Single muscle fiber contractile properties during a competitive season in male runners

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Methods

Experimental Design

This investigation examined the single muscle fiber functional responses during the periodized training periods of a competitive cross-country run season. Testing procedures occurred at three time points, each marking the beginning or end of a training period. Specifically, the runners were tested at T1, T2, and T3.

Subjects and Training Program

Eight members of the men’s varsity cross-country team originally volunteered to participate in this study. Three subjects did not complete the entire study because of running-related injuries. The training over the course of the study duration was developed and implemented by the coaching staff and is presented in Fig. 1. As can be seen, the months preceding the start of the fall season, the runners averaged 97 km/wk total running. In the 8 wk between T1 and T2, total run volume stayed approximately the same (99 km) as interval training was introduced (9% of total weekly volume). From T2 to T3, marking the taper phase, total run volume was decreased ~25%, whereas the

ENDURANCE TRAINING INCREASES the oxidative potential of skeletal muscle and alters its morphological and functional properties (2, 19). Although the changes in muscle fiber type composition with endurance training have been extensively documented (see Ref. 27 for review), less is known regarding the contractile properties of slow (myosin heavy chain (MHC) I)- and fast (MHC IIa)-twitch myofibers from young endurance-trained human muscle. Previous research has shown that myofibers alter their contractile properties (i.e., size, force, shortening velocity, power) in response to training (12, 29, 31, 33, 34, 36–38). However, the use of older subjects (33, 34, 37, 38), nonhuman models (29), and dated techniques (12) substantially limit our understanding of the training-induced alterations on myocellular function, particularly in relation to endurance training.

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amount of interval running continued to increase to 20% of total volume. Interval training was defined as any training faster than 8-km race pace (~3:10 min/km). Before initiating the study, all volunteers were informed of all the risks and procedures associated with the investigation and provided written informed consent according to the guidelines set forth by the Institutional Review Board at Ball State University.

**Treadmill Testing**

To assess aerobic capacity, subjects performed a continuous incremental treadmill test to exhaustion at T1 and T3. Additionally, a submaximal test for measurement of oxygen consumption at 16 km/h (10 miles/h) was performed at T2. Treadmill testing procedures were identical to those previously reported by our laboratory (17).

**Muscle Biopsy and Sample Preparation**

Percutaneous needle biopsies (75–100 mg) were obtained from the lateral head of the gastrocnemius muscle at all three time points (3). To avoid any possible complications in tissue quality arising from multiple biopsies, samples were obtained from the opposite leg with each successive biopsy so that the same leg was sampled at T1 and T3. In this case, T3 specimens were obtained proximal to the T1. The area of the muscle where the biopsy was to be taken was shaved, cleaned with betadine, and subsequently anesthetized with ~1.5 ml of 1% xylocaine. The muscle sample was obtained using a 5-mm biopsy needle. The muscle specimen was immediately divided into longitudinal sections. One section was placed in cold skinning solution (see Single Muscle Fiber Experimental Solutions) and stored at ~20°C for later analysis of permeabilized single muscle fiber physiology and myosin isoform composition, as described below. Each biopsy sample was analyzed for single fiber contractile properties within a 28-day period.

**Single Muscle Fiber Experimental Solutions**

The skinning solution contained (in mM): 125 potassium pro- ponate, 2.0 EGTA, 4.0 ATP, 1.0 MgCl₂, 20.0 imidazole (pH 7.0), and 50% (vol/vol) glycerol. Fibers were kept in this solution for a minimum of 1 day but not longer than 4 wk (22). The compositions of the relaxing and activating solutions were calculated using an iterative computer program described by Fabiato and Fabiato (11). These solutions were adjusted for temperature, pH, and ionic strength using stability constants in the calculations (16). Each solution contained (in mM) 7.0 EGTA, 20.0 imidazole, 14.5 creatine phosphate, 1.0 free Mg²⁺, 4 free MgATP, KCl, and KOH to produce an ionic strength of 180 mM and a pH of 7.0. The relaxing and activating solutions had a free Ca²⁺ concentration of pCa 9.0 and pCa 4.5, respectively (where pCa = −log Ca²⁺ concentration).

