Physiological basis of muscle functional MRI: predictions using a computer model

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Damon, Bruce M., and John C. Gore. Physiological basis of muscle functional MRI: predictions using a computer model. J Appl Physiol 98: 264–273, 2005. First published August 27, 2004; doi:10.1152/japplphysiol.00369.2004.—Muscle functional MRI (mfMRI) has been proposed as a tool for noninvasively measuring the metabolic and hemodynamic responses to muscle activation, but its theoretical basis remains unclear. One challenge is that it is difficult to isolate individually those variables affecting the magnitude and temporal pattern of the mfMRI response. Therefore, the purpose of this study was to develop a computer model of how physiological factors altered during exercise affect the mfMRI signal intensity time course and then predict the contributions made by individual factors. A model muscle containing 39,204 fibers was defined. The fiber-type composition and neural activation strategies were designed to represent isometric contractions of the human anterior tibialis muscle, for which published mfMRI data exist. Sustained isometric contractions at 25 and 40% maximum voluntary contraction were modeled, as were the vascular (capillary recruitment, blood oxygen extraction) and metabolic (lactate accumulation, phosphocreatine hydrolysis, pH) responses. The effects on the transverse relaxation of MRI signal were estimated, and the mfMRI signal intensity time course was measured from simulated images. The model data agreed well qualitatively with published experimental data, and at long exercise durations the quantitative agreement was also good. The model was then used to predict that NMR relaxation effects secondary to blood volume and oxygenation changes, plus the creatine kinase reaction, dominate the mfMRI time course at short exercise durations (up to ~45 s) and that effects secondary to glycolysis are the main contributors at later times.

CONTRAST IN MANY MRI APPLICATIONS is derived from inter- or intratissue variations in certain physiological and biochemical properties, such as water content; the concentrations, natures, and motions of proteins and other macromolecules; pH; and the presence of paramagnetic agents such as deoxyhemoglobin. When these properties vary between organs or between healthy and diseased tissues, a spatially dependent contrast arises that is useful for differentiating tissues for clinical diagnosis or quantitative morphometry. When a physiological event causes these properties to vary over time, a temporally dependent contrast arises. The latter source of contrast is exploited in some physiological MRI techniques, such as brain and muscle functional MRI (mfMRI).

mfMRI is sensitive to several of the metabolic and hemodynamic processes accompanying muscle activation. During intense exercise, the intracellular milieu shows increased tissue water content and acidity; as a result of one or both of these changes, the transverse relaxation time constant (T2) of muscle water increases (14, 25, 43, 45, 47). In addition, increases in blood oxygen extraction, blood flow, and blood volume are expected to cause blood-oxygenation level-dependent (BOLD) contrast changes, similar in mechanism to those that occur during brain activation. The superposition of these phenomena results in a complex signal intensity (SI) time course composed of at least three elements (1, 13, 34, 46). These include 1) an initial rise in SI for up to 10 s after the initiation of exercise; 2) an early dip in SI lasting up to 50 s; and 3) a long-latency, large-magnitude secondary rise in SI. If the physiological basis of the SI time course was sufficiently well understood, it might be possible to use mfMRI to identify regional variations in the metabolic and hemodynamic responses to exercise or as a noninvasive index of the spatial pattern and/or extent of muscle activation.

Currently, there is an incomplete understanding of how individual physiological and biochemical variables contribute to the mfMRI SI time course. One current issue concerns the role played by changes in the vasculature in affecting the mfMRI SI time course, particularly the initial rise and early dip. Capillary recruitment would increase in blood volume within an imaging voxel, increasing proton density and, therefore, SI. Increases in blood oxygen extraction could potentially have two effects. The first is an intravascular effect, because decreases in blood oxygenation decrease the T2 of blood itself (e.g., Ref. 53). This would have a relatively small direct effect on the SI, because the fractional blood volume is small. In addition, blood deoxygenation creates magnetic susceptibility gradients around capillaries, promoting the transverse relaxation of diffusing water molecules. The magnitude of these effects would depend on the orientation of the capillaries with respect to the magnetic field, the relative blood volume, the magnetic field strength, the vessel size, and the diffusion coefficient for water transverse to the capillaries’ long axes (54). Another unresolved issue is the exact relationship between the time-varying fluxes through the creatine kinase reaction and glycolysis and the temporal evolution of mfMRI SI changes.

A complication in trying to resolve these issues is that these factors are interdependent. To quantify the contribution of each process and to identify important areas of further research, we present a computer model of the physiological and biochemical responses to voluntary isometric contraction in the human anterior tibialis (AT) muscle, estimate their effects on NMR relaxation, and then use simulated images to model the mfMRI SI time
interest and vascular water are in rapid exchange. Finally, an intracellular residence time for water of 1.1 s was assumed (37). All relaxation rates were appropriate for a field strength of 1.5 T.

