithm in Sigma Plot 2000, Jandel Scientific) of the response following the first 20 s (i.e., excluding phase I; Ref. 44) to the expression

$$f(t) = BL + A \cdot [1 - e^{-(t - Td)/\tau}]$$
 (1)

where f(t) represents values at *time t*; BL is the baseline value at rest, averaged from the last minute before exercise onset; and A, Td, and τ are the amplitude, time delay, and time constant of the exponential response, respectively. Where this model did not adequately characterize the $\dot{V}o_2$ response (e.g., during heavy and very heavy intensity exercise, where a slow component is expressed), the fitting window was progressively reduced to identify the exponential region a priori. Values of $\dot{V}o_2$ at 3 and 6 min were calculated from the averages of the preceding 30 s and were used to estimate the magnitude of the $\dot{V}o_2$ slow component [$\Delta \dot{V}o_2$ (6-3)] and the relative contribution of the slow component to the overall response amplitude [$\Delta \dot{V}o_2$ (6-3)/ ΔEE ; where ΔEE is the difference between the preexercise baseline and end-exercise values].

T2 distribution characteristics (mean, variance, skewness, and kurtosis) during rest and exercise were estimated from Gaussian fits of probability density plots of T2 values for each muscle in all subjects. The differences in distribution among characteristics were compared between rest and 3 and 6 min of exercise, and the change in T2 variance (a measure of response homogeneity) between 3 and 6 min [Δ T2 variance₍₆₋₃₎] was compared with the magnitude of the slow component, using linear regression. The T2 values of the whole thigh (Σ T2) are reported as the area-weighted sum (i.e., the product of the muscle cross-sectional area and T2).

Statistical treatments. Values are expressed as means \pm SD. The effect of exercise intensity (i.e., moderate, heavy, very heavy) on the variables and parameters was examined by repeated-measures ANOVA (with Tukey's post hoc test where a significant *F*-value was detected). The interaction of exercise intensity and time (i.e., 3 and 6 min) was examined by repeated-measures two-way ANOVA (with a paired *t*-test where a significant *F*-value was detected). Significance was accepted at P < 0.05. All statistical tests were performed with the use of SPSS for Windows (SPSS).

RESULTS

The mean value of $\dot{V}_{02 \text{ peak}}$ during all-out incremental exercise was 1,870 ± 439 ml/min (33.6 ± 3.9 ml·min⁻¹·kg⁻¹; range 28.0–40.7 ml·min⁻¹·kg⁻¹), and the estimated LT averaged 1,014 ± 263 ml/min or 54 ± 5% of $\dot{V}_{02 \text{ peak}}$. The work

rates associated with the LT and CP thresholds averaged 74 \pm 19 and 136 \pm 33 W, respectively. The mean cross-sectional area (in cm² and as percentage of whole muscle area) of each of the muscles of the upper leg and the GM is shown in Table 1. The largest muscle of the upper leg was adductor magnus (AM) (~28%), with the vastus lateralis (VL) and vastus intermedius (VI), which are well known to be extensively recruited during cycling exercise, accounting for ~24 and 15% of the whole area, respectively. Of the other seven muscles, there was none that accounted for >10% of the whole cross-sectional area.

 $\dot{V}o_2$ kinetic responses. The time course of the $\dot{V}o_2$ response to constant-load cycling exercise at the three intensity domains for a typical subject and the average from all 11 subjects are shown in Fig. 1, A and B. The estimated parameters of the $\dot{V}o_2$ kinetics are summarized in Table 2. The phase II $\dot{V}o_2$ time constant (τ) did not differ among the moderate, heavy, and very heavy intensity exercise (moderate, 22.4 ± 6.9 s; heavy, 22.4 \pm 6.9 s; very heavy, 21.8 \pm 3.6 s). As expected, the amplitude of the fundamental $\dot{V}o_2$ component increased (P <0.05) at increasing intensities (moderate, 641 ± 166 ml/min; heavy, $1,182 \pm 277$ ml/min; very heavy, $1,346 \pm 315$ ml/min). Similarly, the $\dot{V}o_2$ slow component [i.e., $\Delta \dot{V}o_{2(6-3)}$ and $\Delta \dot{V}_{O_2 (6-3)} / \Delta EE$] increased with increasing intensity, from functionally zero during moderate intensity (0 \pm 14 ml/min; $-0.2 \pm 2.4\%$) to 74 \pm 40 ml/min (or 5.7 \pm 3.0%) during heavy intensity and to 169 ± 49 ml/min (or $10.9 \pm 1.9\%$) during very heavy intensity exercise.

