

Interrelationships between Muscle Structure, Muscle Strength, and Running Economy

HEIKKI KYRÖLÄINEN¹, RIIKKA KIVELÄ¹, SATU KOSKINEN¹, JEFF MCBRIDE¹, JESPER L. ANDERSEN³, TIMO TAKALA¹, SARIANNA SIPILÄ², and PAAVO V. KOMI¹

¹Neuromuscular Research Center, Department of Biology of Physical Activity, and ²Department of Health Sciences, University of Jyväskylä, Jyväskylä, FINLAND; and ³Copenhagen Muscle Research Centre, Department of Molecular Muscle Biology, Rigshospitalet, Copenhagen, DENMARK

ABSTRACT

KYRÖLÄINEN, H., R. KIVELÄ, S. KOSKINEN, J. MCBRIDE, J. L. ANDERSEN, T. TAKALA, S. SIPILÄ, and P. V. KOMI. Interrelationships between Muscle Structure, Muscle Strength, and Running Economy. *Med. Sci. Sports Exerc.*, Vol. 35, No. 1, pp. 45–49, 2003. **Purpose:** The present study was designed to investigate possible differences in running economy (RE) among elite middle-distance runners by examining muscle structure and maximal isometric force (MVC). **Methods:** Ten young male runners ran at six different running speeds. During the running bouts, respiratory gases, and blood lactate were measured. Muscle biopsies were obtained from the vastus lateralis muscle for analyzing fiber type distribution, muscle fiber area, myosin heavy chain (MHC) composition, activities of a number of metabolic enzymes (citrate synthase, lactate dehydrogenase, phosphofruktokinase, and 3-hydroxyacyl-CoA-dehydrogenase), and titin isoforms. **Results:** Energy expenditure (EE) increased linearly up to the speed of 6.0 m·s⁻¹. The relative distribution of the MHC isoforms was MHC I: 67.0%, MHC IIA: 31.5%, and MHC IIX: 1.5%. The present results demonstrated that higher the area of Type II fibers, higher the MVC ($r = 0.59$, $P < 0.05$). The amount of MHC II correlated inversely with EE when running close to the competition speed ($r = -0.61$, $P < 0.05$). Enzyme activities did not correlate significantly with either RE or EE. Titin analysis revealed that a faster-mobility titin band was observed in all subjects, whereas a lower-mobility titin band was observed only in the most economical runner. **Conclusion:** Differences in RE among homogeneous group of middle-distance runners were observed at various running speeds. This may partly be explained by differences in muscle fiber distribution, MHC composition, and titin isoforms. **Key Words:** ENERGY, MUSCLE FIBER, ENZYME, MYOSIN, TITIN

Numerous studies have been published on the maximal aerobic power of endurance athletes (4,23). These investigations have shown the importance of the maximal oxygen uptake ($\dot{V}O_{2\max}$) for endurance performance. $\dot{V}O_{2\max}$ values have been reported to vary from 70 to 85 mL·kg⁻¹·min⁻¹ among male endurance runners (21). Increased capillarization, activity of oxidative enzymes, and mitochondrial density indicate the importance of the oxidative capacity of skeletal muscle for endurance performance (11).

Anaerobic characteristics may also play an important role in determining endurance performance. It is possible that in middle-distance running a lower oxygen uptake may be compensated for by a greater anaerobic power and capacity (7). However, recent discussion has arisen concerning the role of the neuromuscular system in endurance performance and running economy among middle-distance runners (17). In interindividual comparisons, subjects trained in endur-

ance running are more economical than their untrained counterparts (8). In addition, intra-individual day-to-day variations (2–11%) in running economy have been observed (20). It has been suggested that biomechanical factors may account for a substantial portion of variations in running economy, although it has been concluded that the biomechanical descriptors, in general, are not good predictors of running economy (17).

On a structural level, contractile and elastic structures of a tendomuscular system have been implicated as having some importance concerning this issue. Bosco et al. (6) found a positive correlation between Type II fibers and energy expenditure at the running speed of 3.3 m·s⁻¹; however, the relationship at faster speeds is not known. Furthermore, the role of skeletal muscle myosin heavy chain (MHC) composition in oxygen utilization is unclear. The stored elastic energy capabilities of tendomuscular tissue have recently been of great interest (10). At the molecular level, the large protein called titin, which has significant elastic characteristics and acts as an anchor between the Z-line and myosin within a single sarcomere, may have importance in running energetics.

Further understanding of the interactions between muscle structure, neuromuscular function, and oxygen demand requires additional studies. Therefore, the main purpose of the present study was to investigate possible differences in running economy at different running speeds by examining muscle structure and maximal muscle strength.

Address for correspondence: Dr. Heikki Kyröläinen, Department of Biology of Physical Activity, University of Jyväskylä, FIN-40100 Jyväskylä, Finland; E-mail: heikki@sport.jyu.fi.

Submitted for publication February 2002.

Accepted for publication September 2002.