Step two: Activation of the model muscle. Force production was increased by using MU recruitment and by increasing MU discharge frequency; the force production of an MU (F_{MU}) was calculated as:

$$F_{MU} = \sum A_i \cdot F(P_{a},v)$$

where \(A_i\) is the area of the \(i\)th fiber, and \(F_{a}\) and \(F_{v}\) are a function of the discharge frequency \(v\) and the specific tension \(P_{a}\). To obtain the force-frequency relationship, Fig. 2 from Fuglevand et al. (23) was digitized, and the data were fit by using a Simplex algorithm to:

$$\frac{P}{P_0} = \frac{v}{v_0 + v^e}$$  

where \(P/P_0\) is the relative tension, \(v_0\) is the frequency associated with half-maximal force production, and \(x\) is an empirically determined exponent. This analysis gives estimates of 9.8 and 16.8 Hz for \(v_0\) in slow- and fast-twitch MUs, respectively, and values of 1.8 and 2.1 for \(x\) in slow- and fast-twitch MUs, respectively (\(r^2 > 0.98\)). The \(P_0\) values assumed were 4.38, 6.06, and 6.47 N/cm² for SO, FOG, and FG MUs, respectively (9).

Force production was increased from 0 N to MVC in 100 force-incrementing steps, assuming that the first MUs to be recruited may be either rapidly or slowly contracting, but have small innervation numbers and are fatigue resistant (17, 23, 57). All MUs were recruited at a discharge frequency of \(v_0\) Hz (SD 2). In the first step, the smallest MU was recruited, and F_{MU} and %MVC were calculated. In the next step, the second smallest MU was recruited, and the discharge frequency of the first MU was increased as a linear function of %MVC (17). This procedure was repeated until all MUs were recruited (98 steps). In the final two steps, force increased by rate coding only.

The studies described below were performed at submaximal contraction intensities, requiring less than full recruitment and values for \(P/P_0 < 1\) for each activated MU. To estimate the physiological and NMR consequences of the exercise at the specified contraction intensity, the identities and \(P/P_0\) values of the activated MUs were noted for each step. Physiological and NMR calculations were made only for the activated MUs. Fatigue of individual MUs was not considered.

Step three: Estimation of the physiological and magnetic resonance consequences of the activity. An MU was recruited when any muscle fiber within its spatial boundaries was activated (24). The time course of capillary recruitment (27) was simulated by determining an MU’s recruitment with an exponential function having a time constant of 3 s. Y during exercise (Y_{EX}) was calculated using:

$$Y_{EX} = Y_{R} - \left[ 0.75 \cdot \frac{P}{P_0} \cdot (1 - e^{-\tau_{Y_{R}}}) \right]$$

where \(Y_{R}\) is the resting value for \(Y\), \(P/P_0\) is the mean value of \(P/P_0\) for

| Table 1. Resting values for relaxation rates R_{1} and R_{2} assigned to the intracellular and interstitial spaces, assuming a two-compartment model for the tissue parenchyma |
|-----------------|-----------------|
|                | R_{1}           | R_{2}           |
| Intracellular   | 0.71 (0.03)     | 28.57 (0.625)   |
| Intersitial     | 0.50 (0.04)     | 8.0 (0.21)      |

Values are means (SD) in s^{-1}. R_{1} and R_{2} longitudinal and transverse, respectively, relaxation rate constants. Values are based on published reports (6, 10, 14, 26, 40, 51, 52).
the fibers served by the MVU, \( t \) is the exercise duration, and \( \tau_{\text{pO}} \) is the time constant for increases in blood oxygen extraction (20 s) (4, 5).

In the intracellular space, metabolite concentrations, pH, and volume changed due to the creatine kinase and glycolysis reactions. A resting phosphocreatine concentration ([PCr]) of 27.0 mmol/kg was assumed for all fiber types (58), which approached an end-exercise value of 4.0 mmol/kg at MVC:

\[
\Delta[\text{PCr}] = -23 \text{ mol/kg} \cdot \frac{P}{P_o} \cdot (1 - e^{-\tau_{\text{pO}}})
\]  

(6)

where \( \Delta \) is change, and \( \tau_{\text{pO}} \) is the time constant for PCr hydrolysis (30 s) (3, 50).

