Recruitment of the Thigh Muscles During Sprint Cycling by Muscle Functional Magnetic Resonance Imaging

H. Akima1
R. Kinugasa2
S. Kuno2

Abstract

The purpose of the present study was to investigate recruitment patterns of the thigh muscles during maximal sprint cycling by muscle functional magnetic resonance imaging (mfMRI). Twelve healthy men participated in this study and performed 2, 5, and 10 sets of 6-s supramaximal cycling with a load of 7.5% of their body weight with 0.5 min of rest between the sets. Before and immediately after the exercise, T2-weighted MR images, i.e. mfMRI, of the right-thigh were taken to calculate T2 of eleven thigh muscles. Vastus lateralis, semitendinosus, and sartorius were the highest activated, i.e. had the greatest T2 change, among the quadriceps, hamstring, and adductors, respectively, compared with other muscles. Total power output during 2, 5, and 10 sets of sprint cycling was correlated with percent change in T2 in the quadriceps correlated ($r^2 = 0.507$ to $0.696$, $p < 0.01$), the hamstring ($r^2 = 0.162$ to $0.335$, $p < 0.05$ – 0.001), and the adductor muscles ($r^2 = 0.162$ to $0.473$, $p < 0.05$ – 0.0001). With use of stepwise regression analysis, total power output was significantly correlated with % change in T2 of the vastus medialis (VM) ($p < 0.0001$) and vastus intermedius (VI) ($p < 0.05$) ($r^2 = 0.698$, $p < 0.0001$). We concluded that eleven thigh muscles were activated non-uniformly, and that the VM and VI play a key role during maximal sprint cycling.

Key words
Activation pattern · skeletal muscle · neuromuscular plasticity · exercise · human · recruitment

Introduction

Muscle use during exercise has been evaluated by invasive and non-invasive techniques, e.g. glycogen depletion by muscle biopsy [14,27] and surface electromyography (EMG) [15,16,18], respectively. The muscle biopsy technique has been widely used for determination of recruitment based on the magnitude of glycogen utilization in fast-twitch (FT) and slow-twitch (ST) fibers during exercise. In a classic study, Golnick et al. [14] reported recruitment pattern of FT and ST fibers during endurance bicycle exercise, demonstrating that ST fibers were recruited at the early phase of endurance exercise, and then the contribution of FT fibers gradually increased. Although this type of experiment is useful for determining of fiber type–specific recruitment during a given exercise, quite a small region of muscle (~10 mg), usually with biopsies being taken from the vastus lateralis, was evaluated corresponding to approximately $1.3 \times 10^{-4}$% of muscle volume in the quadriceps femoris [6,7]. There is no doubt that the VL does contribute to bicycle exercises, however, it is unclear, as far as we know, how much the muscle activates in a given exercise according to the previous studies, this being because of the technical difficulty to measuring the activation pattern in several muscles located at surface and deeper regions simultaneously.

Bicycle exercise has been used, due to its easily standardized and measurable resistance, in thousands of exercise physiology and

Affiliation

1 Research Center of Health, Physical Fitness & Sports, Nagoya University, Furo, Chikusa, Aichi, Japan
2 Center for Tsukuba Advanced Research Alliance, University of Tsukuba, Tsukuba, Ibaraki, Japan

Correspondence
H. Akima · Research Center of Health, Physical Fitness and Sports, Nagoya University · Furo, Chikusa · Nagoya, Aichi 464-8601 · Japan · Phone: +81527893954 · Fax: +81527893957 · E-mail: akima@htc.nagoya-u.ac.jp

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Bibliography
biomechanics research articles. During bicycle exercise, muscle groups and individual muscles surrounding the hip, knee, and ankle joints work as primary contributors of power output and mechanical work done against ergometer resistance. According to Ericson et al. [11], the contributions of muscular energy production by the hip extensors, hip flexors, knee extensors, knee flexors, and ankle plantar flexors during cycling (120 W, 60 revolutions per min) were 27%, 4%, 39%, 10%, and 20%, respectively. These results demonstrated that the knee extensors and knee flexors contribute approximately half of the work done during cycling, and that thigh muscle may play a key role in cycling exercise. Therefore, in this study we focused on the thigh muscles during cycling exercise, and determined which individual muscles within the thigh were associated with power output during cycling using muscle functional magnetic resonance imaging (m fMRI). This type of research would be useful in gathering applicable information for applied human physiology.

