Effect of Plyometrics on the Energy Cost of Running and MHC and Titin Isoforms

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ABSTRACT

PELLEGRINO, J., B. C. RUBY, and C. L. DUMKE. Effect of Plyometrics on the Energy Cost of Running and MHC and Titin Isoforms. *Med. Sci. Sports Exerc.*, Vol. 48, No. 1, pp. 49–56, 2016. Several training strategies such as plyometrics have been shown to improve running economy; however, its physiological basis remains elusive. **Purpose**: To examine the effect of plyometric training on the energy cost of running (ECR, $J \cdot kg^{-1} \cdot min^{-1}$), titin, and myosin heavy chain (MHC) isoforms. **Methods**: Subjects were randomly assigned to a 6-wk plyometric treatment (P; $n = 11$) or control group (C; $n = 11$). Preintervention and postintervention outcomes included body composition, vertical jump, sit-and-reach, maximal oxygen consumption ($\dot{V}O_{2max}$), speed at onset of blood lactate, 3-km time trial performance, ECR, and a *vastus lateralis* muscle biopsy for protein analysis. **Results**: Plyometric intervention resulted in improved time trial (P, 2.6% faster, $P = 0.04$; C, 1.6%, $P = 0.17$). $\dot{V}O_{2max}$ improved in the P group (5.2%, $P = 0.03$), whereas the C group increased by 3.1% ($P = 0.20$). The ECR decreased in the P group as the result of 6 wk of plyometric training ($P = 0.02$ for stage 3), whereas it increased in the C group ($P = 0.02$ for stage 3). The ECR correlated strongly with performance at stages 2, 3, and 4 ($r > 0.8$, $P < 0.001$) independent of group. There was no significant main effect of group, time, or interaction on any of the protein isoforms analyzed. A negative correlation was found between the ECR at stage 7 and MHC IIa ($r = -0.96$, $P < 0.001$), and the ECR at stage 6 with titin isoform 1 (T1)/T2 ratio ($r = -0.69$, $P = 0.007$) independent of group. **Conclusion**: Six weeks of plyometric training improved running performance and the ECR despite no measurable changes in MHC and titin isoforms. However, higher MHC IIa and lower T1/T2 isoform ratios correlated to lower ECR. **Key Words**: RUNNING ECONOMY, RUNNING PERFORMANCE, MUSCLE FIBER TYPE, MYOSIN HEAVY CHAIN, NEBULIN, TIME TRIAL

Running economy (RE) has long been demonstrated to relate strongly to performance (8,27,31,33). Despite this strong relationship, mechanisms that result in greater RE have eluded detection. Although fitness per se is not a guarantee for increased RE, various specific training strategies have demonstrated improvements such as intervals (7), altitude training (34), resistance training (32,36), and plyometrics (35). Improvements in RE result in less energy expenditure or oxygen consumption for a given running speed (27). Some have suggested that this is best explained by "free energy" in the elastic recoil of the muscle-tendon unit. Indeed, investigations using varying measurement techniques have shown that increased stiffness in the muscle-tendon unit relates with RE (1,6,8). Spurrs et al. (35) found that 6 wk of plyometrics increased RE, performance, and musculotendinous stiffness, despite no changes in $\dot{V}O_{2max}$.

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We have previously shown that greater RE and performance related to muscle stiffness in a group of well-trained runners (8). What remains to be determined are what changes are occurring with these training strategies, or what inherent properties of the muscle explain the improved running economy and enhanced musculotendinous stiffness.

Muscle fiber isoforms differ in their oxidative properties. Myosin heavy chain (MHC) isoforms (I, IIa, and IIx in human skeletal muscle) have been implicated in the energy expenditure of movement. Myosin heavy chain IIa and IIx, or fast twitch fibers, are considered less efficient with less oxidative capacity, whereas MHC I or slow twitch fibers are more oxidative and therefore are assumed to result in improved economy. This seems to be true in nonelastic recoil modalities such as cycling (5,16,20,30); however, economy in sports that rely on elastic recoil, such as running, may be different. Bosco et al. (3) demonstrated a positive relationship between the energetic cost of running and percent fast twitch fibers at a slow running speed (3.3 $m \cdot s^{-1}$). Conversely, another study found a negative correlation between MHC I fibers and 10-km performance (39). More recently, an investigation found that type IIa fibers relate to an improved stretch shortening cycle and better running economy (22). Relevant to the current proposed study, it was found that 4 wk of speed endurance training resulted in a lower oxygen cost of running, which corresponded with increased

MHC IIx fibers, with no change in the other isoforms (23). Importantly, Kyrolainen et al. (25) investigated the relationship between muscle properties and RE in a group of well-trained runners. They found that MHC II (a and x) isoform related to improved RE at fast running speeds (7 m·s^{-1}). They also were the first and only to show that the structural protein titin may play a role in RE. However, this finding was based solely on one subject who expressed both titin isoforms (T1 and T2) who happened to be an economical runner. Titin, or connectin, is a Z-line connecting protein thought to give skeletal muscle its elastic properties. To date, this is the only study that has investigated both MHC and titin isoforms in relation to running economy in humans. However, it has been shown that titin expression increased in the gastrocnemius muscle of mice after a treadmill training protocol, and this was proposed to increase the stiffness of muscle and improve the efficiency of exercise (2). These studies collectively represent what is known about muscle fiber properties and their relationship to RE and response to short-term training interventions.