**Single Muscle Fiber Experimental Set-up**

On the day of an experiment, a 2- to 3-mm muscle fiber segment was isolated from a muscle bundle and transferred to a small experimental chamber filled with relaxing solution where the ends were fastened securely with 4.0 monofilament posts and 10.0 sutures in small stainless steel troughs that were attached via thin titanium wires to a force transducer (model 400A; Cambridge Technology, Watertown, MA) and a DC torque motor (model 308B, Cambridge Technology), as previously described by Moss (25). The experimental chamber was designed so that the mounted muscle fiber could be transferred rapidly between chambers filled with relaxing or activating solutions. The apparatus was mounted on a microscope (Olympus BH-2) so the fiber could be viewed at ×800 magnification during the experiment.

Unamplified force and length signals were sent to a digital oscilloscope (model 310; Nicolet, Madison, WI) enabling muscle fiber performance to be monitored throughout data collection. Analog force and position signals were amplified (Dual Differential Amplifier, 300-DIF2; Positron Development, Ingelwood, CA), converted to digital signals (National Instruments), and transferred to a computer for analysis using customized software. Servo-motor arm and isotonic force clamps were controlled using a computer interfaced force-position controller (Force Controller, 300-FC1; Positron Development).

All experiments were performed at 15°C. The temperature of the experimental chambers was monitored constantly (copper-constantan thermocouple, PP-T-24S; Omega Engineering, Stamford, CT) during the experiments. To cool the relaxing and activating solutions in the experimental chambers, a bath (RTD 111; Neslab, Portsmouth, NH) constantly circulated water through a milled aluminum plate. A thermoelectric pump Peltier junction current source, with a feedback circuit temperature controller (ES5AX-VAA, sensitivity ±0.3%; Omron), lowered the temperature of the experimental chambers to 15°C and maintained this temperature throughout the experiment.

**Single Muscle Fiber Analysis**

**Single fiber diameter.** The sarcomere spacing for each muscle fiber was adjusted to 2.5 μm using an eyepiece micrometer. A video camera (CCD-IRIS, DXC-107A; Sony) connected to the microscope and interfaced to a computer allowed viewing on a computer monitor and storage of the digitized images of the muscle fibers during the experiment. Fiber diameter was determined from a captured computer image taken with the fiber brieﬂy suspended in air (5, 37). Fiber width (diameter) was determined at three points along the length of the captured computer image using public domain software (NIH Image version 1.61). Fiber cross-sectional area (CSA) was calculated from the mean width with the assumption that the fiber forms a cylindrical cross-section when suspended in air (<5 s; see Ref. 24).

**Force determination.** The output of the force and position transducers was amplified and sent to a microcomputer via a lab-PC+12-bit data acquisition board (National Instruments). Resting force was monitored, and then the fiber was maximally activated in pCa 4.5 solution. Peak active force (P₀) was determined in each fiber by computer subtraction of the force baseline from the peak in the pCa 4.5 solution (37).

**Maximal shortening velocity determination.** Fiber maximal shortening velocity (Vₒ) was measured by the slack test technique, as described by Edman (10). Fibers were transferred from resting state (pCa 9.0) to activating solution (pCa 4.5) and brought to peak tension. The fiber was then rapidly shortened so that tension returned to baseline. The fiber shortened, taking up the induced slack, which was
followed by redevelopment of tension. The fiber was then returned to relaxing solution and its original length. The time between the onset of slack and redevelopment of tension (i.e., period of unloaded shortening) was measured by computer analyses. Four different slack distances (each <15% of FL) were used for each fiber, and the slack length was plotted as a function of the duration of unloaded shortening. Velocity (FL/s) was calculated by dividing the slope of the fitted line by the fiber segment length, and the data were normalized to a sarcomere length of 2.50 μm. Fiber length averaged 2.09 ± 0.01 mm with an average intercept of 14.38 μm resulting in a fiber compliance of 0.7%, which is similar to previous reports from our laboratory (31–34).

