Water exchange induced by unilateral exercise in active and inactive skeletal muscles

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Nygren, Anders T., and Lennart Kaijser. Water exchange induced by unilateral exercise in active and inactive skeletal muscles. J Appl Physiol 93: 1716–1722, 2002. First published August 9, 2002; 10.1152/japplphysiol.01117.2001.—Water exchange was evaluated in active (E-leg) and inactive skeletal muscles by using 1H-magnetic resonance imaging. Six healthy subjects performed one-legged plantar flexion exercise at low and high workloads. Magnetic resonance imaging measured calf cross-sectional area (CSA), transverse relaxation time (T2), and apparent diffusion capacity (ADC) at rest and during recovery. After high workload, inactive muscle decreased CSA and T2 by 2.1% (P < 0.05) and 3.1% (P < 0.05), respectively, and left ADC unchanged. E-leg simultaneously increased CSA, T2, and ADC by 4.2% (P < 0.001), 15.5% (P < 0.05), and 12.5% (P < 0.001), respectively. In conclusion, ADC and T2 correlated highly with muscle volume, indicative of extravascular water displacement closely related to muscle activity and perfusion, which was presumably a combined effect of increased intracellular osmoles and hydrostatic forces as driving forces. A distinguishable muscle temperature rise was initially detected in the E-leg after high workload, and the ensuing recovery of ADC and T2 indicated delayed interstitial restitution than restitution of the intracellular compartment. Furthermore, absorption of extravascular water was detected in inactive muscles at contralateral high-intensity exercise.

diffusion; magnetic resonance imaging; tissue water; transverse relaxation time

MAGNETIC RESONANCE IMAGING (MRI) can be applied on local skeletal muscle regions by analyzing variables pixel by pixel or within multiple local regions, including active as well as less active muscles (31). Therefore, simultaneous evaluation of water exchange in active and inactive muscles during dynamic exercise is valuable to elucidate the signal shift in muscle that is related to exercise and is beneficial to study how different MRI techniques that focus on water displacement relate.

The physics of water exchange in the skeletal muscle is highly complex and multifactorial. The magnetic resonance signal has been shown to be multieponential, which indicates a multicompartamental origin. 1H-MRI using transverse relaxativity focuses on the intrinsic property of water and its exchange between the different compartments as well as the binding capacity of the water molecule to subcellular structures (11). Most present studies have used a monoexponential transverse relaxativity analysis, although a multiexponential behavior has been described (24). The monoexponential proton transverse relaxation time (T2) of skeletal muscle is known to increase by exercise (6, 20). Increased intracellular water content (4, 18) and mechanisms related to aerobic capacity, e.g., net intramuscular accumulation of osmoles (22), are assumed to be the most important factors related to prolonged T2 with exercise. To what degree other factors such as H+, phosphocreatine, or water related to the microvasculature (5) would contribute to the altered T2 still has not been determined. The increased water content in exercising muscles is known to affect both extra- and intracellular volumes (6, 27, 28). However, it is presumed that extracellular water affects the T2 more than total tissue and intracellular water in resting skeletal muscle (19). With diffusion-weighted MRI and calculation of the mean apparent diffusion capacity (ADC), it is possible to estimate water motion related to small and random movements in the tissue. These are probably related to both extra- and intracellular compartments, although the size of the extracellular volume seems to be the most important component. In vitro experiments have shown that ADC decreased when cells swell (1). A decreased ADC has also been related to cell swelling and decreased extracellular volume in the brain (2, 9, 31). Increased ADC, found in exercising skeletal muscles, is accordingly presumed to reflect increased water motion, predominantly in the extracellular compartment, but effects of cytoplasmatic motions are unclear. Furthermore, a temperature-related increase in ADC by ~2%/°C (15) needs to be considered as being due to thermal storage in active muscle. Neurohumoral activity during exercise is known to affect resting skeletal muscle with exposure of increased sympathetic nerve activity (21) and vasoconstriction (3). Although not extensively studied, there are indications that muscle volume could decrease in nonexercising muscles. Reduced cross-sectional area

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(CSA) of nonexercising muscles has been documented in conjunction with dynamic exercise associated with biking (23) and plantar flexion (16). However, evidence of a water shift in nonexercising muscles during exercise has not been detected with isotope techniques (27). It has not been determined whether the decreased volume of nonexercising muscles is a result of reduced tissue water content or mostly of reduced vascular filling as a consequence of vasoconstriction.