Change of muscle T2 during exercise. The resting T2 evaluated before exercise at each of the three intensity conditions was normally distributed and did not widely differ among all muscles (Table 1; the mean coefficient of variation across days and between muscles was 1.1%). An example of MR images before and after 6 min of very heavy intensity cycling exercise in a representative subject is shown in Fig. 2. Following very heavy intensity exercise, the T2 signal intensity change was variable between each of the thigh muscles, even by visual inspection, suggesting a complex muscle recruitment pattern during cycle ergometry. The relative change (%) in muscle T2 from the resting baseline for each muscle in the thigh following 3 and 6 min of cycling at each of the three intensity domains is shown in Fig. 3. During moderate exercise, only one muscle (VM) showed a significant increase in T2 between 3 and 6 min of exercise. However, during heavy intensity exercise, the

Table 1. Resting T2 value at each muscle group in moderate, heavy, and very heavy intensity exercise

		Work Intensity		
Muscle	CSA, cm ² (%)	Moderate	Heavy	Very Heavy
Rectus femoris	9.0±2.2 (8.7±1.5)	29.7±0.3	29.7±0.3	29.6±0.3
Vastus lateralis	25.1±6.6 (23.9±2.8)	29.9 ± 0.3	29.9 ± 0.3	30.0 ± 0.3
Vastus medialis	8.4±1.3 (8.2±1.6)	29.8 ± 0.4	29.8 ± 0.3	29.7±0.3
Vastus intermedius	15.4±4.7 (14.6±2.6)	30.3 ± 0.5	30.0 ± 0.3	30.1 ± 0.4
Sartorius	$2.1\pm0.7~(1.9\pm0.4)$	31.9 ± 0.5	31.9 ± 0.5	31.9 ± 0.5
Gracilius	2.0±0.9 (1.9±0.6)	31.1 ± 0.6	31.1 ± 0.6	31.1±0.6
Adductor magnus	29.7±9.5 (28.0±2.9)	30.4 ± 0.3	30.4 ± 0.3	30.3 ± 0.3
Adductor longus	$8.0\pm3.3(7.5\pm1.8)$	29.6 ± 0.2	29.7 ± 0.2	29.6±0.2
Semitendinosus	$5.3\pm2.0(5.0\pm1.2)$	30.9 ± 0.4	31.0 ± 0.4	31.0 ± 0.4
Gluteus maximum	$0.3 \pm 0.6 \ (0.3 \pm 0.6)$	34.1 ± 0.4	34.0 ± 0.5	34.1 ± 0.4

Values are means \pm SD; n = 11. Transverse relaxation time (T2) values are given in milliseconds. CSA, cross-sectional area. There were no significant differences among the 3 conditions.



Fig. 1. Representative examples of individual subject (A) and group mean (B) responses of pulmonary oxygen uptake ($\dot{V}o_2$) during cycling exercise in the moderate (\bullet), heavy (\blacktriangle), and very heavy (\blacksquare) intensity domains. Data are averaged every 10 s. Values are means and SD in B (n = 11).

three muscles of the vastus group (VL, VM, and VI) each demonstrated a significant (P < 0.05) T2 increase. During very heavy intensity cycling, additional (to those of the vastus group) T2 increases (P < 0.05) were observed in Gr and AM; the remaining four thigh muscles (with the exception of AL) all tended toward an increased T2 (Fig. 3). There was no change in T2 in the AL muscle during cycle ergometry at any of the exercise intensities or time points (Fig. 3).