**Force-velocity and power.** Each fiber segment was fully activated in pCa 4.5 and then subjected to a series of three isotonic load steps. After the third isotonic step, the position motor imposed a slack length step <20% of the original fiber length, and the fiber was transferred back into relaxing solution, where it was reextended to its original fiber length. The entire procedure was performed five to six times at various loads so that each fiber was subjected to a total of 15–18 isotonic contractions.

Shortening velocity and relative fiber force (%P₀) were determined over the final one-third of each isotonic contraction by computer analyses of the slopes of the position and force recordings, respectively. During the isotonic force clamps, the initial phase of each contraction elicited small variations in force and velocity. The small variation lasted approximately one-third (30 ms) of each isotonic load clamp. The variation in force and velocity was stabilized for the final two-thirds of each contraction. Thus the final one-third of each isotonic load clamp was used for analysis.

All shortening velocities were normalized to unit of FL and expressed as fiber lengths per second (FL/s). Because the length of each fiber segment studied varied slightly, it was necessary to normalize all velocity measurements to fiber length to account for the variation in the number of sarcomeres in series. To construct the force-velocity relationships, load was expressed as P/F₀, where P is the force during load clamping and P₀ is the peak isometric force developed before the submaximal load clamps. The Hill equation (18), (P + a)(V + b) = (P₀ + a)b, where P is force, V is velocity, P₀ is peak isometric force, and a and b are constants of force and velocity, respectively, was used to fit the data obtained for an individual by an interactive nonlinear curve fitting procedure. Only individual experiments with r² ≥0.98 were included for analysis.

Fiber power was calculated from the fitted force-velocity parameters and the maximum isometric force observed during the experiment. Absolute power (μN-m/s) was defined as the product of force (in μN) and shortening velocity (Vmax; in FL/s), whereas normalized power (W/l) was defined as the product of normalized force (i.e., force/fiber CSA) and shortening velocity (Vmax; in FL/s).

**MHC Isoform Composition**

After the single muscle fiber physiological measurements, each fiber was solubilized in 80 μl of 10% SDS sample buffer and stored at −20°C until assayed (15). To determine the MHC composition, fibers were run on a Hoefer SE 600 gel electrophoresis system that consisted of a 3.5% (wt/vol) acrylamide stacking gel with 5% separation gel at 4°C. After the gel electrophoresis, the gels were silver stained, as described by Giulian et al. (15). MHC isoforms were identified according to migration rates from the SDS-PAGE/silver staining and compared with molecular weight standards. The MHC were categorized as MHC I, IIA, IIX, I/IIa, I/IIa/IIX, I/IX, and Ia/IIX.

**Statistical Analysis**

Single muscle fiber physiological variables [diameter, P₀, normalized force, V₀, Vmax, absolute power, normalized power, a/P₀, and the percentage of peak force at which peak power occurs (M)] were analyzed using a univariate ANOVA with nested means. The number of studied fibers for a particular individual was nested to represent a mean for MHC I and MHC IIA fibers. Because of the minimal number of hybrid and pure MHC IIX present in these runners, analyses were restricted to MHC I and IIA fibers. Significance was set at P < 0.05, and a Student-Newman-Keuls post hoc test was used when significance was noted. All data are presented as means ± SE.

**RESULTS**

**Subjects**

Subject characteristics (mean ± SE) for the five runners who completed all three time points were as follows: age 20 ± 1 yr, height 178 ± 3 cm, weight 65 ± 4 kg, with a best 8-km run time (minutes and seconds) of 25.51 ± 0.07.

**Performance**

Running performance was measured three times during the 12-wk study duration. The baseline measurement (T1) was taken from the first meet of the season, T2 performance was evaluated from the last meet pre taper, whereas the championship meet served as the performance measure for T3. The competition meets coincided with each of the treadmill testing and muscle biopsy measures. Performance was evaluated from 8-km races, the standard distance for collegiate competition. Performance showed a trend to improve 2.8% from T1 (27.43 ± 0.31 min; P = 0.053) to T2 (26.65 ± 0.20 min) and an additional 1.1% from T2 to T3 (26.36 ± 0.26 min); however, these changes in running performance were not statistically significant.