The time at which glycolytic flux increased was estimated by noting the exercise duration at which intracellular pH began to decrease during isometric contractions (15, 28, 29, 38, 48, 50). Regressing the time of intracellular pH decrease on %MVC, we obtained the following:

\[
t_{\text{glyc}} = -0.63 \cdot \%\text{MVC} + 68 \text{ s}
\]  

(7)

where \( t_{\text{glyc}} \) is the time at which glycolytic flux increases \((r^2 = 0.88)\). For exercise durations greater than \( t_{\text{glyc}} \), the time course of lactate concentration \(([\text{Lac}] \) changes was calculated according to published data (15, 28, 29, 38, 48, 50):

\[
\Delta[\text{Lac}] = ([\text{Lac}_{\text{max}} \text{ mmol/kg}] \cdot \frac{P}{P_o} \cdot [1 - e^{-t_{\text{glyc}}/\tau_{\text{glyc}}}] )
\]  

(8)

where \([\text{Lac}_{\text{max}} \text{ mmol/kg}] \) is the [Lac] at maximal exercise (20, 25, and 30 mmol/kg in SO, FOG, and FG fibers, respectively), and \( \tau_{\text{glyc}} \) is the time constant for [Lac] increase (45 s). Intermuscular lactate accumulation, equal to one-third of the intracellular [Lac], was assumed; this is consistent with the ratio of plasma to intracellular [Lac] observed during maximal exercise (7, 30).

To calculate the intracellular and interstitial volume changes resulting from PCr hydrolysis and lactate accumulation, we assumed muscle cells to be perfect osmometers (19), with access to an infinite supply of vascular water. The latter assumption would hold for isometric contractions in which the intramuscular pressure is less than the systolic pressure (~60% MVC for young adult males performing isometric dorsiflexion; Ref. 60). Water shifts to the intracellular and interstitial spaces were calculated assuming a maintained osmolarity of 300 mmol/kg.

Intracellular pH changes were estimated based on published isometric exercise studies (15, 28, 29, 38, 48, 50). Flux through the creatine kinase reaction was assumed to cause alkalosis of up to 0.20 pH units. Glycolysis was assumed to cause acidosis such that the maximal pH changes were ~0.6, ~0.8, and ~1.0 in SO, FOG, and FG fibers, respectively. For exercise durations less than \( t_{\text{glyc}} \), the pH change due to the creatine kinase reaction in each fiber was calculated as:

\[
\Delta pH = 0.2 \cdot \frac{P}{P_o} \cdot (1 - e^{-t_{\text{glyc}}/\tau_{\text{glyc}}})
\]  

(9a)

For exercise durations greater than \( t_{\text{glyc}} \), the pH change \((\Delta pH_{\text{glyc,max}})\) was calculated as:

\[
\Delta pH = 0.2 \cdot \frac{P}{P_o} \cdot (1 - e^{-t_{\text{glyc}}/\tau_{\text{glyc}}}) - \Delta pH_{\text{glyc,max}} \cdot \frac{P}{P_o} \cdot [1 - e^{-t_{\text{glyc}}/\tau_{\text{glyc}}}] 
\]  

(9b)

where \( \Delta pH_{\text{glyc,max}} \) has values of ~0.8, ~1.0, and ~1.2 in SO, FOG, and FG fibers, respectively.

Changes in intracellular \( R_2 \) \((R_{2,\text{Intra}})\) were calculated based on independent and linear dependences of \( R_{2,\text{Intra}} \) on intracellular volume and pH (14). In maximally exercised human muscles studied at 1.5 T, \( R_{2,\text{Intra}} \) changes from ~28 to ~20 Hz (47, 49). Based on the above assumptions concerning pH and osmolyte changes, the corresponding physiological conditions are an intracellular decrease of 1.0 pH unit and an intracellular volume increase of 17.67% (100% × 53 mmol/kg ÷ 300 mmol/kg). \( R_{2,\text{Intra}} \) changes were then calculated with:

\[
\Delta R_{2,\text{Intra}} = \frac{4 \text{ s}^{-1}}{17.67\% \text{ Increase in Volume}} \cdot 100\% \cdot \Delta[\text{Osmolites, mosmol/kgH}_2\text{O}] \cdot \frac{300, \text{ mosmol/kgH}_2\text{O}}{100\%} \cdot \Delta pH
\]  

(10)

An analogous calculation was used for the change in intercellular \( R_2 \) \((R_{2,\text{Inter}})\), except that it was based solely on the volume change due to interstitial lactate accumulation.

**Step four: Calculation of \( T_2\)-weighted SI changes.** To account for transaxial and longitudinal water exchange, apparent relaxation rates and volume fractions \((R_1, R_{1,\text{Extra}}\) etc.) were calculated according to the two-site exchange equations (61), using the actual intra- and extracellular volumes, the intracellular \( R_1 \) \((R_{1,\text{Intra}}, R_{1,\text{Extra}}, R_{1,\text{Extr}}, \text{ and } R_{2,\text{Extra}}\) intracellular residence time for water, and the interstitial residence time. Finally, simulated echo-planar images were created based on a single-slice acquisition with an in-plane resolution of 3 mm × 3 mm, a 1-cm slice thickness, and repetition time (TR) and echo time (TE) appropriate to the studies used to test the model (see below). The SI in each voxel was calculated using:

\[
\text{SI} = \sum_{\text{f}} \left(100 \cdot f_{\text{Intra}} \cdot (1 - e^{-t_{\text{R}1,\text{Intra}}/R_{1,\text{Intra}}}) \cdot e^{-t_{\text{TE}}/R_{2,\text{Intra}}} + (100 \cdot f_{\text{Extr}} \cdot (1 - e^{-t_{\text{R}1,\text{Extr}}/R_{1,\text{Extr}}}) \cdot e^{-t_{\text{TE}}/R_{2,\text{Extr}}} \right)
\]  