T2 distribution characteristics and their relationship with $\dot{V}o_2$ kinetics. There were significant correlations between the magnitude of the change in T2 in individual muscles between the 3rd and 6th min [$\Delta T2_{(6-3)}$] and the magnitude of the slow component [$\Delta \dot{V}o_2$ (6-3)/ ΔEE]. $\Delta T2_{(6-3)}$ was well correlated with $\Delta \dot{V}o_2$ (6-3)/ ΔEE for AM (R = 0.53, P < 0.01), VI (R = 0.42, P < 0.01), and VL (R = 0.35, P < 0.01). Together, these resulted in a significant correlation between the area-weighted sum of T2 values [$\Delta \Sigma \% T2_{(6-3)}$] and $\Delta \dot{V}o_2$ (6-3)/ ΔEE (R = 0.51, P < 0.01).

The magnitude of the $\dot{V}o_2$ slow component was also closely related to the distribution of T2 values among the leg muscles, both within individuals and for the group as a whole. Characteristics of the variance (distribution) of T2 within each individual were similar among subjects (Table 3), and, therefore, the subjects were grouped, allowing detailed distribution plots to be made (Fig. 4). The probability density of T2 values was well modeled by a Gaussian distribution and was not significantly skewed in any condition (Fig. 4; skewness ranging from -0.02 to 0.41). T2 variance (the width of the Gaussian curve at one-half height) between muscles averaged 3.1 ± 0.6 ms at rest and was not appreciably increased during moderate exercise $(3.7 \pm 0.3 \text{ and } 4.2 \pm 0.1 \text{ ms at } 3 \text{ and } 6 \text{ min, respectively;}$ Fig. 4). After 3 min of heavy exercise, T2 variance was similar $(3.6 \pm 0.2 \text{ ms})$ to the resting value, but it was approximately doubled by 6 min of heavy exercise (to 6.5 ± 1.7 ms) with a concomitant fall in kurtosis from 0.21 to -0.73, reflecting a flatter, wider distribution. The T2 distribution at 3 min of very heavy exercise was already increased above resting levels $(5.5 \pm 0.8 \text{ ms})$ and reached $12.3 \pm 5.4 \text{ ms}$ by 6 min. The 6-min time point in both heavy and very heavy intensity exercise was associated with a reduced confidence of the Gaussian fit (R^2 = 0.86 in both conditions) and a progressively platykurtic (flatter and wider) distribution (reflected in the negative kurtosis values; Table 3). The mean increase in T2 variance between minutes 3 and 6 was significantly correlated with the magnitude of the $\dot{V}o_2$ slow component for each individual (R^2 = 0.78; P < 0.05) as well as the group mean ($R^2 = 0.98$; P <0.05; Fig. 5).

DISCUSSION

The present study investigated the relationship between $\dot{V}o_2$ kinetics and the pattern of muscle recruitment evaluated by T2 MRI in moderate, heavy, and very heavy intensity cycling exercise. As expected, the fundamental $\dot{V}o_2$ time constant was unchanged among the three exercise domains (7, 27) but was

Table 2. Pulmonary $\dot{V}o_2$ response characteristics during moderate, heavy, and very heavy intensity exercise

	Moderate	Heavy	Very Heavy				
BL, ml/min	238±43	242 ± 41	242 ± 40				
Monoexponential fitting							
"Effective" τ, s A, ml/min	22.4 ± 6.9 641 ± 166	33.6±8.6* 1,272±272*	36.7±7.6*† 1,446±298*†				
	Primary compon	ents					
τp, s Ap, ml/min Ap/W, ml·min ⁻¹ ·W ⁻¹	10.9±0.3 (A/W)	22.4 ± 6.9 $1,182\pm277$ $10.1\pm0.5*$	21.8±3.6 1,346±315† 9.2±0.4*†				
·····	Slow componen	nts					
$\Delta \dot{V}O_{2}$ (6-3), ml/min $\Delta \dot{V}O_{2}$ (6-2)/ ΔFE %	0.01 ± 14 -0.2 + 2.4	$74 \pm 40^{*}$ 5 7 + 3 0	169±49*† 10 9+1 9				

Values are means \pm SD; n = 11. BL, baseline $\dot{V}o_2$; "effective" τ and A, time constant and amplitude of the overall response estimated by monoexponential fit, respectively; τp , Ap, and Ap/W, time constant, amplitude of the primary component response, and the increase in $\dot{V}o_2$ relative to the work rate, respectively; $\Delta \dot{V}o_2$ (6-3) and $\Delta \dot{V}o_2$ (6-3)/ ΔEE , the increment in $\dot{V}o_2$ between the 3rd and 6th min of exercise and the relative contribution of the slow component to the overall amplitude, respectively. *Significantly different from moderate exercise (P < 0.05). †Significantly different from heavy exercise (P < 0.05).