**Treadmill Testing**

Absolute (l/min) and relative (ml·kg⁻¹·min⁻¹) maximum oxygen consumption were 4.6 ± 0.3 l/min, 4.7 ± 0.3 l/min, 70.5 ± 0.7 ml·kg⁻¹·min⁻¹, and 71.7 ± 1.2 ml·kg⁻¹·min⁻¹ at T1 and T3, respectively. Additionally, running economy as measured by oxygen consumption (l/min) at 16 km/h was 3.5 ± 0.2, 3.6 ± 0.3, and 3.6 ± 0.3 at T1, T2, and T3, respectively. No differences existed for any of these variables.

**Single Fiber MHC Composition**

On average, 22 fibers were studied from each subject at each time point (Table 1). Of the total number of fibers examined, 66% were identified as MHC I fibers and 33% were MHC IIA fibers. Only three hybrid fibers were studied, representing <1% of the fibers used for analysis. No MHC IIX fibers were examined, which is representative of this population.

**Table 1. Number of fibers analyzed for functional properties for each MHC isoform at each time point during the study duration**

<table>
<thead>
<tr>
<th></th>
<th>MHC I</th>
<th>MHC I/IIa</th>
<th>MHC IIA</th>
<th>MHC I/IIa/IIX</th>
<th>MHC I/IX</th>
<th>MHC IIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>69</td>
<td>0</td>
<td>38</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>82</td>
<td>1</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T3</td>
<td>65</td>
<td>1</td>
<td>41</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>2</td>
<td>109</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

MHC, myosin heavy chain; T1, base training phase; T2, 8-wk intense training period; T3, 4-wk taper phase.
Single Fiber Diameter

Diameter of MHC I fibers was 3% smaller (P < 0.05) at T2 compared with T1 (Table 2). After the taper phase (T3), MHC I fibers were 4% smaller (P < 0.05) than pretaper (T2) and 7% smaller (P < 0.05) compared with T1. The training periods did not alter MHC IIa fiber diameter.

Single Fiber Peak Force

\(P_0\) increased (P < 0.05) 18 and 11% for MHC I and IIa fibers, respectively, from T1 to T2 (Table 2). MHC IIa fibers were 9% stronger (P < 0.05) at T3 compared with T2. Conversely, \(P_0\) decreased 9% (P < 0.05) with the taper phase (T2-T3) in MHC I fibers. At T3, MHC IIa fibers were 21% stronger compared with T1, whereas no significant differences existed for MHC I fiber \(P_0\) between T1 and T3.

Peak force normalized for fiber size (specific force; \(P_0/CSA\)) increased (P < 0.05) 38 and 26% for MHC I and IIa fibers, respectively, with 8 wk of high-volume/high-intensity training (T1-T2; see Table 4). The taper phase did not alter specific tension for either fiber type (T2-T3). Specific tension for MHC I and IIa fibers was 32 and 31% higher (P < 0.05), respectively, at T3 compared with T1.

Single Fiber \(V_o\)

MHC I fiber \(V_o\) decreased (P < 0.05) 23% from T1 to T2 (Table 3). Additionally, \(V_o\) for MHC I fibers at T3 was 17% slower (P < 0.05) compared with T2 and 36% slower (P < 0.05) than baseline (T1). Contractile velocity of MHC IIa fibers was unaltered during the 12-wk study duration. Frequency distributions for MHC I and IIa fiber \(V_o\) at T1, T2, and T3 can be seen in Fig. 2. As can be observed in Fig. 2, at T1, 32% of MHC I fibers had a shortening velocity below 1.5 FL/s.