It remains to be determined how training modalities such as plyometrics affect muscle isoform expression and if this relates to an improvement in RE and performance. It was therefore the aim of the current study to determine whether the improvement in RE and performance via plyometric training corresponds with changes in titin and/or myosin heavy chain isoform expression.

METHODS

Subjects' characteristics. Twenty-five volunteers from local running clubs and races in Missoula, Montana were recruited for the study. All subjects were screened for health history, current and prior training status, and injury history. All subjects were experienced runners with no previous experience in plyometric training for at least the previous 3 months. Before testing, subjects read and signed an informed consent form approved by the University of Montana Institutional Review Board for the ethical use of human subject research and met the standards of this journal and the Declaration of Helsinki.

Experimental design. After screening, the subjects were preliminarily tested for both anthropomorphic and dependent variables of $\dot{V}O_{2max}$, running economy, body composition, vertical jump (VJ), sit-and-reach (SR), muscle isoform expression, and 3000-m running time trial (TT) performance. The subjects were randomly assigned to either a control group or a plyometric training intervention and stratified for sex. The subjects then participated in 6 wk of plyometric training supervised by the same researcher throughout the intervention. *Vastus lateralis* muscle biopsies were taken before and after plyometric intervention. A food diary supplied on the first visit for the 24 h preceding the muscle biopsy was collected and redistributed before posttesting to eliminate dietary impact. Training outside of the study was recorded, but not controlled. The subjects were asked to not significantly change their training over the intervention. All measures were repeated after the 6-wk intervention, with all data collection completed within 11 d of the final plyometrics session. Order of testing, time of day, and diet were matched to that of pretesting. Twenty-two ($n = 11$ in each group) subjects completed all aspects of the study.

Physiological/fitness testing. During the first laboratory visit, both anthropomorphic and physiological fitness parameters were assessed. Because of the importance of $\dot{V}O_{2max}$ and lactate threshold on running performance and the established relationship between muscle tendon stiffness and running economy, parameters were collected both before and after intervention. The subjects underwent hydrostatic weighing for body composition, and VJ was tested with a Vertec (Grand Rapids, MI). A standard SR test was used to assess flexibility. Subjects completed a discontinuous treadmill session used for determination of onset of blood lactate (OBLA), $\dot{V}O_{2max}$, and running economy (RE) at several speeds. The protocol consisted of 3-min run bouts with 1-min rest intervals at progressively higher speeds until volitional fatigue. Stages 1–9 were run at a 1% grade to mimic over-ground running, starting at 2.15 m·s^{-1} and increased by 0.402 m·s^{-1} until a speed of 5.36 m·s^{-1} was reached. At this point, speed was maintained, and incline was increased by 2% each stage. Gas collection was carried out continuously for calculation of a $\dot{V}O_{2max}$ and RE (True One Metabolic Cart, Parvomedics, Sandy, UT) using 15-s averaging within a mixing chamber. Metabolic cart calibration was carried out repeatedly according to manufacturer's instructions. Blood was collected from a finger prick during each 1-min rest interval into a 50-μL capillary tube, immediately transferred to a lysis buffer, and stored at $-80°C$ until subsequent analysis of blood lactate (YSI 1500 Sport, Life Sciences, Yellow Springs, OH) for determination of OBLA. The oxygen cost at a given running speed (RE), and the energy cost of running (ECR) are accepted expressions of RE (27). Others have used the ECR to include the energy equivalent of lactate production, to include both aerobic and anaerobic systems in the estimation of total energy expenditure (25). Because of our interest in capturing RE both below and above the lactate threshold, RE was analyzed as ECR (J·kg^{-1}·min^{-1}). The ECR was calculated based on the energy equivalent of 20,202 J for each liter of oxygen, and added to the lactate values (when ≥2 mmol·L^{-1}) based on the energy equivalent of 60 J·kg^{-1}·mmol·L^{-1} for each running speed (25).