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(AM), adductor longus (AL), and semitendinosus (Semi). supplemented by a $\dot{V}o_2$ slow component during supra-LT exercise (15, 30). In addition to this, however, concomitant MRI of thigh muscles during cycling exercise revealed *1*) a marked heterogeneity between each of the individual muscles of the thigh (as well as GM), such that the exercise-induced T2 increases became increasingly more marked with increases in exercise intensity; 2) a progressive broadening (between muscles) of T2 increases between the 3rd and 6th min of exercise during heavy and very heavy intensity exercise; and 3) that the

Fig. 2. Magnetic resonance images across right thigh of a representative subject before (A) and after (B) performance of 6 min of cycling exercise in the very heavy intensity domain. C: the resulting anatomic segmentations of the rectus femoris (RF), vastus lateralis (VL), vastus medialis (VM), vastus intermedius (VI), sartorius (Sar), gracilis (Gr), adductor magnus

increase in T2 variance between the 3rd and 6th min of exercise was significantly correlated with the magnitude of the $\dot{V}o_2$ slow component. These findings are consistent with the notion that the $\dot{V}o_2$ slow component is a manifestation of progressive muscle recruitment during supra-LT exercise.

MR T2 imaging and muscle recruitment. During large muscle mass exercise, such as cycle ergometry, the contribution of individual muscles to total power output is difficult to determine using standard surface EMG or biomechanical approaches. Recently, MRI has been used to quantify the degree and pattern of muscle activation during whole body exercise (e.g., Refs. 3, 36, 39). Exercise-induced increases in the T2 of muscle protons in the MR image result in increased image contrast and are sustained for at least 10-20 min following exercise cessation (1, 2, 3, 12, 25, 33, 34, 35, 36, 39). While the mechanism of this exercise-induced T2 increase is somewhat controversial (25), it is thought to be due, at least in part, to increases in extracellular fluid accumulation and/or increases in intracellular osmotic potential from metabolite accumulation within the recruited regions (29). Investigations of this process have shown that muscle T2 is well correlated with surface integrated EMG (iEMG) and workload (1, 34). This exerciseinduced T2 increase has been shown to be manifest only in activated muscles (either voluntarily or by electrical stimulation), with no change in muscles that were not activated (1, 3, 3)33). T2 may, therefore, allow estimation of the diversity of muscle activation during exercise. Although this is an indirect measurement of muscle activity, this MRI technique does have some advantages over standard techniques such as EMG. For example, it offers the opportunity to investigate all the muscles engaged in the external work rather than those regions proximal to surface electrodes and allows spatial mapping of muscle "activity" during exercise. In the present study, we used this property to investigate activity patterns (2, 4, 18, 25, 33) in a cross section of 10 leg muscles during cycle ergometry over a range of intensities.

Richardson et al. (36) have previously shown that the pattern of muscle activation during cycle ergometry (at 90% of maximum work rate for 2–2.5 min) results in a complex and diverse (heterogeneous) T2 increase. This was in contrast to