### Table 2. Diameter, \(P_0\), and peak tension of MHC I and MHC IIa individual fibers before (T1) and after (T2) a period of high-volume and intense training and after a 4-wk taper phase (T3).

<table>
<thead>
<tr>
<th>Diameter, (\mu m)</th>
<th>(P_0), mN</th>
<th>(P_{0s}) kN/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>IIa</td>
</tr>
<tr>
<td>T1 100±8</td>
<td>101±8</td>
<td>0.57±0.08</td>
</tr>
<tr>
<td>T2 97±12*</td>
<td>97±8</td>
<td>0.67±0.11*</td>
</tr>
<tr>
<td>T3 93±10*†</td>
<td>99±8</td>
<td>0.61±0.08†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 at all time points. \(P_0\), peak force. * P < 0.05 compared with T1. †P < 0.05 compared with T2.

### Table 3. \(V_o\) and \(V_{max}\) of MHC I and MHC IIa single fibers before and after a period of high-volume and intense training and after a 4-wk taper phase

<table>
<thead>
<tr>
<th>(V_o), FL/s</th>
<th>(V_{max}), FL/s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>T1 1.66±0.20</td>
<td>3.42±0.43</td>
</tr>
<tr>
<td>T2 1.27±0.17*</td>
<td>3.72±0.50</td>
</tr>
<tr>
<td>T3 1.05±0.13†</td>
<td>3.49±0.46</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 at all time points. \(V_o\), maximum unloaded shortening velocity; \(V_{max}\), loaded shortening velocity; FL, fiber length. * P < 0.05 compared with T1. †P < 0.05 compared with T2.

Fig. 2. Frequency distribution of myosin heavy chain (MHC) I and MHC IIa fiber shortening velocities (\(V_o\)) at T1 (A), T2 (B), and T3 (C). The x-axis distribution is in increments of 0.5 fiber length (FL)/s. For example, the value at 1.0 FL/s represents all fibers with a \(V_o\) ranging from 1.0 to 1.49 FL/s.
pared with T2 and 91% of MHC I fibers at T2 and T3, respectively. No significant changes in the frequency distribution of MHC Ila fibers across each time point were noticed.

**Single Fiber Absolute Power**

From T1 to T2, absolute power (μN·FL·s⁻¹) of MHC I fibers decreased 20% (P < 0.05; Table 4). Power of MHC I fibers decreased an additional 25% (P < 0.05) with the taper phase (T3) so that absolute power of MHC I fibers at T3 was 39% (P < 0.05) lower compared with T1. Absolute power of MHC Ila fibers was unaltered throughout the 12-wk study duration. Composite force-power curves for MHC I and Ila fibers at each time point are shown in Fig. 3.

**Single Fiber Normalized Power**

From T1 to T2, no changes occurred for normalized power (W/l) of either MHC I or MHC Ila fibers. After the taper phase (T3), normalized peak power of the MHC I fibers was 15 and 24% (P < 0.05) lower than T2 and T1, respectively. MHC Ila normalized power at T3 was not different compared with T1 and T2.

**Force-Velocity Parameters**

MHC I $V_{\text{max}}$ was 18% (P < 0.05) lower at T3 compared with T1 (Table 3). $V_{\text{max}}$ for MHC I fibers was not significantly different at T2 compared with T1 or T3, although a nonsignificant trend (P = 0.085) existed from T2 to T3. Fiber $V_{\text{max}}$ for MHC Ila fibers was not altered from T1 to T2 or with the taper phase (T2-T3). $aP/\alpha$, which describes the curvature of the force-velocity curve, decreased (P < 0.05) at each time point, T1 (0.039 ± 0.006) to T2 (0.030 ± 0.007), T2 to T3 (0.026 ± 0.003), and T3 compared with T1, illustrating an increased curvature. $aP/\alpha$ for MHC Ila fibers was lower (P < 0.05) at T2 (0.035 ± 0.007) and T3 (0.026 ± 0.003) compared with T1 (0.044 ± 0.006), but no different at T3 compared with T2. M, defined as the percentage of peak force at which peak power occurs, for MHC I fibers were 0.16 ± 0.01, 0.14 ± 0.01, and 0.13 ± 0.01 at T1, T2, and T3, respectively. M values for MHC Ila fibers were 0.17 ± 0.01, 0.15 ± 0.01, and 0.15 ± 0.01 at T1, T2, and T3, respectively. Statistical differences for M values of both fiber types followed the same trends as $aP/\alpha$.