Time trial. To assess running performance, a 3000-m time trial (TT) was completed on an indoor 200-m nonbanked tartan (rubber) surface running track. The subjects were placed in groups of 5–8 by ability based in part on self-reported running ability (recent 5-km time) and in part on the results of the treadmill test. All runners were instructed to treat the TT as a race and were instructed to cover the distance as fast as possible. They were encouraged to pace themselves evenly throughout and allowed a self-selected warm-up. Split times for each subject were recorded, but not supplied to the runners,

TABLE 1. Subjects' characteristics.

	Controls	Plyometrics
N	11	11
Male/Female	7/4	7/4
Age, yr	34.2 ± 2.6	32.5 ± 2.0
Height, cm	170.5 ± 2.2	171.8 ± 2.7
Weight, kg	71.0 ± 3.6	68.2 ± 3.9
BMI, kg·m^{-2}	24.3 ± 0.9	24.0 ± 1.7
Body fat, %	22.1 ± 2.4	21.2 ± 2.4
$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	47.7 ± 2.3	48.0 ± 1.8

Data are mean ± SEM.
BMI, body mass index; $\dot{V}O_{2max}$, maximal oxygen consumption.

so as not to influence their pacing. Verbal encouragement was offered by the timers. Similar competitive groupings were used before and after intervention.

Plyometric training. The progressive plyometric training consisted of 15 sessions over 6 wk. Both total contacts per session, from 60 to 228, and intensity of the jumps increased throughout the 6 wk. The routine was a modified version of that used by Spurrs et al. (35) to induce a positive change in RE and performance in a group of runners. To protect our subjects from the potential of plyometric injury, the Spurrs et al. (35) protocol was slightly modified to include a slower progression, which led to an initial reduction in contacts in the first 2 wk. In addition, depth jumps were modified to deep knee bend box jumps landing on a mat.

Muscle biopsy. Muscle biopsies were collected from the *vastus lateralis* using the percutaneous needle biopsy technique with the aid of suction. For the preintervention biopsy, leg selection was randomized. The sample was flash frozen in liquid nitrogen and stored at −80°C until analysis. All biopsies were completed 48 h after any vigorous activity including the treadmill testing and the last plyometric training bout. The postintervention muscle biopsy was performed at the same site on the contralateral leg.

Titin and MHC analysis. Muscle samples were homogenized electrophoresed on a 1% agarose gel (38) with a Biorad Mini Protean gel system (Biorad, Hercules, CA) for titin analysis. Gels were subsequently Coomassie stained and underwent densitometric analysis using ImageJ analysis software (NIH). A 4-point volume loading technique was implemented as a linearization check with a minimal r^2 value of 0.90. The slope of this line obtained from linear regression was taken as the titin content of the homogenate as previously described (38). Titin ratios were computed from this point. Total content were scaled to a control sample run on each gel and normalized to total MHC for the individual sample.

Myosin isoforms were separated using the same total muscle homogenates via 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis on a Biorad Mini-Protean gel system (Biorad). Gels were stained with Coomassie blue and each isoform (I, IIa, and IIx) expressed as a percent of total densitometry. Densitometry was performed using ImageJ analysis software (NIH).

Statistical analysis. Group-by-time mixed-model 2 × 2 ANOVAs were run for all measures using SPSS version 22.0. Pairwise comparisons reported are for group, time, and group–time interaction differences unless otherwise specified. Main effects are reported when $P \leq 0.05$. Pearson correlations were computed for cross-sectional data. All results are reported as mean ± SEM, with significance set at an alpha level of $P \leq 0.05$.

RESULTS

The subjects' characteristics and sex distribution are found in Table 1. No differences were identified between groups. There was not a significant effect of group or time on training volume (Table 2). There was a significant main effect of time on $\dot{V}O_{2max}$. The P group showed a 5.2% improvement ($P = 0.03$), whereas the C group increased by 3.1% ($P = 0.20$). Vertical jump demonstrated a main effect of time ($P = 0.045$), and a trend for a group–time interaction ($P = 0.08$). The C group showed a significant decrease in VJ ($P = 0.01$), whereas the P group did not change. A main effect of group and time was found for SR flexibility (Table 2). This suggests that both the C ($P = 0.003$) and P ($P = 0.05$) groups lost flexibility over the course of the study. The P group was not different from C before the plyometric intervention ($P = 0.09$) but was significantly lower ($P = 0.03$) after. The running speed at OBLA was different between groups ($P = 0.05$) but did not significantly change with time. The 3-km TT demonstrated a significant main effect of time. The P group showed a 2.6% improvement ($P = 0.04$) in the performance trial, whereas the C group increased by 1.6% ($P = 0.17$).