the finding that knee extensor exercise led to a relatively homogeneous T2 increase in the four muscles of the quadriceps. Reid et al. (34) have also demonstrated a complex muscle activity pattern during cycle ergometry using T2 imaging, but they showed that T2 became progressively more uniform during high intensity cycle ergometry (90% of the maximal work rate during an incremental test) than during low intensity exercise. In other words, the number of active muscles was increased with intensity, rather than simply the magnitude of activation within muscles initially recruited [in accordance with the suggestions of Ray and Dudley (34)]. Their data suggests a large degree of activation in 8 of the 10 leg muscles measured during high intensity exercise, whereas low intensity work rates were predominantly met by energetic contributions from the three vastus muscles. In contrast, during the present study, moderate intensity exercise produced some degree of T2 increase in most of the leg muscles investigated. However, similar to the study of Reid et al. (35), the largest T2 increases at any exercise intensity in the present study were seen in the vastus group, as well as the Sar and AM muscles [although it may be of interest that in the present study and that of Richardson et al. (36), the RF muscle was one of the least "active" muscles during cycle ergometry, unlike for Reid et al. (35)]. However, the present study showed that the range of T2 values became progressively more diverse as supra-LT exercise continued, suggesting broader muscle recruitment after 6 min of cycle ergometry that after 3 min.

MR T2 imaging and $\dot{V}o_2$ kinetic responses. Saunders et al. (38, 39) have also combined T2 imaging with pulmonary gas exchange measurements to elucidate the relationship between the late (i.e., after 3 min) increase in T2 and the magnitude of the $\dot{V}o_2$ slow component (defined as the difference in $\dot{V}o_2$ from the 3rd to 15th min after the onset of exercise). They showed a significant positive correlation between the magnitude of the exercise-induced T2 in the whole lower extremity and the \dot{V}_{02} slow component (the degree of both measures reducing with exercise training; Ref. 38). These results are consistent with the many previous suggestions (10, 31, 35, 41) that an increase in muscle recruitment is responsible for the $\dot{V}o_2$ slow component. However, Reid et al. (35) showed (in subjects exhibiting a wide range of maximal aerobic capacities) that exercise-induced T2 increases were dependent on the relative intensity and not absolute work rate per se, highlighting the relationship between metabolic "stress" and T2 increases. It is important, therefore, when investigating these responses to assign exercise into domains in that are defined by similar (relative) metabolic stress profiles. In the present study (unlike Refs. 38, 39), work rates were assigned to intensity domains [moderate, heavy, and very heavy, as defined by Whipp (42)] based on known Downloaded

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Fig. 3. Percent change in transverse relaxation time (T2) following cycle ergometer exercise for 3 min (solid bars) and 6 min (hatched bars) in each of the individual muscles measured over a range of exercise intensities: moderate intensity (*top*), heavy intensity (*middle*), and very heavy intensity (*bottom*). Bars are relative (%) increase in T2 from preexercise resting values. Values are means \pm SD; n = 11. *Significantly greater \hat{v}_{02} compared with the 3-min value of the same muscle and intensity (P < 0.05).

physiological stress profiles and delineated by LT, CP, and $\dot{V}o_{2 \text{ max}}$. We could be confident, therefore, that T2 values were compared within domains of the same relative metabolic stress. Similar to Saunders et al. (39), we found a positive correlation between the magnitude of the $\dot{V}o_{2 \text{ max}}$ slow component and the activation of the muscles in the whole thigh over the same time period. In addition, however, we also demonstrated that the T2

increase was not simply isolated to any one muscle (or the limb as a whole) but that an increase in the diversity of T2 values among the 10 muscles was manifest during intensities engendering a $\dot{V}o_2$ slow component. This progressive increase in heterogeneity of muscle "recruitment" was linearly correlated with the $\dot{V}o_2$ slow component magnitude over the three intensity domains examined (Fig. 5).