**DISCUSSION**

This is the first investigation to study myocellular physiology in a group of young competitive runners and to monitor changes in cellular function with consecutive phases of training consisting of varying volumes and intensities. The unique findings from this study are that the contractile properties of myofibers from trained athletes are sensitive to alterations in training intensity. Furthermore, it appears that variations in distance run training alter the contractile function of slow-twitch (MHC I) fibers, whereas the fast-twitch (MHC Ila) fibers appear to be unchanged with intense endurance training and taper.

**Myofiber Diameter**

Modest, yet significant, decreases in MHC I fiber size were observed at each time point during the season, whereas MHC Ila fiber diameter was unaltered. This is contrary to earlier reports showing no change in myocellular size after run training in rodents (29); however, no interval type training was used in the treadmill running protocol; thus, the contrasting results may be because of differing training protocols or a differential response between species. Fast-twitch (MHC Ila) fiber size appears to be more responsive to changes in training volume and intensity in competitive swimmers (12, 31). Short-term high-volume and intense training can reduce fast-twitch fiber size by 15% (12), whereas reduced training elicits robust increases in the diameter of fast-twitch fibers only (31). It is difficult to ascertain if the small reduction in slow-twitch fiber size directly translated into a physiological advantage.

**Myofiber Force**

Interestingly, the decrease in cell size did not translate into a reduction in $P_o$. $P_o$ of both fiber types increased after the 8-wk high-volume and intense training phase, and MHC Ila fibers continued to increase force production after the taper phase. This increase in strength coupled with a decrease (MHC I) or no change (MHC Ila) in fiber size resulted in an increase in force per CSA of both fiber types. This was surprising, since alterations in strength are typically proportional to changes in cell size (12, 31, 33, 34, 36, 37). More importantly, the increase

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**Table 4. Peak absolute power and peak normalized power of individual MHC I and MHC Ila fibers before and after a period of high-volume and intense training and after a 4-wk taper phase**

<table>
<thead>
<tr>
<th></th>
<th>Absolute Power, μN·FL·s⁻¹</th>
<th>Normalized Power, W/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>Ila</td>
</tr>
<tr>
<td>T1</td>
<td>13.2±2.5</td>
<td>48.1±12.6</td>
</tr>
<tr>
<td>T2</td>
<td>10.6±2.2*</td>
<td>49.9±10.2</td>
</tr>
<tr>
<td>T3</td>
<td>8.0±1.4†</td>
<td>46.2±5.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 at all time points. *P < 0.05 compared with T1. †P < 0.05 compared with T2.
in force indicates that the decrease in cell size was not detrimental to the force-generating capacity of the cell. It is not likely that peak force is limited in endurance run performance, especially in response to base training, such as at T1. At baseline (T1), normalized force (P₀/CSA) in the current set of runners was substantially less (~30%) than values we have previously reported for single muscle fibers of the vastus lateralis of young sedentary men (32) and from the gastrocnemius of middle-aged (42 yr) untrained men (37). The higher values observed at T2 and T3 were in agreement with normalized force measurements previously reported in untrained men. This suggests that chronic, repetitive low-force contractions such as with aerobic run training may cause a reduction in P₀/CSA and that the added stress of interval training was sufficient to return the force-generating capacity of the myofibers to normal (23). It should be noted that the drastic (>30%) increase in normalized force from T1 to T2 was largely influenced by changes observed in one subject (Fig. 4). However, because each subject showed a general trend for an increase in P₀/CSA at T2, these data indicate that this functional muscle parameter is altered with large volume run training.