(11)

where \( N \) is the number of fibers in the voxel, \( f_{\text{Intra}} \) is intracellular fraction, and \( f_{\text{Extr}} \) is extracellular fraction. Gaussian noise was added to create a signal-to-noise ratio of 175, which we have observed previously in images obtained with similar TR, TE, and field strength (1.5 T; Ref. 13). In the first image of exercise, partial saturation effects were accounted for using muscle architectural data reported by Mangarais and Baltzopoulos (41) and standard partial saturation equations.

**Model Trials**

**Evaluation of model performance.** Six model trials were performed by using contractions sustained for 2 min at 25% MVC; these model results were compared with published experimental data (46). The TR and TE for these studies were 4,000 and 30 ms, respectively. Model results from six trials at 40% MVC (TR/TE = 4,000/35 ms) were also compared with experimental data (13); the data in the experimental study were reanalyzed such that a region of interest was drawn around the entire AT, rather than for individual mMRI data clusters.

**Individual contributions of the creatine kinase reaction, glycolysis, and vascular factors.** To identify the contribution of the creatine kinase reaction as a function of exercise duration, model trials were run at 40% MVC with glycolysis inactivated, no capillary recruitment, and no increase in blood oxygen extraction. The relative contribution of the creatine kinase reaction \((\%\text{Contribution}_{\text{CK}})\) was determined as a function of exercise duration using:

\[
\%\text{Contribution}_{\text{CK}} = 100\% \cdot \frac{\Delta S_{\text{SIT}}}{\Delta S_{\text{SIT, total}}} 
\]  

(12)

where \( \Delta S_{\text{SIT}} \) is the change in SI from baseline during the experimental trial, and \( \Delta S_{\text{SIT, total}} \) is the change in SI during the control trials. Analogous procedures were conducted to identify the individual contributions of glycolysis and vascular factors (blood volume and blood oxygen extraction changes). In all cases, six model trials were performed.
run. To clarify the contributions of the metabolic and hemodynamic factors, partial saturation effects were ignored in the first exercise image; other parameters were the same as in the trials that evaluated model performance.

Statistics. Mean and SD were calculated for all data. For tests of model performance, the mean SI was recorded as a function of exercise duration for a region of interest drawn around the resting muscle. Model predictions of physiological and biochemical parameters were also characterized as a function of exercise duration. For variables in which fiber-type-specific responses were observed ([PCr], [Lac], total osmolyte accumulation, pH, $R_2_{\text{Intra}}$, $R_2_{\text{Inter}}$), the end-exercise mean values were compared by using a two-way ANOVA (exercise intensity $\times$ fiber type). For other variables (relative blood volume, absolute blood volume, intracellular volume, interstitial volume, blood oxygenation, and SI), the end-exercise mean values at 25 and 40% MVC were compared by using Student’s t-test. For tests of individual contributions of specific factors, trials were compared with control trials by using a two-way ANOVA (exercise duration $\times$ factor). In trials that evaluated the contributions of individual physiological factors, repeated-measures ANOVAs were performed because the same six muscles were used in each of the trials, and only the factor under study was varied. Significant ANOVA results were followed with Tukey’s post hoc test. Statistical comparisons were considered significant at $P < 0.05$.

RESULTS

Tests of Model Performance

Physiological and biochemical properties. The force and discharge frequency behavior of the model muscle during the 100 force-incrementing steps are illustrated in Fig. 1. The recruitment and rate-coding strategies resulted in a smooth build-up of force (Fig. 1A); the whole-muscle force-frequency relationship for the entire ramp-up of force is shown in Fig. 1B. For all exercise intensities up to $\approx 78$% MVC, only SO and FOG MUs were involved. Above this value, the FG MU was also recruited; thus all MUs were recruited at $\approx 85$% MVC (Fig. 1A). In trials performed at 25% MVC, 60 SO MUs [8,341 fibers (SD 321)] and 17 FOG MUs (SD 455) were active. In trials performed at 40% MVC, 65 SO MUs [9,414 fibers (SD 422)] and 20 FOG MUs [2,837 fibers (SD 460)] were active.