To acquire the physiological and biochemical characteristics of human skeletal muscle using exercise-induced change in T2, i.e. m fMRI [1, 2, 8, 22, 31, 32] has been used. It has been demonstrated that a T2 change correlates with integrated EMG activity [1], related to isometric torque induced by electromyostimulation [2], increasing with exercise intensity [1, 2, 20, 22], and the metabolic state [30, 31] of the skeletal muscle. Moreover, this technique can utilize for determination of the recruitment plasticity between neuromuscular compartments within the tibialis anterior muscle during dynamic dorsiflexion exercise in humans [4]. Thus, this technique would be ideal for investigating the neuromuscular activation patterns with a high spatial resolution of exercising human skeletal muscles [20].

The purposes of this study are to investigate activation patterns of eleven thigh muscles using m fMRI signal changes and the relationship activation level and power output during 2, 5, and 10 sets of sprint cycling.

**Materials and Methods**

**Subjects**

Twelve healthy men participated in this study and all subjects considered themselves to be in good to excellent physical condition. Before the experiment, the procedures, purposes, and risks associated with the study were explained and written informed consent was obtained. The experimental protocol had been approved by the Human Ethics Committee of the University of Tokyo. Physical characteristics of the subjects are as follows: age: 22.3 ± 1.2 yr, height: 172.4 ± 1.6 cm, weight: 64.0 ± 2.2 kg (means and SE).

**Sprint cycling exercise**

After a few minutes of warming up, subjects performed 2, 5, and 10 sets of 6-s supramaximal exercise interspersed with 30-s rest periods on an electrically braked bicycle ergometer (Powermax VII, Combi Cop., Tokyo, Japan) with semi-upright posture equipped with toe-clips [13]. The load was set 7.5% of subjects’ body weight and for each 6-s exercise period, subjects were encouraged to perform with maximum effort. The power data (peak power, mean power, and pedaling rate) were sampled (200 Hz) and stored on a personal computer (Dyna Book, Toshiba Cop., Tokyo, Japan) via a specially designed software (Combi Cop., Tokyo, Japan) for calculation of mechanical output during cycling. We calculated total mean power output, simply summed mean power output in each set, during 2, 5, and 10 sets of sprint cycling. To avoid the effect of muscle fatigue on performance and the other measurements, 2 and 5 sets of sprint cycling were randomly performed on a same day at least 1.5 hours apart between the bouts, and 10 sets of exercise were performed on a different day.

**Muscle functional magnetic resonance imaging**

Standard MR images of the right-thigh were taken using a 0.2-T magnet (AIRIS II, Hitachi Medical Cop., Tokyo, Japan) essentially as done previously [2, 3, 8]. Five 10-mm-thick transaxial T2-weighted images (fast spin-echo, repetition time = 1500 ms; echo time = 30 and 90 ms) of the right-thigh spaced 30 mm apart were collected with an extremity coil. The third image was corresponded to the middle of length of the thigh. A 256 × 128 matrix was acquired with one excitation and a 32-cm field of view. Ink marks on the thigh aligned with cross-hairs of the imager allowed for similar positioning in the magnet bore over repeat scans. The subjects were fully rested before the first scan. We needed approximately 1 min from the end of the sprint cycling exercise to the beginning of the second scan.

