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

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To date, no data are available characterizing the contractile properties of single fibers from young competitive runners. Competitive athletes are unique in that they possess genetic traits that underlie their aptitude for a particular activity. Subjecting the muscle to many years of run training may result in adaptations at the cellular level that are not manifest by short-term training programs. Furthermore, it is likely that these athletes are capable of training at higher intensities and volumes, which may lead to different adaptations. To further comprehend the response at the myocellular level to training, it is important to examine muscle that has been exposed to prolonged and varied training.

Endurance athletes typically undergo phases of intense training followed by periods of reduced training (taper) in an attempt to optimize performance. The cellular adaptations that occur with these training phases have been examined independently in collegiate swimmers (12, 31), illustrating that myocellular function is sensitive to changes in training volume and/or intensity. However, no investigation has studied the alterations in the contractile characteristics of individual muscle fibers that occur during multiple training phases. Therefore, the intent of this research was to measure the single muscle fiber functional adaptations to different phases of run training during a competitive season in male runners. Specifically, these runners were tested after a period of high-volume base training (T1), after 8 wk of high-volume/high-intensity training (T2), and after a 4-wk reduced training phase designed to prepare runners for the championship meet (T3).

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

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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.

The purpose of this investigation was to examine the contractile properties of individual myofibers in response to periodized training periods throughout a collegiate cross-country season in male runners. Muscle biopsies of the gastrocnemius were taken after a summer base training phase (T1), an 8-wk intense training period (T2), and a 4-wk taper phase (T3). Five runners (n = 5; age = 20 ± 1 yr; wt = 65 ± 4 kg; ht = 178 ± 3 cm) completed all three time points. A total of 328 individual muscle fibers [myosin heavy chain (MHC) I = 66%; MHC IIa = 33%; hybrids = 1%] were isolated and studied at 15°C for their contractile properties. Diameter of MHC I fibers was 3% smaller (P < 0.05) at T2 compared with T1 and an additional 4% smaller (P < 0.05) after the taper. Cell size was unaltered in the MHC IIa fibers. MHC I and IIa fiber strength increased 18 and 11% (P < 0.05), respectively, from T1 to T2. MHC I fibers produced 9% less force (P < 0.05) after the taper, whereas MHC IIa fibers were 9% stronger (P < 0.05). Specific tension increased 38 and 26% (P < 0.05) for MHC I and IIa fibers, respectively, from T1 to T2 and was unchanged with the taper. Maximal shortening velocity (V_s) of the MHC I fibers decreased 25% (P < 0.05) from T1 to T2 and 17% (P < 0.05) from T2 to T3, whereas MHC IIa V_s was unchanged. MHC I peak power decreased 20% (P < 0.05) from T1 to T2 and 25% (P < 0.05) from T2 to T3, whereas MHC IIa peak power was unchanged. Power corrected for cell size decreased 15% (P < 0.05) from T2 to T3 and was 24% (P < 0.05) lower at T3 compared with T1 for the MHC I fibers only. These data suggest that changes in run training alter myocellular physiology via decreases in fiber size, V_s, and power of MHC I fibers and through increases in force per cross-sectional area of slow- and fast-twitch muscle fibers.
Fig. 1. Total weekly training volume (separated into interval training and base running) during the 12 wk study duration. Also shown is the mean training volume for the 8 wk preceding baseline testing (T1).

The 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 propionate, 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-245; Omega Engineering, Stamford, CT) during the experiments. To cool the relaxing and activating solutions in the experimental chambers, a bath (RTE 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 (Vmax) 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

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followed by redevelopment of tension. The fiber was then returned to
to the relaxed 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 shorten-
ing. 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 compli-
ance 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
variable 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
analysis of the slopes of the position and force recordings, respec-
tively. 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 nor-
malize 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/P₀, where P is
the force during load clamping and P₀ is the peak isometric force
elicited at 4°C. After the gel electrophoresis, the gels were silver
staining gel at 4°C, and then subjected to a series of three isotonic load
steps. The small variation in force and velocity 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.