As expected, the 10 muscles in the lower limb showed an increase in T2 during moderate, heavy, and very heavy intensity cycling exercise. Importantly, however, the distribution of the increased T2 values was not greatly altered during moderate intensity exercise (Fig. 4), reflecting a relatively homogeneous contribution from different muscles (presumably from slow-twitch motor units) to the overall power output. Similarly, after 3 min of heavy intensity exercise, the exercise-induced T2 increase was relatively homogenous between muscles of the leg (i.e., the variance of T2 values throughout the leg was similar to the resting value). However, when heavy exercise was continued to 6 min, the distribution of T2 values became broader and more heterogeneous. This, we suggest, reflects an increasingly diverse recruitment pattern that occurs concomitantly with the expression of the $\dot{V}o_2$ slow component. During very heavy intensity exercise, the distribution of T2 values was already increased by 3 min of exercise, suggestive of a heterogeneous recruitment profile even at this early stage. By 6 min, however, this heterogeneity was extremely profound, with an approximate fourfold increase in T2 variance compared with rest (Fig. 4). These increases in T2 variance manifest during heavy and very heavy intensity cycle ergometry were significantly correlated with the magnitude of the Vo₂ slow component (Fig. 5). By analyzing the cross-sectional images of 10 muscles in the exercising limb, we were able to elucidate a very strong correlation (c.f., Ref. 39) between the diversity of muscle T2 increase (reflecting the diversity of muscle recruitment; Refs. 2, 4, 25, 36, 39, 40) and the magnitude of the \dot{V}_{02} slow component. A progressively increasing diversity of recruitment would, if reliant on less-efficient fibers, i.e., fibers with poor P:O₂ or high energy cost of force production characteristics, result in a progressively increasing "O2 cost" of the external power output and result in the expression of a V_{02} slow component. These data are therefore consistent with the progressive recruitment hypothesis for the Vo₂ slow component mechanism. In addition, for this to be the case, the magnitude of the Vo₂ slow component would be expected to be dependent on the degree of recruitment diversity (as demonstrated in Figs. 4 and 5), i.e., a greater degree of fiber recruitment, leading to a larger magnitude of the Vo₂ slow component, as was seen in comparison of the very heavy intensity exercise responses with those during heavy intensity.

It has been suggested that additional recruitment of "fresh" muscle may also contribute to the increases in muscle recruitment during supra-LT exercise (13). In the present study, we were unable to identify increases in T2 at 6 min from any muscle that was not already "recruited" by 3 min (Fig. 3). This finding is consistent with the notion that fatigue within muscle fibers may be the initial event that leads to additional recruitment within the same muscle and a consequential increase in \dot{V}_{02} .

Progressive recruitment and the $\dot{V}o_2$ *slow component.* It has been repeatedly demonstrated that the muscle and pulmonary $\dot{V}o_2$ responses during heavy and very heavy intensity exercise

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		Мо	Moderate		Heavy		Very Heavy	
	Rest	3 min	6 min	3 min	6 min	3 min	6 min	
			Individ	luals				
Variance, ms	2.5 ± 0.9	3.3 ± 1.3	3.9 ± 1.4	$4.9 \pm 1.7*$	6.5±2.7*†	7.6±3.3*	12.4±3.3*†	
			Gro	ир				
Variance, ms	3.1 ± 0.6	3.7 ± 0.3	$4.2 \pm 0.1 *$	3.6 ± 0.2	6.5±1.7*†	$5.5 \pm 0.8 *$	12.3±5.4*†	
R^2 of Gaussian fit	0.96	0.98	0.99	0.99	0.86	0.96	0.86	
Skewness	0.81	0.41	0.15	0.18	0.11	0.14	-0.02	
Kurtosis	0.29	0.42	0.14	0.21	-0.73	-0.42	-0.85	

Table 3. Distribution characteristics of lower limb T2 during moderate, heavy, and very heavy intensity exercise

Values are means \pm SD. Individual variances are based on n = 10 muscles per subject. Group values are derived from the Gaussian fit of all muscle T2 values for the whole group (n = 109). *Significantly different from rest (P < 0.05). †Significantly different from 3-min value within the same intensity (P < 0.05).

manifest an additional excess component supplementing the underlying fundamental exponential response kinetics (e.g., Refs. 7, 8, 14, 16, 17, 19, 27, 28, 37, 40). While many mechanisms for this $\dot{V}o_2$ slow component have been suggested (30), the most commonly proposed view is that of ongoing



Fig. 4. Probability density distribution of muscle T2 at rest and after 3 or 6 min of cycle ergometer exercise in the moderate, heavy, and very heavy intensity domains. Each curve is fitted with a Gaussian distribution. Values are resting (gray), 3 min of exercise (black), and 6 min of exercise (dashed). Moderate intensity (*A*): mean T2 increased slightly after 3 min (black) of moderate intensity cycling compared with rest (gray) but did not increase further after 6 min (dashed). Heavy intensity (*B*): increases in mean muscle T2 during heavy intensity exercise were accompanied by an increasing variance (broader distribution) after 6 min. Very heavy intensity (*C*): both the mean and distribution of T2 were increased after 3 min of very heavy intensity cycle ergometry compared with rest. This was further exacerbated following 6 min of cycling.