m fMRI were transferred to a personal computer (Power Mac G4, Apple Computer Inc., Cupertino, CA, USA) as the DICOM (Digital Imaging and Communications in Medicine) file format for calculation of T2 by using a modified version of the public domain National Institute of Health (NIH) Image program (written by Wayne Rasband at the NIH and available from the Internet by anonymous ftp from zippy.nimh.nih.gov), as previously done [2, 3, 8, 22]. Original m fMRIs were shown in Fig. 1 obtained at rest and after 2, 5, and 10 sets of the exercises. Muscles investigated were as follows: vastus lateralis (VL), vastus intermedius (VI), vastus medialis (VM), rectus femoris (RF), biceps femoris long head (BFL), semitendinosus (ST), semimembranosus (SM), gracilis (Gr), sartorius (Sr), adductor longus (AL), and adductor magnus (AM). After spatial calibration, two to three regions of interest (ROIs: 1 to 1.2 cm²) per individual muscles were defined to calculate mean T2 value at five slices. Muscle T2 was calculated on a pixel by pixel basis in the ROIs from the fomular T2 = (ta − tb)/ln(ia/ib), where ta and tb are spin-echo collection times and ia and ib are signal intensities [2, 3, 22]. We paid attention to exclude visible aponeurosis, vessels, fat, and nerves from the ROIs. The reproducibility of T2 measurements using this procedure has been reported elsewhere [3, 8].

**Statistics**

Relative change in each muscle T2 was analyzed by using two-way (3 × 11: number of sets × muscles) analysis of variance (ANOVA) with repeated measures. Significant main effects and interactions were compared using the least squares difference (LSD) post hoc test. The correlation between power output and muscle activation (i.e. % change in T2) in eleven muscles across all sprint exercise intensities was determined by stepwise regression (forward step) for the dependent (power output). Seven independent variables (% change in T2 of each seven thigh muscles) were entered into the stepwise regression if they repre-
sented a significant contribution to the explained variance (F to enter ≥ 4.00, F to remove ≤ 3.996) corresponding to an alpha level of p < 0.05. The level of significance was set at p < 0.05 for all analysis. Data are presented as mean ± SE.

Results

Mean power output during 2, 5, and 10 sets of sprint cycling were 575 ± 25 W, 545 ± 22 W, and 495 ± 22 W, respectively. The power output gradually decreased with the increase in sets (2 sets vs. 5 sets, p < 0.01; 5 sets vs. 10 sets, p < 0.0001). Total mean power output during 2, 5, and 10 sets of sprint cycling were 1150 ± 50 W, 2722 ± 111 W, and 4947 ± 220 W, respectively (2 sets vs. 5 sets, p < 0.0001: 5 sets vs. 10 sets, p < 0.0001, 2 sets vs. 10 sets, p < 0.0001).

Fig. 2a shows that the % change in T2 of the quadriceps femoris after 2, 5, and 10 sets of sprint cycling. In the RF, VL, VI, and VM, the % change in T2 linearly increases with the number of sets. The % change in T2 of the VL was significantly higher than the VI and VM for 2, 5, and 10 sets of sprint cycling. With the 5 sets, the RF shows a significant higher % change in T2 than the VI, and with the 10 sets the % change in T2 of the RF is higher than the VI and VM and lower than that of the VL.

Fig. 2b shows that the % change in T2 of the hamstrings after 2, 5, and 10 sets of sprint cycling. The aspect of the % change in T2 is different with that of the quadriceps. In all three muscles, a sharp increase in T2 is found with the 2 sets of sprint cycling, and then it linearly increases. The ST shows a significant higher T2 with all three tested during the exercises (2 sets: p < 0.05 vs. SM, 5 sets and 10 sets: p < 0.05 vs. BFI, p < 0.001 vs. SM). With the 5 and 10 sets of sprint cycling, the BFI shows a significant higher T2 than the SM (p < 0.001).

Fig. 2c shows that the % change in T2 of the adductor muscles after 2, 5, and 10 sets of sprint cycling. In the adductor muscles, a unique activation pattern is found: T2 of the Gr, Sar, and AM increases linearly with the number of sets, however, no activation is observed in the AL. The T2 change in the Gr is significantly higher than the AM at 5 and 10 sets of sprint cycling (all p < 0.01). The AL is significantly lower than the other three muscles with all the sets tested (all p < 0.0001).