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% sepa-
rating 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, Ila, IIX, I/Ila, I/Ila/IIX, I/IIX, and Ila/IIX.

Statistical Analysis

Single muscle fiber physiological variables [diameter, P₀, nor-
malized force, Vₓ, 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 Ila fibers. Because of the minimal number
of hybrid and pure MHC IIX present in these runners, analyses were
restricted to MHC I and Ila fibers. Significance was set at P < 0.05.
and a Student-Newman-Keuls post hoc test was used when signifi-
cance 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 champi-

onship 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);
hence, 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 Ila
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 (17).

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 Ila</th>
<th>MHC Ila/IIX</th>
<th>MHC IIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>69</td>
<td>0</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>T2</td>
<td>82</td>
<td>1</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>T3</td>
<td>65</td>
<td>1</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>216</td>
<td>2</td>
<td>109</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_o 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_o 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_o between T1 and T3.

Peak force normalized for fiber size (specific force; P_o/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.

Table 2. Diameter, P_o, 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, μm</th>
<th>P_o, mN</th>
<th>P_o, kN/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>IIa</td>
</tr>
<tr>
<td>T1</td>
<td>100±8</td>
<td>101±8</td>
</tr>
<tr>
<td>T2</td>
<td>97±12*</td>
<td>97±8</td>
</tr>
<tr>
<td>T3</td>
<td>93±10†</td>
<td>99±8</td>
</tr>
</tbody>
</table>

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

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.

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</td>
<td>1.66±0.20</td>
</tr>
<tr>
<td>T2</td>
<td>1.27±0.17*</td>
</tr>
<tr>
<td>T3</td>
<td>1.05±0.13†</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 IIa 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 IIa fibers was unaltered throughout the 12-wk study duration. Composite force-power curves for MHC I and IIa 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 IIa 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 IIa normalized power at T3 was not different compared with T1 and T2.

**Force-Velocity Parameters**

MHC I V_max was 18% (P < 0.05) lower at T3 compared with T1 (Table 3). V_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_max for MHC IIa fibers was not altered from T1 to T2 or with the taper phase (T2-T3). a/P_o, 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. a/P_o for MHC IIa 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 IIa 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 a/P_o.

**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 IIa) 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 IIa 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 IIa) 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 IIa fibers continued to increase force production after the taper phase. This increase in strength coupled with a decrease (MHC I) or no change (MHC IIa) 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

![Fig. 3. Mean absolute power curves (μN·FL·s⁻¹) for MHC I and IIa muscle fibers before (T1) and after (T2) a period of high-volume and intense training and after a 4-wk taper phase (T3). Measurements were made using isotonic load clamps while maintained at 15°C. P_o, peak tension.](http://ajpregu.physiology.org/Downloadedfromhttp://ajpregu.org/)

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**Table 4.** Peak absolute power and peak normalized power of individual MHC I and MHC IIa 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>IIa</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 (\(P_o/CSA\)) 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 \(P_o/CSA\) 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 \(P_o/CSA\) 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 \(V_o\) 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 \(V_{\text{max}}\), another index of shortening velocity, displayed a similar trend, although to a lesser magnitude. \(V_{\text{max}}\) is extrapolated from the force-velocity test and is known to underestimate \(V_o\) (22), thus accounting for the lower \(V_{\text{max}}\) (compared with \(V_o\)) at each time point. Previous research has demonstrated that \(V_o\) 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 \(V_o\) (12). Likewise, run training leads to an increase in \(V_o\) of MHC I fibers with a trend toward a decreased \(V_o\) 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 \(V_o\) 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 \(V_o\) 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 \(V_o\) of <1.5 FL/s compared with 71 and 91% at T2 and T3, respectively. Additionally, each subject showed a decrease in MHC I \(V_o\) throughout the study duration, illustrating a unidirectional shift consistent with all the runners (see Fig. 4). The lack of a training effect on the \(V_o\) 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.
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 IIa 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 $a/P_e$ 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 IIa 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 IIa 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 and in 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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