The study was designed to simultaneously evaluate exercising and nonexercising skeletal muscles during graded dynamic exercise and, moreover, to study whether MRI was able to measure altered extravascular and transverse relaxativity relates to muscle bulk volume. MRI was used to measure calf CSA, regional muscle T2, and water diffusion measured as the mean ADC. The primary aim of this study was to define whether dynamic exercise with a small muscle mass could induce a water shift in inactive muscles. The second aim was to evaluate how the responses of muscle transverse relaxativity, diffusion, and volume are related to graded dynamic exercise and during recovery in both active as well as inactive muscles.

METHODS

Subjects

Six healthy male students were included with a mean age of 25 yr (range 23–26 yr). The Ethics Committee of the Karolinska Institutet approved the study.

Exercise Protocol

A specially designed foot ergometer in a nonmagnetic material constructed for plantar flexion exercise was used (16). Subjects were familiarized with the exercise setup within 1 wk before exercise in the magnetic resonance imager by performing unilateral graded plantar flexion exercise with the right foot and keeping the contralateral left leg resting. The highest workload sustained for 9 min was tried out. Two subjects exercised at the highest workload of 12 kg, and four subjects exercised at 22 kg. Exercise in the magnetic resonance imager was thereafter performed on 2 different days. The highest workload sustained for 9 min was tried out. Two exercise setups with the (4 kg) or high workload (12 or 22 kg) on the first day and the other workload on the next day. Two exercise setups with the same workload were performed with a 50-min interval between bouts (Fig. 1). T2 acquisition was repeatedly scanned during the 45 min after the first stop, and diffusion weight imaging (ADC) was scanned during the 15 min after the second stop.

MRI

Before imaging, all subjects had been resting in a supine position for at least 30 min. Single-slice imaging was performed with a 1.5-T magnet at 63 MHz by using a birdcage quadrature head coil with a uniform field (Signa, General Electric, Milwaukee, WI). Transaxial imaging was performed at the largest CSA of the calf. Padding was placed under the knees in the birdcage to prevent changes in calves’ position during scanning. However, the padding did not ensure an identical angle of the knees between imaging days. The diagonal of the measured slice could therefore be different on the 2 imaging days and probably affected the measured CSA at rest. Accordingly, the percent change from rest was used when comparing exercise levels achieved on different days.

T2 images were obtained by a multiple spin-echo sequence [repetition time (TR)/echo time (TE): 1,500/15, 30, 45, 60 ms; slice thickness: 10 mm; field of view: 40 × 20 mm, matrix 256 × 128 mm, which gave the in-plane resolution of 1.6 × 3.1 mm²], with a 2-min interval between points. CSA was measured with manual planimetry on the image with a TE of 15 ms performed by one examiner without knowing the subject’s name and date of the study. Signal intensity values were obtained within a region of interest (ROI) of ~2.2 cm² on the lateral portion of the gastrocnemius, excluding visible vessels and fascia. T2 was calculated with the least square method by fitting four echoes to monoexponential decay by using the equation (regression coefficient) 10⁻¹⁻¹⁰³ as T2. Diffusion-weighted spin echo echo-planar imaging using Stejskal-Tanner diffusion was applied with tetrahedral gradients (29) with special concern to achieve a high temporal resolution. Acquisition parameters were b value = 600 s/mm², TR/TE = 4,000/63 ms, field of view = 40 × 40, matrix = 128 × 128, and slice thickness = 10 mm, with an interval between points of 0.33 min. ROI was chosen to be large, placed within the lower half of the calf, including gastrocnemius, soleus, and peroneus longus, because the signal-to-noise ratio was presumed to be low. The outer and central parts (vessels, fibula, and tibia) of the calf border were excluded. The diffusion coefficient was also calculated according to a monoeponential model. When the immediate postexercise value was presented, a mean of the three initial values within the first minute was used.