Figs. 3–5 show the relationship between power output during sprint cycling and the % change in T2 in the quadriceps (Fig. 3), hamstring (Fig. 4), and adductors (Fig. 5), respectively. Total power output during 2, 5, and 10 sets of sprint cycling was linearly correlated with the percent changes in T2 of the VL (r² = 0.569, p < 0.0001), RF (r² = 0.507, p < 0.0001), VI (r² = 0.566, p < 0.0001), VM (r² = 0.696, p < 0.0001). In the hamstring, total power output was linearly correlated with the percent changes in T2 of BFI (r² = 0.335, p < 0.001), ST (r² = 0.226, p < 0.01), and SM (r² = 0.162, p < 0.05). Finally, in the adductor muscles, total power output was linearly correlated with the percent changes in T2 of Gr (r² = 0.162, p < 0.05), and Sar (r² = 0.362, p < 0.0001), and AM (r² = 0.473, p < 0.0001).

Fig. 6 shows that a comparison of the T2 change among eleven thigh muscles at each sprint cycling during the three different numbers of sets. Overall, the response of the thigh muscles to
All independent variables (% change in T2 in eleven thigh muscles) were used in a multiple stepwise regression analysis to select variables influencing power output during 2, 5, and 10 sets of sprint cycling. Table 1 shows that summary of the regression analysis. The only variables that significant correlated with power output in the final model were VL (p = 0.0466) and VM (p < 0.0001). The final regression equation was:

Total power output = 47.0 × (VL) + 133.6 × (VM) + 295.5

The correlation coefficient (R) and adjusted R² for this model was 0.846 and 0.698, respectively.

Discussion

The aim of this study is to clarify the activation patterns in eleven thigh muscles during maximal sprint cycling by fMRI. The main results of this study are that T2 linearly increased in all of the tested thigh muscles excluding the AL (Figs. 2 and 6), and that the significant predictors of power output during sprint cycling were the VL and VM as a result of a stepwise regression analysis (Table 1, R² = 0.698). Richardson et al. [26] reported that muscle activation patterns in fatigue cycle exercise lasted from 2 to 2.5 min with 90% of the maximal work rate using fMRI. In the study, they stated that activation patterns appeared to be similar across ten thigh muscles (% increase in T2 ranged from 9 to 20%), however, the VL and AM were the most activated muscles during this type of exercise, which is consistent with the present study. The present study shows that the VL is one of the most activated among the tested muscles across 2, 5, and 10 sets of sprint cycling. The total power output during maximal sprint cycling correlated to the % change in T2 in ten muscles out of eleven (Figs. 3–5), suggesting that these muscles have increased activation according to the increase of work. In all individual muscles in the quadriceps, we found very high and significant correlation coefficients between the total power output and % change in T2 (r = 0.712–0.834). On the basis of this result shown in Fig. 3 and stepwise linear regression analysis (Table 1), we showed that the quadriceps, especially the VL and VM, primarily contributed to this type of exercise as has been suggested in the previous studies [15, 18].

The VL has been evaluated biochemically and physiologically as one of the working muscles during movement such as running, cycling, and knee extension in the numerous studies [8, 22, 27]. The primary reason of taking a biopsy from the VL is that this muscle is large, has a relatively simple anatomy, and is one of the working muscles with lower limb exercise in the field of exercise physiology. As far as we know, however, there is no well-established data on how the VL contributes to a given exercise. We found that the VL is one of the most activated during repeated sprint cycling exercise in this study, because the VL had the higher T2 than the other thigh muscles (Fig. 6) and the volume of this muscle is the largest within the thigh [5–7]. Is this finding sprint cycling specific? Three studies have been reported muscle activation pattern during bicycle exercise found by fMRI [12, 25, 26] and in two of these studies they showed that the VL represented the highest exercise-induced T2 change [25, 26], how-

sprint cycling varied, however a similar activation pattern is found across the three different numbers of sets. In Fig. 6, all significant differences are represented comparing them with the VL, because this muscle shows the highest % change in T2 across the number of sets. There is no significant difference with 2 sets of sprint cycling, except for the AL (p < 0.001). With 5 sets of sprint cycling, T2 of the VL (p < 0.01), SM (p < 0.01), and AL (p < 0.001) show significantly lower values than the VL, and the AL (p < 0.01), VM (p < 0.05), BFI (p < 0.05), SM (p < 0.001) and AL (p < 0.001) show significantly lower muscle T2 with 10 sets of sprint cycling.