The modeled hemodynamic responses to exercise included MVU recruitment and a decrease in $Y$. In both the 25 and 40% MVC trials, $\approx 6$% of the capillaries were perfused during the preexercise rest, which resulted in a mean blood volume of 0.71 mm$^3$ (SD 0.18). During exercise, the blood volume increased ($>99$% of all capillaries were perfused at each intensity). As a result, the final absolute blood volume was 13.46 mm$^3$ (SD 0.04) for the entire muscle at each contraction intensity (25% MVC: Fig. 2A). However, the larger muscle parenchyma volume at 40% MVC (see below) caused the end-exercise relative blood volume to be greater ($P < 0.01$) at 25% MVC [0.86% (SD 0.02)] than at 40% MVC [0.84% (SD 0.01)]. Figure 2B shows $Y_{\text{EX}}$ during simulated exercise at 25% MVC. The final values of $Y_{\text{EX}}$ were 0.57 (SD 0.01) (25% MVC) and 0.46 (SD 0.01) (40% MVC). These values differed significantly from each other ($P < 0.01$). Note that for these and all subsequently reported data, similarly shaped, but different in magnitude, time courses existed for 40% MVC but are not shown.

The [PCr] decreased immediately and to the same extent in SO and FOG units (Fig. 2C), but the end-exercise values were greater at 40% MVC than at 25% MVC ($P < 0.01$). After glycolysis was activated, [Lac] increased (Fig. 2D). The end-exercise [Lac] was greater in FOG than in SO fibers ($P < 0.01$) and at 40% MVC than at 25% MVC ($P < 0.01$). Lactate also accumulated in the interstitial space (data not shown). Coincident with the time courses of the creatine kinase reaction and glycolysis, the intracellular pH first increased and then decreased (Fig. 2E: 25% MVC). At both exercise intensities, the end-exercise pH change was greater in FOG than in SO fibers ($P < 0.01$); also, the pH change was greater at 40% MVC than at 25% MVC ($P < 0.01$).

Exercise-induced increases in osmolyte content at 25% MVC are shown in Fig. 2F. Osmolyte accumulation was greater in FOG than SO fibers ($P < 0.01$) and at 40% MVC than 25% MVC ($P < 0.01$). The end-exercise increase in total muscle intracellular volume (Fig. 2G) was greater at 40% MVC than at 25% MVC ($P < 0.01$). The whole muscle interstitial volume is shown in Fig. 2H; the end-exercise values at 25 and 40% MVC did not differ significantly. The relative increases in the total muscle intracellular and interstitial volumes are less than the relative increases in intracellular and interstitial osmolyte contents of activated fibers, because only a subset of fibers was activated.

![Fig. 1. Neuromuscular performance of model. A: force development as a function of step number. Dark gray zone indicates recruitment and rate coding of slow oxidative (SO) and fast oxidative glycolytic (FOG) units. Medium gray zone indicates recruitment and rate coding of fast glycolytic (FG) unit and rate coding of SO and FOG units. Light gray zone indicates rate coding of all units. B: force-discharge frequency relationship for the entire model muscle.](http://jap.physiology.org/DownloadedFrom/10.1152/jappl.99921.2004)
NMR relaxation rates and image SI. The transverse relaxation rates of the intracellular, interstitial, and vascular spaces changed according to Eqs. 2 and 10 (data not shown). The mean SI time courses for 25 and 40% MVC are shown in Fig. 3, A and B, respectively. The end-exercise SI change was greater at 40% MVC than at 25% MVC (P < 0.01). At each intensity, the modeled SI time courses reflected the initial rise, early dip, and secondary rise temporal pattern. Also shown are experimental mfMRI time courses reported previously (13, 46). Price et al. (46) reported only selected error estimates, and so these have not been included in Fig. 3A. However, the errors that were reported indicate an SD of ~0.0015 normalized SI units at end exercise. The SD for the data reported by Damon et al. (13) are indicated in Fig. 3B. Thus for exercise durations >60 s for both 25 and 40% MVC, and also for short exercise durations at 40% MVC, all of the simulated mfMRI data points fall within 1 SD of the experimental data.

**Individual Contributions of the Creatine Kinase Reaction, Glycolysis, and Vascular Factors**

Figure 4 shows the contributions of capillary recruitment and blood oxygenation changes (A and B), the creatine kinase reaction (C and D), and glycolysis (E and F). Figure 4A shows that vascular factors cause a small rise in normalized SI (~0.005 units) that lasts for the first ~10 s of exercise. This positive effect of the vasculature on SI then starts to diminish, eventually making a negative contribution to SI beginning at an exercise duration between 24 and 28 s and being maximally negative at an exercise duration of ~40 s. Figure 4C and D, shows the predicted absolute and relative contributions of the creatine kinase reaction to SI, respectively. The absolute contribution is small at all exercise durations, being responsible for a ~0.01 unit normalized SI increase at end exercise. However, the relative contribution (Fig. 4D) is large initially (~25%
Physiological and biochemical events of exercise are estimated, the effects of these events on NMR relaxation are predicted, and the mfMRI SI time course is obtained from simulated images. After describing the strengths and limitations of the model, we use it to address the question of the relative contributions made by various physiological factors to the mfMRI SI time course.