The mechanism(s) responsible for the enhanced force per CSA cannot be deduced from these data. Possible explanations include an augmented absolute number of cross-bridges acting in parallel (13) or an improved mechanical efficiency (i.e., higher force output per cross-bridge; see Ref. 7). Increases in the myofilament density or in the concentration of contractile proteins could result in an increase in force production; however, no training studies have supported this theory (1, 9). Alterations in the myosin light chains (MLC) have been shown to affect force production in skeletal muscle cells (30) and cardiac myocytes (39) and therefore remain a possible mechanism accounting for the changes in normalized force. Although the manner by which the force-generating capacity of single muscle fibers was increased in response to the training phases is unclear, this remains an exciting finding, and future studies will likely examine this issue.

**Myofiber Shortening Velocity**

The decrease in unloaded V₀ of the MHC I fibers at T2 and T3 indicates that changes in the training protocol elicited alterations in cross-bridge cycling kinetics in a fiber-type-specific manner. It should be noted that Vₘₐₓ, another index of shortening velocity, displayed a similar trend, although to a lesser magnitude. Vₘₐₓ is extrapolated from the force-velocity test and is known to underestimate V₀ (22), thus accounting for the lower Vₘₐₓ (compared with V₀) at each time point. Previous research has demonstrated that V₀ is sensitive to endurance training, since slow-twitch fibers from trained collegiate swimmers contract faster compared with untrained subjects while fast-twitch fibers have a slower V₀ (12). Likewise, run training leads to an increase in V₀ of MHC I fibers with a trend toward a decreased V₀ of MHC IIa fibers in rats (29), which is in agreement with what was reported among master’s age runners (37). At baseline, the present group of runners displayed similar trends. A V₀ of 1.65 FL/s for MHC I fibers, as observed at T1, is higher than what is typically reported from untrained individuals (32). As can be seen in Fig. 2, the decrease in V₀ of the MHC I fibers throughout the competitive season led to less overlap between the fiber types. At T1, 32% of MHC I fibers had a V₀ of <1.5 FL/s compared with 71 and 91% at T2 and T3, respectively. Additionally, each subject showed a decrease in MHC I V₀ throughout the study duration, illustrating a unidirectional shift consistent with all the runners (see Fig. 4). The lack of a training effect on the V₀ of MHC IIa fibers is in contrast to reports in swim athletes, which is likely the result of the differing training natures of the activities. Competitive swim training requires a higher-power component that would result in a greater recruitment of fast-twitch fibers. It is probable that the repetitive nature of prolonged running at a similar

![Fig. 4. Individual subject responses to run training at each time point for MHC I fiber diameter, peak force, specific tension, shortening velocity, peak (absolute), and normalized (Nrm) power.](http://ajpregu.physiology.org/)

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submaximal pace during the base training phase preceding T1 elicited adaptations specific to the MHC I fibers only. The change in $V_o$ of MHC I fibers suggests that run training may lead to an uncoupling of protein structure and function, since shortening velocity has been shown to be highly dependent on MHC isoform (28). Such an uncoupling has also been reported in humans with swim (31) and resistance training (33), postspaceflight (35), and after short-term run training in rodents (29). It is possible that alterations in the MLC profile can account for the changes in $V_o$ (29); however, we have previously reported changes in $V_o$ with training that were independent of the MLC profile (34). The collective results from these studies support the notion that $V_o$ may not be as tightly regulated by MHC isoform as was initially thought (4).

Myofiber Power

Peak power is perhaps the most relevant physiological parameter in relation to performance, since it accounts for peak force and contractile velocity. Whole muscle power output has been positively linked to run performance by enhancing running economy (e.g., energy expenditure at a given running velocity; see Refs. 8, 20, and 26). Running economy was unaltered at each time point in the present study, and no significant correlation was observed between cellular peak power and energy expenditure during submaximal running; however, such a relationship may have been present at intensities closer to performance pace. The decline in peak power at T2 and T3 of the MHC I fibers is most likely attributed to the decline in contractile velocity. Corrected for cell size, MHC I normalized power is still lower at T3 compared with T2 and T1. This indicates that the addition of interval training and racing diminishes absolute and normalized power preferentially of the slow-twitch fibers.