Fig. 2a to c Percent increases in T2 of the quadriceps femoris (a), hamstring (b), and adductor muscles (c) after 2, 5, and 10 sets of sprint cycling. Values are means and SE. At panel (a), *p < 0.05, **p < 0.01 vs. VI and VM, #p < 0.05 vs. VI, †p < 0.05 vs. VL, VI, and VM. At panel (b), *p < 0.05 vs. SM, #p < 0.05 vs. BFI, p < 0.001 vs. SM, †p < 0.0001 vs. SM. At panel (c), *p < 0.01 vs. AM, †p < 0.001 vs. Gr, Sar, and AM. VL: vastus lateralis, VI: vastus intermedius, VM: vastus medialis, RF: rectus femoris, BFI: biceps femoris long head, ST: semitendinosus, SM: semimembranosus, Gr: gracilis, Sar: sartorius, AL: adductor longus, AM: adductor magnus.
Fig. 3  Relationship between % change in T2 of the quadriceps femoris and total mean power output during 2, 5, and 10 sets of sprint cycling. Open circle, closed circle and open circle with bold line represented 2, 5 sets, 10 sets of sprint cycling, respectively. VL: vastus lateralis, VI: vastus intermedius, VM: vastus medialis, RF: rectus femoris.

Fig. 4  Relationship between % change in T2 of the hamstring and total mean power output during 2, 5, and 10 sets of sprint cycling. Open circle, closed circle and open circle with bold line represented 2 sets, 5 sets, and 10 sets of sprint cycling, respectively. BFI: biceps femoris long head, ST: semitendinosus, SM: semimembranosus.
ever, they tested constant work load cycling exercise lasting 2 to 5 min, not sprint cycling.

The metabolic capacity of muscle is important for understanding of an mfMRI study because the mfMRI signal change is related to the metabolic capacity of the working muscles [30]. According to Johnson et al. [17], they demonstrated that there seems to be no marked difference in muscle fiber types in the human thigh muscles, especially, within the quadriceps femoris, there seems to be no apparent fiber type differences among individual muscle. Thus, the higher T2 change after sprint cycling would be due to the activation level and/or quantity rather than the fiber type related the metabolic difference. From these results, we would like to emphasize that the VL is one of the most activated muscle during maximal sprint cycling as evaluated by mfMRI signal change. Furthermore, we found that the VM appears to be much more important for this type of exercise from the result of regression analysis (Table 1).

The hamstring (BFI, ST, and SM) also demonstrated higher muscle activation in this study. In particular, the ST demonstrated one of the highest % change in T2 compared with the other muscles in the hamstring after 2 and 5 sets of sprint cycling. In biomechanical studies [15,18], the hamstring was recruited from just after starting the downstroke, i.e. knee extension, to the middle of the upstroke, i.e. knee flexion, of pedaling phases. Hautier et al. [16] suggested that the knee flexor muscle (BF) was not fatigued after 15 sets of maximal 5-s sprint cycling by EMG signal analysis, being very similar experimental settings to the present study, however, the result is conflicted with the determination of our study. In our study, the hamstring, e.g. BF and ST, showed high % change in T2 after 2 to 10 sets of exercise, implying this muscle group is greatly involved in sprint cycling and would be fatigued to be similar to the quadriceps at the end of the exercise. Because, there was no significant difference in % change in T2 between the VL and ST across all experimental conditions (2 to 10 sets of sprint cycling), and there seemed to be no difference in % change in T2 between the other individual muscles in the quadriceps (i.e. VI, VM, and RF) and those in the hamstring (i.e. BFI and SM) (see Fig. 6). Jorge and Hull [18] reported that when greater cycling power production was found, EMG activity of the quadriceps femoris (RF, VL, and VM) and the hamstring (BF and SM) increased, suggesting that these two muscle groups were involved in the cycling. Furthermore, as pointed out in the above, fiber type is one of the affecting factors in exercise-induced T2 change, i.e. type II fibers showed a higher T2 change [24]. Johnson et al. showed that the % type I fibers in the BFI was approximately 20% higher than that of the VL and VM. These results suggested that the hamstring would be fatigued similar to the quadriceps at the end of the sprint exercise independent of the number of sets. The discrepancy would be partly due to differences of methodology (mfMRI vs. EMG), physical activity of subjects (untrained vs. trained), protocol (2, 5, and
Sar: 41 and 25 cm, respectively), acting adduction of the thighs, and biarticular muscles crossing the hip and thigh joints. Van Bolhuis et al. [9] suggested that the biarticular muscles thought to control the external force direction. However, it is not well known how these two biarticular muscles, i.e. the Gr and Sar, contribute to this type of exercise as well as to human movement, further studies are needed on this point.