**DISCUSSION**

We have developed a computer model of voluntary isometric contraction in which force production is increased realistically, physiological and biochemical events of exercise are estimated, the effects of these events on NMR relaxation are predicted, and the mfMRI SI time course is obtained from simulated images. After describing the strengths and limitations of the model, we use it to address the question of the relative contributions made by various physiological factors to the mfMRI SI time course.

**Evaluation of Model Performance**

**Physiological and biochemical considerations.** The model employed several realistic considerations with regard to MU organization, recruitment, and discharge behavior during human voluntary isometric contractions. First, MU size distributions include many small MUs and few large MUs (e.g., Ref. 57) and have been described by using an exponential function (Eq. 1, as reported in Ref. 20). A second feature is that the first MUs to be recruited may be either rapidly or slowly contracting, but always have small-twitch and tetanic forces and are fatigue resistant (17, 23, 57). These properties were considered in the initial assignment of MU types and the force-incrementing strategy. Third, muscle force output can be increased by using both rate coding of already active MUs and recruitment of new ones (e.g., Refs. 17, 23, 57). The specific assumptions concerning rate coding were that 1) the initial discharge frequency of an MU approximately equals $v_0$; 2) the discharge rate of an MU increases as a linear function of contraction intensity; and 3) the maximum discharge rate will approximately equal the value associated with 90% maximal force production. The smooth build-up in force and the similarity of the whole muscle force-frequency relationship (Fig. 1B) to that reported by Connelly et al. (12) are evidence that the model considered these factors appropriately.
The primary limitation of the model with regard to MU behavior is that we did not model fatigue. Because the $T_2$ recovery after exercise is slow ($\sim 35$–45 min for full recovery; Refs. 49, 59), the $T_2$ of an MU derecruited due to fatigue would remain elevated and contribute positively to SI for the remainder of the exercise bout. Simultaneously, the $T_2$ of a newly recruited MU would begin to increase and add further to the SI. Thus this omission probably biased the SI negatively, particularly at long exercise durations. It is not possible to quantify the amount of error introduced by this limitation, but the effect is likely to be small because only fatigue-resistant MUs were recruited and their activity would be sustained by at least partial blood flow (60).

The model also performed well with regard to the biochemical and physiological responses to isometric contraction in the AT. Because ATP synthesis by the creatine kinase (8, 42), glycolytic (8), and oxidative (8, 11, 56) reactions each depend linearly on exercise intensity, a strength of the model was the use of $P/P_0$ to scale the changes in $Y$, [Lac], [PCR], and pH on an MU-specific basis. While we were unable to find reports of a well-resolved time course of [Lac] or $Y_{\text{EX}}$ for sustained isometric dorsiflexion at these intensities, the modeled pH and [PCR] changes were similar to those reported for isometric contractions in vivo by most (28, 50), although not all (29), works.

This agreement resulted from the use of empirical data when calculating physiological and biochemical effects. Alternatively, the ATP cost of contraction could be combined with blood flow; ATP synthesis rates from the creatine kinase reaction, glycolysis, and oxidative phosphorylation; and buffer capacity estimates to determine metabolite levels, pH, and blood oxygenation. While this would have provided a more robust model with respect to muscle biochemistry, the intent of our simple metabolic model was solely to generate realistic time courses of metabolite and pH levels, so that relaxation effects could be calculated. Thus our simple strategy for developing these time courses did not detract from the overall objective, which was to simulate the mfMRI time course produced by normally metabolizing muscles. Also, because the metabolic calculations were independent of one another, this allowed us to null the contributions of individual variables, so that the unique contributions made by others could be determined.

A final simplification in the model was in the time course of MVU recruitment. We determined the recruitment of an individual MVU by using a random number generated by an exponential weighting function. This simplification made it unnecessary to model the factors controlling precapillary sphincter muscles. But because the time course of blood volume changes was appropriately modeled on the whole muscle level (27) and because of the inclusion of many MVUs within each imaging voxel, we consider that this probably had little quantitative impact on the SI time course.

Therefore, we conclude that the model simulated the physiological and biochemical aspects of voluntary human isometric contractions in the AT well, and in particular with regard to force-increasing strategies and the metabolic and physiological responses to contraction. We consider next the appropriateness of the NMR responses.

**NMR considerations.** Overall, the model simulated the mfMRI SI time course well. Qualitatively, the initial rise, early dip, and secondary rise were each observed. Quantitatively, at both exercise intensities, the modeled SI time course fell within 1 SD of the experimental data for exercise durations greater than $\sim 60$ s. At 40% MVC, the quantitative agreement was also good for exercise durations shorter than 60 s. One possible reason for quantitative disagreement at short exercise durations is illustrated by work reported by Damon et al. (13), who observed that, in most voxels, the initial rise occurred in the first exercise image but that, in other voxels, the initial rise did not occur until the second exercise image. This is consistent with a differential time course for MU activation across the AT muscle and for the whole muscle experimental data would be responsible for the higher SI in the second exercise image than in the first exercise image. Thus, part of the reason for the model’s disagreement with the experimental data at short exercise durations may have been that a more complex time course of muscle activation may have existed in experiments than was considered in the model. We will also argue below that the quantitative disagreement at short exercise durations may further result from an incomplete understanding of how BOLD contrast affects the relaxation of muscle water.