At both the beginning and end of the intervention, performance in the time trial strongly correlated to $\dot{V}O_{2max}$ ($r > -0.8$, $P < 0.001$) and the speed at OBLA ($r > -0.8$, $P < 0.001$). Vertical jump values from both before and after data also correlated negatively with 3-km performance ($r = > -0.55$, $P < 0.005$). Sit-and-reach flexibility related positively with the ECR at several stages ($r > 0.4$, $P < 0.03$).

Significant main effects of group were found for the ECR for stages 2, 3, and 6 (with a trend in stage 4) (Table 3). The

TABLE 2. Performance measures in C and P groups.

	Controls		Plyometrics		Main Effects		
	Pre	Post	Pre	Post	Group	Time	G × T
Training, km·wk^{-1}	29.5 ± 4.6	31.3 ± 4.3	36.2 ± 4.6	34.4 ± 4.3	0.4	1.0	0.5
$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	47.7 ± 2.3	49.2 ± 2.1	48.0 ± 1.8	50.5 ± 2.1	0.8	0.02	0.5
VJ, cm	48.8 ± 4.2	45.5 ± 4.6	44.7 ± 4.1	44.4 ± 4.0	0.7	0.04	0.08
SR, cm	38.5 ± 2.1	42.4 ± 2.1	33.3 ± 2.1	35.6 ± 2.5	0.04	0.001	0.3
OBLA speed, m·s^{-1}	3.48 ± 0.12	3.50 ± 0.14	3.83 ± 0.14	3.88 ± 0.14	0.05	0.6	0.8
3-km TT, s	830.4 ± 35.6	817.2 ± 39.8	780.9 ± 29.9	760.8 ± 29.1	0.3	0.02	0.6

Data are mean ± SEM.

TABLE 3. Energy cost of running ($J\cdot kg^{-1}\cdot min^{-1}$) in C and P groups.

	Controls		Plyometrics		Main Effects		
Stage	Pre	Post	Pre	Post	Group	Time	G × T
1 (2.15 m·s⁻¹)	673 ± 17	685 ± 20	646 ± 17	643 ± 20	0.2	0.7	0.6
2 (2.55 m·s⁻¹)	746 ± 17	763 ± 18	703 ± 17	696 ± 18	0.03	0.6	0.2
3 (2.95 m·s⁻¹)	857 ± 19	882 ± 28	789 ± 19	779 ± 28	0.01	0.5	0.2
4 (3.36 m·s⁻¹)	998 ± 30	991 ± 31	927 ± 28	920 ± 29	0.09	0.6	0.9
5 (3.76 m·s⁻¹)	1163 ± 41	1131 ± 37	1070 ± 39	1095 ± 35	0.2	0.9	0.3
6 (4.16 m·s⁻¹)	1383 ± 59	1340 ± 41	1220 ± 50	1217 ± 34	0.05	0.3	0.4
7 (4.56 m·s⁻¹)	1518 ± 168	1451 ± 105	1337 ± 84	1415 ± 52	0.5	0.9	0.4

Data are mean ± SEM.

ECR decreased in the P group as the result of 6 wk of plyometric training ($P = 0.02$ for stage 3), whereas it increased in the C group ($P = 0.02$ for stage 3). A significant group–time interaction did not reach significance for ECR. The ECR correlated strongly with 3-km performance at stages 2, 3, 4, and 6 independent of group (Fig. 1). Similarly, the ECR related negatively with both the speed at OBLA ($r > -0.6$, $P \leq 0.001$) and $\dot{V}O_{2max}$ ($r \geq -0.6$, $P \leq 0.03$).

Rating of perceived exertion demonstrated a significant main effect of time ($P < 0.05$) at stages 1, 4, 5, and 6, indicating a lower perceived effort after the 6-wk plyometric intervention. The P group showed a lower rating of perceived exertion after plyometrics at stage 6 (17.3 ± 0.5 vs 16.7 ± 0.5; $P = 0.02$), whereas the C group did not significantly change (16.2 ± 0.6 vs 16.3 ± 0.8; $P = 0.42$).

Vastus lateralis muscle protein isoforms are found in Table 4. There was not a significant main effect of group, time, or interaction on any of the protein isoforms analyzed. However, a trend ($P = 0.06$) for a group–time interaction was shown for nebulin/MHC. A strong negative correlation was found between ECR and MHC IIa at stage 7 (4.56 m·s⁻¹) (Fig. 2A) independent of group. Correspondingly, a strong positive relationship exists between ECR and MHC I at stage 7. The ratio of titin isoforms T1/T2 related negatively to ECR at stage 6 ($r = -0.69$, $P = 0.007$) (Fig. 2B). Additionally, the T1/MHC related negatively to ECR at stage 6 ($r = -0.57$, $P = 0.03$). This suggests that the greater the amount of T1 (lower mobility), either normalized to MHC or as a ratio to T2 (faster mobility), and the greater percentage of MHC IIa results in improved RE (lower ECR). Despite these relationships, none of the muscle isoforms related to 3-km TT performance or $\dot{V}O_{2max}$. Myosin heavy chain IIa did show a positive relationship with VJ ($r = 0.59$, $P = 0.008$).