Another surprising result was that the AM also showed higher activation among the adductor muscle group during sprint cycling. The exact function of the AM during human movement such as walking, running, and cycling is not well known. Richardson et al. [26] using fmMRI technique showed that T2 in the AM increased by 20%, which was the highest among the ten thigh muscles, after bicycle cycling at 90% of the maximum work rate. Moreover, in exhaustive horizontal and uphill (10% grade) running the AM demonstrated a higher degree of use when evaluated by fmMRI [29]. These studies suggested that the AM would be highly involved in human movement such as bicycling and running. According to our human unloading study, muscle volume of the AM decreased by approximately 7% after 20 days of bed rest (Akima et al. unpublished observations), this reduction is comparable to that of the quadriceps (7%) [5,6]. These results suggested that the AM would work as one of the activating muscles in physical activities such as running and cycling as well as our daily life activities. No activation was found in the AL even after 10 sets of sprint cycling, this is clearly shown in Fig. 1. The result is also interesting. Fleckenstein et al. [12] have shown that the AL is an inactive muscle during several intensities (20, 50, 70, and 100% of peak VO2) of 5-min constant workload cycling exercise. The mechanisms of non-uniform activation patterns among the individual adductor muscles do not account for the fmMRI data. This may be due to biomechanical (i.e. moment arm, force-length curve during sprint cycling etc.) and muscle architectural (penetration angle and fiber length etc.) factors among the individual muscles in the adductor muscle group.

In stepwise linear regression analysis, % change in T2 in the VL and VM accounted for 70% (i.e. adjusted R2 = 0.698) of the total variance in total power output during sprint cycling. The rest of nine muscles evaluated in this study were not selected by the stepwise regression analysis. The final regression equation showed higher regression coefficient in the VM than in VL, suggesting activation of the VM would be better variable for explanation of the total variation in total power output. As far as we

10 sets of 6-s exercise with 30-s rest vs. 15 sets 5-s exercise with 25-s rest), and differences of posture during exercise [18].

The surprising result of this study is that the Gr and Sar showed very high activation during maximal sprint cycling through 2 sets to 10 sets of exercise. Interesting characteristics of the two muscles found in a small physiological cross-sectional area (both approximately 3 to 4 cm2) [5,6] was long fiber length (Gr and

![Graph](image)

Fig. 6  Percent increases in T2 of eleven thigh muscles after 2, 5, and 10 sets of sprint cycling. * p < 0.05, ** p < 0.01, # p < 0.001 vs. VL. VL: vastus lateralis, VI: vastus intermedius, VM: vastus medialis, RF: rectus femoris, BFl: biceps femoris long head, ST: semitendinosus, SM: semimembranosus, Gr: gracilis, Sar: sartorius, AL: adductor longus, AM: adductor magnus.

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VL: vastus lateralis, VM: vastus medialis. SE: standard error

Table 1 Stepwise regression analysis
In conclusion, we investigated activation pattern of eleven thigh muscles after 2, 5, and 10 sets of maximal sprint cycling using mfMRI. The level of activation in individual muscles varied but showed a similar pattern across the number of sets. These results suggested that the thigh muscles activated non-uniformly during sprint cycling from mfMRI observation.

References