muscle recruitment during the slow component phase, presumably mediated through ongoing muscle fatigue. In particular, recruitment of motor units and/or muscle fibers that manifest a poor force production-to-O₂ consumption ratio is often implicated (6, 10, 11, 39): progressive recruitment of such fibers (and/or a progressive reduction in efficiency of the fatiguing fiber pool) leads to an increase in O_2 cost of the external power output and results in the expression of a Vo₂ slow component. Consistent with this notion, Barstow et al. (6) reported that human subjects with a high percentage of type II fibers in the vastus lateralis (from muscle biopsy) also manifest a larger Vo₂ slow component during cycling exercise. Because initial efficiency of type II (murine) muscle is thought to be low compared with that of type I (5, 11), it may be that the $\dot{V}o_2$ slow component is a manifestation of progressive type II muscle fiber recruitment. The results of the present study are consistent with this progressive recruitment hypothesis; however, the nature of the fibers recruited during the $\dot{V}o_2$ slow component (i.e., whether type I or type II) has yet to be determined.

Various approaches to demonstrate a causal or even a correlative relationship between muscle activation and the $\dot{V}o_2$ slow component have been implemented, but they have not resulted in a consistent picture. Attempts to elucidate additional recruitment of fast-twitch muscle fibers concomitant with the onset of the $\dot{V}o_2$ slow component using iEMG and/or



Fig. 5. Relationship between the increase in T2 variance $[\Delta T2 \text{ variance}_{(6-3)}]$ and $\dot{V}O_2$ $[\Delta\dot{V}O_2$ $(_{6-3)}]$ between 3 and 6 min of cycle ergometry: values reflect the increase in T2 distribution and $\dot{V}O_2$, respectively, during the slow component region. Linear regression shows a significant (P < 0.05) correlation between the increasing variance of T2 (thought to reflect an increasingly diverse muscle recruitment) and the magnitude of the $\dot{V}O_2$ slow component.

the mean power frequency of the surface electromyogram have resulted in either a good correlation (41) or no significant relationship (10, 40).

Recent demonstrations from Krustrup et al. (21, 22) have shown that muscle glycogen and PCr breakdown became increasingly more pronounced and diverse between 3 and 20 min of high intensity exercise. These findings, analyzed from a range of individual fibers from biopsy samples, are consistent with both the present data and the notion of progressive recruitment causing the $\dot{V}o_2$ slow component. The data of Krustrup et al. (21, 22) were derived from biopsy samples and are assumed to be representative of each of the muscles engaged within the exercising limb. Presumably, therefore, the increasing T2 variance in the present data reflects the increasing metabolic reliance on diversely distributed motor units and muscle fibers. Of course, the present findings do not have the resolution required to make measurements at the fiber level, but they do have the benefit of being derived from measurements of all the muscles in the limb. Together, these findings add considerable weight in confirming the progressive recruitment hypothesis of the Vo₂ slow component, which may operate at the level of individual motor units throughout the entire exercising limb.

Conclusion. In conclusion, the present study investigated the proportionality between the magnitude of Vo₂ slow component and muscle activity (estimated from MR T2 imaging) during moderate, heavy, and very heavy intensity cycling exercise. We found that T2 diversity was not significantly altered during moderate exercise where no Vo2 slow component was manifest. In contrast, however, supra-LT exercise in the heavy or very heavy intensity domains resulted in an increasing Vo₂ slow component magnitude between 3 and 6 min of exercise that was associated with a progressive increase in the variance of T2 values, reflecting, we suggest, an increasingly diverse recruitment profile. Overall, there was a significant positive correlation between the variance of thigh muscle T2 values and the magnitude of the $\dot{V}o_2$ slow component. These data support the notion that progressive muscle recruitment within the muscles engaged in the external work is a major determinant of the magnitude of the Vo₂ slow component during supra-LT exercise.

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