DISCUSSION

The main findings of the current study are that although 6 wk of plyometric training resulted in improved running performance and ECR, it did not significantly affect MHC or titin isoform expression. We modeled our plyometrics intervention from Spurrs et al. (35) who found nearly identical (2.7%) increases in 3-km running performance. Spurrs et al. concluded that increases in muscle stiffness attributed to the observed increases in RE and performance. Indeed, it is becoming well understood that muscle stiffness relates well to RE (1,6,8). Although we did not include a true measure of

muscle stiffness, we did demonstrate a relationship between VJ and performance and flexibility with the ECR. The positive relationship between SR and the ECR is consistent with others (27), suggesting reduced flexibility, and perhaps enhanced stiffness, resulting in improved ECR. Our results also suggest that a greater VJ results in improved performance. It was also true that MHC IIa positively related to VJ. Together these data are consistent with the finding that MHC IIa related to better ECR and faster 3-km performance. Little has been done previously on VJ and economy; however, this finding is consistent with both strength and elastic recoil of the legs relating to economy (1,6,8). In our study, plyometric training did not increase VJ. This is likely due to the forward motion (single- and double-legged bounds and hops) run focus of the plyometric training versus vertical (drop jumps for height). Our plyometric protocol was nearly identical to that of Spurrs et al. (35), which did demonstrate a modest (5 cm) but significant increase in countermovement jump. The reasons for this discrepancy may be due to their faster and younger subjects, or our slight modification of the depth jump. In our study, it seems that plyometric training protected the subjects from a decrease in VJ shown in the C group. The decrease in VJ, increase in SR and $\dot{V}O_{2max}$ in the C group, suggests additional run training performed by the subjects outside of the intervention. Our subjects were instructed to not change their run training during the intervention, indeed the kilometer per week did not show statistical significance (Table 2); however, large variances in free living humans may have masked
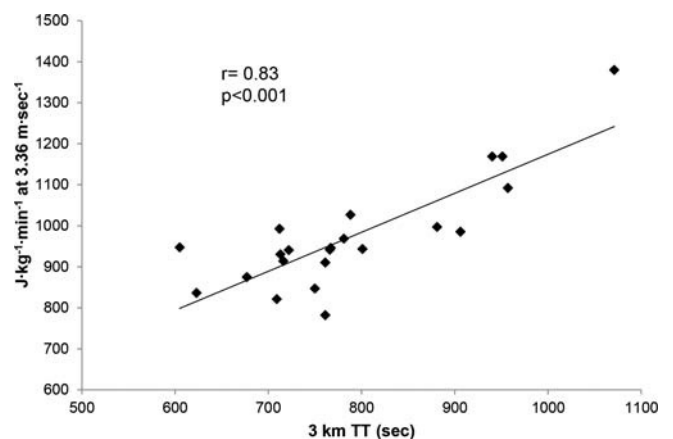

FIGURE 1—Correlation between the ECR ($J\cdot kg^{-1}\cdot min^{-1}$) and 3-km TT performance ($r = 0.83$, $P < 0.001$).

TABLE 4. Protein isoforms of the *vastus lateralis* in C and P groups.

| | Controls | | Plyometrics | | Main Effects | | |
	Pre	Post	Pre	Post	Group	Time	G × T
MHC IIx, %	3.9 ± 1.3	1.8 ± 0.3	4.1 ± 2.1	3.6 ± 1.5	0.6	0.1	0.3
MHC IIa, %	41.5 ± 3.8	35.3 ± 4.9	32.3 ± 5.1	30.7 ± 3.4	0.2	0.2	0.4
MHC I, %	54.5 ± 4.0	62.9 ± 2.5	63.6 ± 6.6	65.7 ± 3.9	0.3	0.2	0.3
Total titin	4.46 ± 0.42	4.37 ± 0.45	3.70 ± 0.47	4.20 ± 0.50	0.3	0.6	0.5
Titin/MHC	0.364 ± 0.036	0.335 ± 0.022	0.342 ± 0.039	0.357 ± 0.025	0.9	0.8	0.4
T1/MHC	0.27 ± 0.03	0.25 ± 0.02	0.26 ± 0.03	0.28 ± 0.02	0.7	0.9	0.4
T2/MHC	0.087 ± 0.011	0.082 ± 0.008	0.078 ± 0.012	0.075 ± 0.008	0.5	0.6	0.9
T1/T2	3.53 ± 0.48	3.42 ± 0.50	3.78 ± 0.54	4.17 ± 0.55	0.5	0.7	0.5
Nebulin/MHC	0.094 ± 0.01	0.092 ± 0.01	0.095 ± 0.11	0.12 ± 0.01	0.3	0.1	0.06