Statistical Analysis

The statistical significance was evaluated by t-test for independent and dependent samples, and one-, two-, and three-way ANOVA and Pearson’s product-moment correlation with regression line and 95% confidence interval were graphically displayed. Values from the whole acquisition were used in the ANOVA analysis. Significant statistical level was considered at P < 0.05, and values were expressed as means ± SD. All analyses used Statistica 5.5 software (StatSoft, Tulsa, OK).

RESULTS

MRI

Changes during exercise indicated by the first postexercise value. Immediate postexercise values are presented in Table 1. CSA, T2, and ADC at rest did not differ significantly between the 2 days. However, CSA differed numerically by ~3% in the active skeletal muscle between low and high workloads, probably be-
cause of different knee padding in the coil. With exercise, a progressively decreased CSA was found in the inactive skeletal muscle because CSA increased in the active leg, which caused a significant interaction between the legs. T2 did not change in either leg at low workload, but an interaction between the legs was found at high workload (P < 0.05) because T2 shortened by 3.1 \pm 2.4\% in the inactive leg and was prolonged by 15.5 \pm 11.5\% in the active leg. Motion artifacts at high workload probably affected some ADC values in one subject; therefore, all values in the inactive leg and the 12 last values in the active leg were excluded. ADC did not change significantly in the inactive leg at either workload but increased significant in the active leg by 7.1 \pm 3.0\% at low workload and by 12.5 \pm 6.9\% at high workload.

**Correlation between CSA, T2, and ADC.** Both CSA and ADC, but not T2, increased at low workload. A strikingly highly positive correlation was found between CSA and T2 in the exercising calf (r = 0.94, P < 0.001; Fig. 2A), and a nearly significant correlation was found in the nonexercising calf (r = 0.54, P = 0.07). Moreover, ADC correlated highly with CSA (r = 0.86, P < 0.001); however, this was mostly related to the correlation within the active leg (r = 0.66, P < 0.05), but a decreased or unchanged CSA, as seen in the inactive leg, was related to a decreased diffusion in half of the subjects (Fig. 2B). Furthermore, a correlation of ADC with T2 was found (r = 0.70, P < 0.001).

**Postexercise recovery period.** Both CSA and T2 had a significant three-way interaction with ANOVA regarding leg, load, and time. ADC had fewer interaction terms but presented significant changes between loads and time (Table 1). After low workload, there were only minor, nonsignificant changes of T2 and CSA in the inactive leg, probably because of a rather large variation within the group. Significant differences between loads were present during the initial 8 min for CSA and during the first 2 min for T2 (P < 0.05). The immediate postexercise ADCs in the inactive leg were not significantly changed from resting values (Table 1).