There are three specific limitations of the model with regard to NMR, and each of them points to the need for further investigation into the physiological basis of the mfMRI time course. First, extravascular BOLD effects were not considered. As discussed below, this omission would bias the modeled time course positively, because decreases in $Y$ and increases in blood volume should decrease the $T_2$. Second, the published titrations of $R_{2,\text{Intra}}$ vs. pH (14, 25) and $R_{2,\text{Intra}}$ vs. intracellular volume (14) in skeletal muscle cannot be directly applied to the imaging condition modeled here. An improved understanding of how these variables affect $R_{2,\text{Intra}}$ at TE values used in imaging and at the field strengths used in humans needs to be developed. Finally, we made the simplifying assumption that interstitial and vascular water exchange rapidly, to be able to account for the intermediate exchange across the sarclemma using the two-site exchange model. Whereas the transcapillary exchange is actually slow, the quantitative impact of this assumption is lessened by the small-volume fractions of these compartments and because, at the TE values used in this study, the relaxation of intracellular water dominates the image SI equation.

We conclude that the model predicted well the mfMRI time course and that it can be used validly to predict the physiological basis of mfMRI and to highlight important areas for future study.

**Predicted Physiological Origin of the mfMRI Time Course**

The initial rise. The difference in SI in exercise image 1 in Fig. 3B and Fig. 4 (control condition, Fig. 4, A, C, and E) was the inclusion (Fig. 3) or exclusion (Fig. 4) of partial saturation and muscle shortening effects. When these effects were included, the initial rise was $\sim 0.015$ normalized SI units; when excluded, it was $\sim 0.005$ units. Thus this model predicts that the major contribution to the initial rise under these exercise and image-acquisition conditions was an MRI artifact related to muscle shortening.

A second determinant of the initial rise in modeled SI was vascular in origin and produced a normalized SI increase of $\sim 0.003$ units for exercise durations of up to $\sim 10$ s. The
positive SI contribution resulted from capillary recruitment, which increases proton density. Simultaneously, the modeled increase in oxygen extraction increased $R_{2,\text{Blood}}$ and tempered this effect somewhat. However, capillary recruitment’s effect on proton density was the dominant vascular effect during the initial rise.

The final contributor to the initial rise was the creatine kinase reaction. PCr hydrolysis results in the net production of an osmolyte, causing water to enter the cell and increasing the free intracellular water content. This decreases the $R_{2,\text{Intra}}$ and thereby decreases the SI. In our view, the $R_{2,\text{Intra}}$ change also depends on the intracellular pH such that acidosis would cause a further decrease in $R_{2,\text{Intra}}$ and alkalosis would cause an increase in $R_{2,\text{Intra}}$ (14; see also Ref. 25). Thus the osmotic effect of decreasing $R_{2,\text{Intra}}$ would be countered by the fact that the creatine kinase reaction partially buffers a proton and causes a net alkalosis. However, others have provided evidence that pH changes do not enhance the whole muscle $R_2$ change over what could be explained by osmotic effects alone (43, 47). Whichever model for the $R_{2,\text{Intra}}$ change is correct, the overall effect of the creatine kinase reaction is to decrease $R_{2,\text{Intra}}$ (14) as an immediate consequence of exercise, it makes a small absolute but large relative contribution to SI during the initial rise (25–50% of the total SI change).

**Predicted physiological origin of the early dip in SI.** The early dip in SI, which we define as occurring between exercise durations of 10 and 40 s for this exercise intensity, was also determined by the flux through the creatine kinase reaction and by vascular factors. However, the relative contributions of these factors were quite different than during the initial rise. The absolute and relative contributions of vascular factors diminished and eventually became negative over this time period, with a relative contribution that was maximally negative at an exercise duration of ~40 s. This results from continued increases in oxygen extraction over this interval, which counter and eventually overwhelm the proton density effect of capillary recruitment. Conversely, the absolute contribution of the creatine kinase reaction grew throughout this period. Eventually, the SI contribution from this reaction exceeded the overall SI change, making its relative contribution >100%.

A notable exclusion from this model was an extravascular BOLD effect. Muscle BOLD effects have been clearly demonstrated during cuff occlusion experiments (18, 39, 55), and analysis of these effects using analytical models (54) shows that they could make a negative normalized SI contribution of up to ~0.01 units. Like the other factors, these effects would evolve as a function of exercise duration, but would probably have their greatest relative importance during the early dip as well, when the total normalized change is on the order of 0.01–0.03 units. An indirect implication of the model is, therefore, the need to expand our understanding of how the extravascular BOLD effect influences the SI in mMRI.