Units are arbitrary density units unless otherwise expressed. Data are mean ± SEM.
T1, titin isoform 1; T2, titin isoform 2.

additional run training in several of the subjects. Although we demonstrated a significant relationship between $\dot{V}O_{2max}$ and performance in this heterogeneous group of runners, other investigations with homogenous groups of better runners do not show this correlation (8). Similar to this previous investigation, $\dot{V}O_{2max}$ was positively related to the ECR at submaximal intensities. This suggests that those runners with a higher $\dot{V}O_{2max}$ are less economic.

The main purpose of our investigation proposed that MHC and titin isoforms may shift to explain improvements in economy after a plyometrics intervention; however, we were unable to detect these changes. Previous reports demonstrating
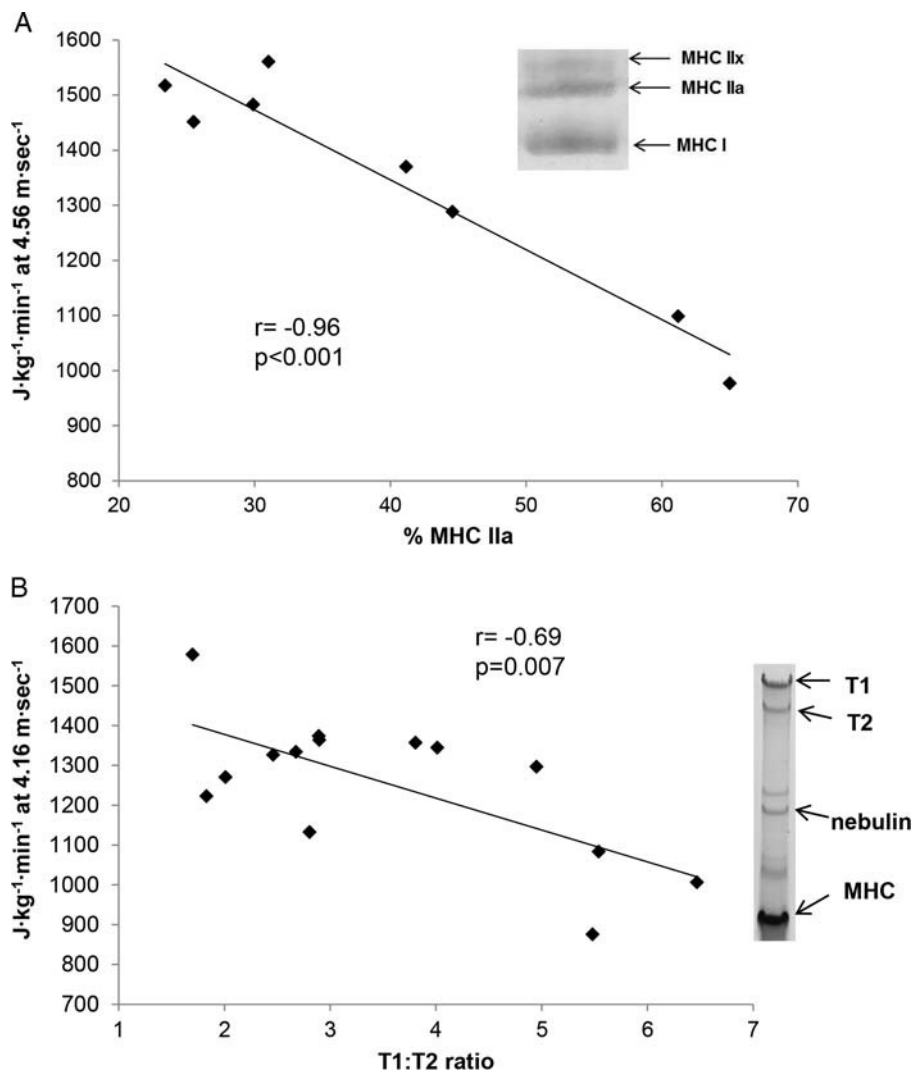
FIGURE 2—A, Correlation between the ECR (J·kg^{-1}·min^{-1}) and percent MHC IIa of the *vastus lateralis* ($r = -0.96$, $P < 0.001$). B, Correlation between the ECR (J·kg^{-1}·min^{-1}) and T1/T2 titin isoform ratio of the *vastus lateralis* ($r = -0.69$, $P = 0.007$).

relationships between muscle stiffness and RE arguably do not isolate the muscle stiffness properties from the muscle-tendon unit (1,6,8,35). However, using the free oscillation technique, we previously found that muscle stiffness related better than tendon stiffness (8) in a group of well-trained runners. Conversely, Arampatzis et al. (1) found that triceps surae tendon stiffness related well to RE. Therefore, it remains to be determined whether improvements in RE and performance with plyometric training may afford changes in tendon or other extramuscular properties.