The extent to which the decrease in MHC I fiber power influences whole muscle power output is unknown. Myocellular power production is fiber type dependent, following a continuum from slow- to fast-twitch fibers in which power output is highest in the fast-twitch fiber population (6), which our laboratory has shown with various exercise regimens (31, 33, 34). Indeed, MHC Ila fibers generated four to six times greater power compared with the MHC I fibers throughout the study duration; therefore, it is possible that whole muscle peak power may not have been altered during the course of the study. It is likely that sustained submaximal power outputs are essential to distance running and that peak power is not a limiting factor in performance. Additionally, a lower $a/P_o$ at T2 and T3 for both fiber types indicates a shift in the force-velocity curves, signifying that, at any given load, the fibers contract at a slower velocity. Figure 3 displays a leftward shift in force-power curves, suggesting that peak power is reached at a lower relative force. It is unclear if these adaptations are favorable for muscle performance or a consequence of a suboptimal training program.

The relative lack of adaptations among the MHC Ila fibers compared with the MHC I fibers suggests that the fast-twitch fiber population is less affected by run training compared with swim and resistance training. Another possibility is that the changes in the training periods were not sufficient to elicit adaptations in the MHC Ila fibers. As can be seen in Fig. 1, the runners in the current study averaged ~100 km/wk of total run volume for the 8 wk preceding T1 and during the 8-wk training phase of the study (T1-T2). However, before T1, all running was aerobic in nature, consisting of no interval training, whereas intervals comprised 9% of the total run volume during the T1-T2 phase. With the taper phase (T2-T3), total run volume was reduced by 25%, with the absolute and relative amount of interval training increasing to 20% of total run volume. Therefore, the taper period was characterized more by an increase in interval training than a dramatic volume reduction. In the current study, 8-km run time did not significantly improve in response to the 4-wk taper, which is in contrast to previous reports. However, the trend ($P = 0.053$) for faster run times at T3 does indicate that the cellular changes observed in the current set of runners most likely contributed to the modest improvements in running performance. It should be noted that performance was assessed using cross-country races, which can be influenced by environmental factors. Performance events at T1 and T3 were conducted on the same course, which differed from T2. For this reason, it is difficult to compare run performance.

Taper periods consisting of ~85% reduction in training volume have elicited 3% improvements in 5-km run performance (21) while swimmers have shown a 4% improvement in performance (31), suggesting that the taper in the current study was less than optimal. It is possible that the increase in the relative and absolute amount of interval training with the taper phase counteracted the volume reduction and prevented any positive adaptations in myocellular function. The modest reduction in training volume with the taper phase in the current set of runners may explain, in part, the differential adaptations in myocellular function in run and swim training. Additionally, it should be noted, as shown in Fig. 4, that the alterations in myocellular physiology were consistent among each athlete, signifying that the trends represented by the mean data are truly indicative of the individual response.

In conclusion, this is the first investigation to examine single muscle fiber contractile properties in response to multiple run training periods throughout a competitive season. One limitation to this study is the lack of control group examined at each time point. However, we are confident that these data accurately reflect the influence of the training phases on myocellular function, since the coefficient of variation for our force transducer and servo-mechanical lever mechanism assessed at each time-point were <1%. Additionally, we have previously reported the validity of our experimental analyses with the longitudinal examination of multiple biopsies from an individual (14). The present results indicate that myofiber function is receptive to changes in run training and that interval training is a potent stimulus for eliciting changes in cellular function. Furthermore, it appears that these alterations are unique to run training, since changes in force per CSA have not been shown previously with swim (31) or resistance training (36). The lack of robust changes in single fiber power and run performance with the taper, as has been previously reported (31), suggests that a 25% reduction in training volume was not enough to overcompensate for the 67% ($9–15$ km/wk) increase in interval training; thus, the training program may have not been optimal.

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REFERENCES