**Physiological origin of the secondary rise in SI.** At 40% MVC, the secondary rise began at an exercise duration of ~40 s and continued to the end of the modeled exercise bout. The results shown in Fig. 4 illustrate that this phase of the time course reflects, almost exclusively, the increased flux through cellular energy metabolism pathways. The creatine kinase reaction’s absolute contribution continues to be positive during this time (Fig. 2C), but its relative importance diminishes rapidly after the onset of glycolysis (Fig. 2D). The secondary rise is thus essentially a glycolytic phenomenon brought about by the effects of lactic acid production.

This proposal agrees well with a number of other observations. First, it is evident from the results of Price et al. (46) that the time until the onset of the initial rise is exercise intensity dependent. The model’s prediction that the secondary rise depends on glycolysis explains this result, because the time until the onset of pH decreases during isometric contractions (our $t_{\text{glyc}}$) depends inversely on exercise intensity (15, 28, 29, 38, 48, 50). Also, in patients with myophosphorylase deficiency, the $T_2$ change with exercise is smaller than in healthy subjects (16) or absent altogether (22); we later reported analogous results for iodoacetate-poisoned frog muscles (14). In each of these studies, end-exercise changes in $R_{2,\text{Intra}}$ or whole muscle $T_2$ were reported, but in all cases the exercise duration was long enough that glycolytic flux would have increased in a normal muscle. In the present study, if the SI changes caused by vascular factors were combined with those of the creatine kinase reaction, a crude model of myophosphorylase deficiency would result. Combining the results in Fig. 4, A and C, in this way indicates only a small positive SI change. These model results are, therefore, consistent with both naturally occurring and experimentally perturbed glycolytic conditions.

**Predictions Following From the Model**

There are a number of testable predictions and practical implications that follow from this model, as follows.

1) The proposed major dependence of the initial rise on muscle shortening indicates that, as the TR is incremented from very short to very long values, the magnitude of the initial rise should decrease. Furthermore, adequate knowledge of the longitudinal relaxation time constant ($T_1$) could be combined with such an experiment to obtain a general indication of muscle deformation during contraction.

2) When partial saturation effects are removed by using very long TR values ($5 \times T_1$), a small initial rise, resulting from capillary recruitment, should remain. This could potentially be used as a rough index of vascular function in conditions known to capillary density or MVU recruitment, such as diabetes mellitus (36).

3) The correspondence between the maximum negative relative contribution of vascular factors to SI and the time at which the nadir of the early dip occurred in mMRI data clusters in the AT (13) suggests that the magnitude and/or the curvature of the early dip would be impacted by the adequacy of muscle oxygenation during exercise.

4) The proposed dependence of the early dip on BOLD phenomena and the secondary rise on glycolysis suggests that the early dip should be deeper, and the secondary rise less pronounced, in predominantly SO muscles.

5) Further refinements to this model, including more sophisticated metabolic modeling and consideration of exercise intensities above that at which blood flow is completely occluded, might allow the fitting of a family of single-factor SI curves such as those shown in Fig. 4 to the mMRI SI time course to estimate the extent and time course of specific metabolic and hemodynamic events of exercise.
Summary and Conclusions

We have developed a model of human voluntary neuromuscular function and used it to model the mMRI SI time course. The model employed realistic strategies to increase the force production of the muscle and used prior empirical observations to produce reasonable estimates of the extent and time course of the physiological and biochemical responses to this activation. Based on established relationships between these variables and NMR relaxation rates, we formed simulated T2-weighted echo-planar images and compared the model predictions to published experimental data. For exercise intensities at which muscle perfusion is either unimpaired or only partially occluded, the model performance was outstanding qualitatively and, in most cases, was also quantitatively accurate.

We then applied the model to the question of how the absolute and relative contributions of the creatine kinase reaction, glycolysis, and hemodynamic factors evolve as a function of exercise duration. The model predicts that the initial rise is determined primarily by positive SI contributions of capillary recruitment and the creatine kinase reaction, with an additional contribution of muscle shortening under certain data-acquisition and exercise conditions. The model also predicts that, during the early dip, the contributions of vascular factors rapidly decrease and partially counteract the growing positive contribution of the creatine kinase reaction. Finally, the model predicts that osmotic and/or pH effects of lactic acid production make the primary contribution to the secondary rise, with a continued contribution from the creatine kinase reaction. The model needs to be expanded to include other phenomena altered during exercise, such as the factors contributing to the extravascular BOLD effect, and highlights the need to investigate further the mechanistic basis of R2_intra changes, especially with regard to the dependence on intracellular pH.

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