A possible limitation of the current study is that 6 wk may not have been long enough for MHC and titin isoform changes to be detected. Only one study previous to ours has investigated how training interventions may alter MHC isoforms and RE (23). Iaia et al. (23) demonstrated that a series of 30-s running sprints for 4 wk improved RE and the percentage of MHC IIx fiber type but not 10-km run performance. This 4-wk study showed an increase from 9.7% to 14.0% in MHC IIx fibers. This study supports the belief that ≥4 wk is sufficient to observe changes in MHC isoforms. Our subjects demonstrated similar percentages of MHC I isoforms but greater IIa and less IIx compared to the Iaia et al. investigation. It is important to mention that the subjects in the Iaia study experienced significantly reduced overall training volume when undertaking the speed training intervention. This may have contributed to the observed shift in isoforms. The subjects in our study were instructed to continue their current level of run training outside of the plyometric intervention. Another possible limitation of the current study is the biopsy sampling of the *vastus lateralis* muscle. Previous investigations relating muscle stiffness and economy typically measure the lower leg, or triceps surae muscle groups. We are not aware of any investigation that has sampled the gastrocnemius or soleus muscles of runners to relate muscle properties to RE. All the previous investigations relating muscle isoforms to RE have also sampled the *vastus lateralis* (3,22, 23,25,39). Our study, like others, looked at whole muscle isoform expression and did not consider changes in MHC expression within single fibers, or hybrid fibers. How hybrid fibers are affected by training remains unclear. In a cross-sectional comparison, distance runners (competing >3 km) had greater MHC I expression, whereas middle distance runners (800–1500 m) expressed greater MHC IIa fibers (17). Both groups of runners in this study had a reduced amount of hybrid fibers compared to recreationally active controls (17). However, this reduced amount of hybrid fibers with training is not a universal finding (24). It remains to be seen how plyometric training may alter hybrid fibers from single muscle fiber preparations.

The current data do contribute to the understanding of how muscle isoforms contribute to the ECR. We found a significant negative relationship between the amount of MHC IIa fibers and the ECR. This is consistent with previous work demonstrating a relationship between MHC II fibers and RE (17,22,23,25,39). Most recently, Hunter et al. (22) found that RE related to MHC IIa fibers and increased

stretch shortening cycle in a group of trained runners. Perhaps importantly, the subjects in this study contained higher amounts of MHC II fibers (IIa and IIx of ~60%) than the current study (~35%–40%). Kyrolainen et al. (25) similarly found that a greater amount of MHC II fibers resulted in reduced ECR in a group of competitive middle distance runners. This relationship was at a similar ECR (~1000–1600 $J \cdot kg^{-1} \cdot min^{-1}$) despite running at a faster pace. The study only reported a significant relationship at 7 $m \cdot s^{-1}$ and a 0% grade compared to our study (Fig. 2A) at 4.56 $m \cdot s^{-1}$ and 1% grade. Because this is greater than the maximal aerobic capacity in either group of subjects, there was a significant contribution to the ECR from anaerobic sources. This lends importance to expressing the ECR as $J \cdot kg^{-1} \cdot min^{-1}$ (or $J \cdot kg^{-1} \cdot m^{-1}$) as is done in both the Kyrolainen and the current investigation. Indeed, only eight of our 22 subjects completed this 3-min stage, whereas in the Kyrolainen study their discontinuous test required only 1 min at 7 $m \cdot s^{-1}$. This was the only speed at which they report a significant relationship between MHC II fibers and the ECR. Although our study showed a strong relationship between MHC IIa and ECR at 4.56 $m \cdot s^{-1}$ and 1% grade, significance was approached at several other submaximal speeds including 2.15 $m \cdot s^{-1}$ ($r = -0.4$, $P = 0.07$), and 2.55 $m \cdot s^{-1}$ ($r = -0.45$, $P = 0.06$). Indeed, ECR at all stages had negative relationships with MHC IIa fiber expression, whether it reached statistical significance or not. Other investigations report MHC relationships at only one measured speed, either walking (1.34 $m \cdot s^{-1}$) (21), slow running (6 mph, or 2.68 $m \cdot s^{-1}$) (22), or fast running (>10 mph, or 4.47 $m \cdot s^{-1}$) (25). Others report indirect MHC relationships, such as a negative correlation between MHC I fibers and 10-km performance (39), or changes in MHC IIx associated with improvements in economy (23).