The recovery of CSA in the inactive leg after high workload was defined as an exponential growth (Fig. 3). The half-time (t_1/2) could be calculated, when considering a monoexponential function, and equaled 24.2 \pm 12.6 min. Likewise, the decay of CSA and transit time was considered monoexponential in the active leg after high workload with a t_1/2 of 21.9 \pm 12.6 min (range 4.3–36.5 min) and 22.3 \pm 12.3 min (range 9.8–40.8 min), respectively, with closely correlated recovery times (r = 0.80, P = 0.05). The slow recovery of ADC in both legs, especially in the exercising leg, probably explained the higher values in the active leg at rest before the second exercise bout. When calculating the 50% recovery of ADC in the active leg at high workload, the extrapolated late ADC value obtained 45 min after the first exercise bout but before the ADC exercise bout was used. This gave a t_1/2 of 65 \pm 36 min (range 27–116 min), a significantly longer recovery time than that of both CSA and T2 (P < 0.05), which was still significantly prolonged if the suspect initial temperature-related values were excluded.
The effect from the prior exercise bout preceding the T2 acquisition was probably not fully normalized at the time when resting ADC values were obtained. ADCs in the inactive leg at low workload were therefore chosen to be the reference resting value for both legs when the percent change was described. However, there could be a slight overestimation of resting ADC values because $t_{1/2}$ was 172 ± 152 min in the inactive leg at low workload, but with low ADCs the error would be small. If ADC values in active leg during the initial 4-min postexercise recovery were excluded, the level was still elevated by ~6% at low workload and by ~10% at high workload (Fig. 4), with a mean difference between loads of 74% (range 45–99%) during the recovery period (Fig. 5B). Because of the large variation of ADC values, no significances were found between workloads in the immediate postexercise value. However, a significant interaction was found between workloads (Table 1), which indicated a workload-dependent elevation of ADCs in the recovery period that was indicative of a response to graded exercise.

DISCUSSION

This study presents a novel correlation of skeletal muscle T2 with ADC and how they are related to muscle bulk volume during recovery from exercise in both active and inactive muscles. Furthermore, evidence of extravascular water absorption from inactive muscles at contralateral high-intensity dynamic plantar flexion exercise was found.

Absorption of extravascular water in inactive muscles was indicated by decreased muscle CSA and shortened monoexponential T2 compared with resting values with known, nonsignificantly changed blood flow to the nonexercising calf during high-intensity contralateral plantar flexion exercise (16). The nearly significant correlation between T2 and CSA in the inactive muscle suggests a response to graded exercise.
unchanged or decreased CSA (Fig. 2). Indicated small or even decreased water motion with correlation between changes in ADC and CSA that muscles between workloads, there was a significant activation. Despite unchanged mean ADC in inactive tralateral exercise intensity and neurohumoral ac-

Therefore, increased oncotic pressure was not considered a dominant cause of dehydration of inactive mus-

Therefore, fluid absorption from inactive tissues seems to be needed (13); a mechanism that includes inactive mus-

Furthermore, there are some interesting results related to exercising muscles and especially with reference to the diffusion of water that could give some additional insight to water exchange in skeletal muscle as a result of exercise. It is known that water flux can be considered to be driven by a concentration gradient described by Fick’s law (particle flux = \(-\)diffusion coefficient \(\times\) concentration gradient) and not necessarily by an osmotic gradient, but the Fick’s law equation follows a result of random motion that is measured with diffusion-sensitive MRI and is calculated as ADC. It is not only the size of the interstitial space that may affect ADC, although it may dominate, but also the altered flux in the compartment. Therefore, lymphatic flow could also, if considered random, affect the random motion of water and measured ADC since its flow is presumed to be hundreds to a thousand times slower than resting perfusion in skeletal muscles (7). To what degree lymphatic flow could affect tissue-water motion and calculated ADC is not known but cannot so far be neglected. Moreover, increased temperature is known to elevate random water motion; therefore, temperature-dependent elevated ADC values are expected in exercising muscles. The initial postexercise effect of hyperemia can be neglected because of nonrandom high velocities in vessels and would not give significantly false elevated values with our pulse sequence. However, tissue motion due to pulsations could be a problem. We did not measure local muscle temperature per second, but the presumed increased muscle temperature of \(-1^\circ \text{C}\) (Fig. 4) is within the range of previously reported findings (8, 12, 25). Furthermore, González-Alonzo et al. (8) showed that during 6 min of recovery, \(-60\%\) of stored muscle temperature was released with an exponential decline. Therefore, increased water motion by temperature is likewise ex-

![Image](http://jap.physiology.org/)

J Appl Physiol • VOL 93 • NOVEMBER 2002 • www.jap.org
pected in our study, and maybe throughout recovery, and is overlaid on the effect from the extravascular compartments, predominantly the interstitium. The temperature-related effect on ADC after high-intensity exercise could after 5 min of recovery probably only explain an additive effect by <0.5%. Moreover, we did not have any signs of transferred heating of the non-exercising calf because ADC was not significantly elevated in the inactive leg. It is reasonable to suggest that with prolonged elevated temperatures in exercising muscles, transferred heating would be expected in nonexercising muscles as previously described after heavy knee extension (10).