Together these data support negative relationships between MHC II fibers and RE or the ECR, and positive relationships between MHC I fibers and RE. This relationship is reverse of what is found in cycling, where MHC I fibers relate positively to gross efficiency and improved performance (5,16,20,30). We would like to suggest that economy of movement be considered in at least two different types of movements, elastic recoil (running, jumping), and nonelastic recoil type events (cycling and rowing). Elastic recoil modalities seem to benefit by greater MHC II isoforms, whereas the traditionally efficient MHC I isoforms favor economy in nonelastic recoil modes. What remains to be determined is whether muscle properties of the MHC II fibers themselves explain improved RE, or if some other aspect of the muscle-tendon unit associated with greater MHC II fibers is important for the reduced ECR.

To our knowledge, this is the first study to investigate changes in titin isoforms with plyometric training in runners. Although we found no changes after 6 wk of plyometric training in titin isoform expression, we did find a modest relationship between T1/T2 ratio and the ECR. The relationship we demonstrated between T1/T2 ratio and the ECR suggests that subjects with little or no T2 isoform have improved RE. In fact, a significant negative relationship was

found between total T1 normalized to MHC and the ECR. One previous investigation has attempted to cross-sectionally relate titin to RE (25). They found that only one of their 10 subjects expressed two titin bands, or both T1 and T2 isoforms. This one subject was also an economic middle distance runner, which led the authors to speculate to the importance of titin in RE. Unlike this investigation, most (20 of 22) of our subject population expressed both titin isoforms. The presence of two titin isoforms is consistent with previous findings in humans (9,25,28,29,37). Fry et al. (10) showed that five of 15 subjects expressed both T1 and T2 isoforms. Important to the current study, they also showed that expression of isoforms was consistent across *vastus lateralis*, *gastrocnemius*, and *soleus* muscles. Additionally, as we found, there was no relationship between titin isoform expression and MHC isoforms. McGuigan et al. (29) also found that subjects who expressed both titin isoforms did not change their isoform pattern after 8 wk of jump squat training. Despite the apparent lack of evidence of changes in titin isoforms in *vastus lateralis* and other peripheral skeletal muscle, there is a healthy body of research investigating titin as it relates to stiffness of the heart and diaphragm (11,13, 14,18,19). Exercise training increased the titin/MHC ratio, and thus stiffness, in the diaphragm but not in the left ventricle of mice (19). In humans, McBride et al. (28) showed that the percentage of T2 was lower in strength and power athletes compared to nonathletes. In the paucity of research on titin and exercise performance, this is consistent with our observed negative relationship of T1/T2 and T1/MHC with the ECR.

A confounding factor in titin isoform analysis is the belief that T2 may be a spliced product of T1 (12,26). Splicing, or degradation of the T1 to create alternative protein fragments, may be a result of *in vivo* proteolytic activity, or *ex vivo* inappropriate handling of muscle tissue. For this reason, strict care was taken in the processing of muscle samples as explained previously by Granzier et al. (15). We feel confident that our muscle processing resulted in optimized native titin isoform composition, since the appearance of two bands in human skeletal muscle is similar to that found by others (9,28,29,37). Another property of titin that may have been ignored by the current, and other studies of exercise training

in humans, is that titin phosphorylation can also affect stiffness of this protein (18). To date, there remains no evidence of titin isoform influence on nonelastic recoil modalities such as cycling. Because of the contributions of total titin, T1 or T2 isoforms, and phosphorylation can have on titin stiffness, a better understanding of titin's role in exercise requires further investigation.

Even less is known about the role nebulin plays in RE and adaptations to exercise training. It is known as a filamentous protein involved in the length of actin, but a recent surge in research using knockout mice implicates it in the generation of force, adaptations to strength training, and the energy cost of muscle contraction (4). Far less is known in humans. We show a trend toward greater nebulin in the plyometric training group, but not in the controls. Previously, Trappe et al. (37) demonstrated that as the result of one bout of muscle damaging exercise, both total titin and nebulin decreased. Others showed that nebulin may be important in the remodeling of sarcomeres after eccentric contractions (40). These suggestive data support further work on the intriguing role of nebulin in movement economy.

This study demonstrates that whereas plyometric training increases running performance, it does not correspond with measurable changes in titin or MHC isoforms. However, cross-sectionally, the ECR seems to be lowest in those with higher amounts of MHC IIa isoforms and a larger portion of T1 to T2 isoforms. This is opposite of what has previously been shown in cyclists, where higher economy seems to occur in individuals with greater MHC I fibers (5,16,20,30). We thus propose that the contribution of muscle fiber isoforms to economy/efficiency is different across elastic recoil versus nonelastic recoil modalities.

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