The obtained ADC values in the exercising calf need to be interpreted cautiously, especially because the values showed a large signal variation with extrapolated resting values only. However, the immediate postexercise ADC value in exercising muscles was during high-intensity exercise increased in accordance with a previous study (14), but a graded response could also be documented in this study. A workload-dependent elevation of ~7 and 12.5% during low- and high-intensity exercise, respectively, was found, and thereafter, there was a slow decline during 15 min of recovery (Figs. 4 and 5B). The increased ADC, like T2, correlated in active muscles to muscle volume, presumably primarily to oxidative metabolic rate (e.g., extravascular accumulation of osmoles), and, to a presumably lesser extent, to hydrostatic forces (31); there was no attempt in this study to discriminate their relative importance. Furthermore, the delayed recovery of ADC relative to T2 could affirm the presumed slower restitution of fluids within the interstitial space than it could the contribution of water and its binding property within the intracellular compartment (26, 31), a presumption also supported by the numerically shorter $t_{1/2}$ of T2 than of CSA in the inactive calf (Fig. 3). Both Sjögaard (27) and Ward et al. (31) recognized a slower capillary to extravascular exchange than intracellular to the interstitium, although with a different outcome of the size of the interstitium during exercise. It is, nevertheless, obvious that, in our study, increased ADC was detected at the first midaquisition time, 15 s after cessation of high-intensity exercise, and was affected by temperature as well as extravascular volume. None of the evaluated parameters in the exercising calf was fully normalized after high workload, which indicated that extravascular edema was still present after 45 min of recovery.

The restoration rate of displaced water could to some degree depend on the positioning of the leg with a slightly bent knee and the foot slightly below knee level and above central veins. The evaluated parameters were, unfortunately, neither obtained within the same ROI nor evaluated within the same demarcated individual muscle. Different activation patterns of the calf muscles among subjects could therefore conceal significant changes and correlations. However, it was previously shown that both gastrocnemius and soleus muscles are activated by using this exercise setup (17). Measured ADC including both muscle groups would therefore reflect an average diffusion of the calf during this plantar flexion exercise. CSA of the calf would likewise be an adequate measure of exercise-induced volume change since muscles other than gastrocnemius and soleus are not activated to any substantial degree. However, results from a previous study (17) showed that tibialis anterior in the nonexercising calf was activated during high-intensity contralateral exercise; it was probably activated unintentionally to stabilize the pelvis during exercise. Regional CSA of tibialis anterior increased by ~15%, and the true volume reduction of inactive muscles was therefore underestimated most likely by ~0.3% when calf CSA was measured (unpublished data from Ref. 16), and this is probably also applicable to this present study.

It is concluded that a combined MRI approach was feasible and capable of measuring water fluxes related to skeletal muscle exercise, and partly distinguished different compartments, however, were overlaid because of a multicompartmental origin of the measured parameters. During high-intensity dynamic exercise with a small muscle group, there was a small, although clearly reduced, volume of the inactive calf. This extravascular water absorption was probably induced primarily by sympathoadrenal vasoconstriction in inactive muscles, although minor contribution by osmosis cannot be excluded. Water diffusion had, just as T2, a graded response to exercise and correlated highly with muscle volume, which is indicative of extravascular water accumulation linked to perfusion and is likely a combined effect dominated by metabolic activity as driving forces and to a lesser extent by hydrostatic pressure. Furthermore, our findings support a faster restitution of intracellular water content and its binding property within the cell than that of extracellular water.

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