0195-9131/03/3501-0045/\$3.00/0

MEDICINE & SCIENCE IN SPORTS & EXERCISE®

Copyright © 2003 by the American College of Sports Medicine

DOI: 10.1249/01.MSS.0000046149.03322.BB

METHODS

Subjects

Ten male national level middle-distance runners (age 23 ± 3 yr, height 1.81 ± 0.06 m, body mass 66.4 ± 5.8 kg, and body fat $7.8 \pm 1.6\%$) volunteered as subjects for the present study. The $\dot{V}O_{2\max}$, maximal heart rate, and peak blood lactate (B-La) were measured in 7 of 10 subjects during treadmill running with progressively increased load until exhaustion, and their values were 74.5 ± 5.1 mL \cdot kg $^{-1}\cdot$ min $^{-1}$, 194 ± 4 beats \cdot min $^{-1}$, and 12.1 ± 2.8 mmol \cdot L $^{-1}$, respectively. All the runners were fully informed of the procedures and possible risks of the experiment, and they gave their written agreement to participate in this project, which was part of their normal testing. The Ethical Committee at the University of Jyväskylä approved this study.

Procedure

After a 30-min warm-up (running and stretching according to the individual procedure), the subjects were asked to perform two maximal isometric contractions (MVC) of knee extensor muscles. The knee angle was set at 107° .

After 10-min recovery, the subjects performed five submaximal running bouts on an indoor track (Jyväskylä, Finland). First, they ran for 3 min at a predetermined constant speed of 4.0 m \cdot s $^{-1}$ ($48 \pm 3\%$ of the maximal speed), 5.0 m \cdot s $^{-1}$ ($58 \pm 4\%$), and 5.5 m \cdot s $^{-1}$ ($66 \pm 3\%$) with 8-min recovery between each 3-min bout. After the 8-min recovery, they ran 1 min at the constant speed of 6.0 m \cdot s $^{-1}$ ($73 \pm 4\%$) and 7.0 m \cdot s $^{-1}$ ($82 \pm 4\%$), having again 8 min of recovery between each bout. Finally, after a 15-min recovery, they performed three maximal sprints over a distance of 30 m, the run-up phase having been individually selected by the subject. The mean (\pm SD) maximal speed was 8.50 ± 0.57 m \cdot s $^{-1}$.

During submaximal running, the subjects ran around the 200-m-long track at the predetermined constant speed by following the flashing lights of a speed control system (Protom, Naakka Inc., Lappeenranta, Finland).

Measurements

In all running tests, the average speed was measured by photocells (Newtest, Oulu, Finland). The respiratory gases were measured continuously by the breath-by-breath method (K4b², Cosmed, Rome, Italy). The instrument was regularly calibrated with known gas mixtures, and the measured values were corrected automatically into STPD. To determine the physiological loading of the subjects, the heart rate was recorded with a heart rate monitor (Polar Vantage, Kempele, Finland). Blood samples were drawn from a fingertip for blood lactate (B-La) analysis at rest and immediately after each running bout.

Approximately 2 h after the running tests, needle biopsies (a sample size of 100–150 mg) were taken from the middle portion of the vastus lateralis muscle. Local anesthetics (2-mL lidocaine-adrenalin, 1%) were admin-

istered subcutaneously before incision of the skin. The biopsy was divided into two portions. One portion for enzyme activity measurements was frozen immediately in liquid nitrogen and the other part for histochemical analysis was mounted on Tissue-TEK (Miles Inc., Elkhart, IN) and frozen rapidly in isopentane, which was cooled to -160°C in liquid nitrogen. The samples were stored at -80°C until analyzed.

Analysis

Energy expenditure. In the expired-air analysis during running, only 30 s of the steady state phase of the 3-min runs and the last 20 s of the 1-min run were used for analysis. To calculate the energy expenditure, an energy equivalent of 20202 J \cdot L $^{-1}$ oxygen was applied when respiratory exchange ratio (R) was 0.82. The change of ± 0.01 in R-value corresponds to ± 50 J change in energy expenditure (19). This method was utilized when B-La was negligible (<2.0 mM). When B-La exceeded the mentioned threshold, its energetic value was then calculated on the basis of an equivalent of 60 J \cdot kg $^{-1}\cdot$ mM $^{-1}$ (3 mL O₂ \cdot kg $^{-1}\cdot$ mM $^{-1}$) (22). Finally, this value was added to the oxygen consumption and aerobic energy cost obtained as described above. This sum value can be referred to as the oxygen requirement or the equivalent oxygen consumption (17). The heart rate values were determined by averaging their values during the respective steady state phase.

Muscle biopsy. From the freeze-dried muscle samples, citrate synthase (CS), lactate dehydrogenase (LDH), phosphofructokinase (PFK), and 3-hydroxyacyl-CoA-dehydrogenase (HAD) activities were determined as described by Saltin et al. (23). Enzyme activities at 37°C are expressed as $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g}^{-1}$ (dry weight).

For histochemical analysis, serial sections (10 μm) of the muscle biopsy samples were cut in cryostat (-24°C), and routine ATPase analysis was performed after preincubation at pH of 4.37, 4.60, and 10.30 (9). Four different fiber types were defined (Types I, IIA, IIAB, and IIB) according to Staron et al. (24). To localize the muscle cell borders, the sections were incubated with a 1:500 dilution of mouse monoclonal antidystrophin (dys 2, Novocastra Laboratories, Newcastle, UK) overnight at $+4^\circ\text{C}$. After washing with TRIS-buffered saline (TBS, pH = 7.5) the bound primary antibody was visualized by avidin-biotin peroxidase kit (Vectastain PK-4002, Vector Laboratories, Burlingame, CA) using diaminobenzidine (Sigma, St. Louis, MO) as a chromogen.

The serial sections were visualized and analyzed using an Olympus BX50 microscope (Olympus Optical Co., Tokyo, Japan), a Sanyo Hi-resolution Color CCD camera (Sanyo Electronic Co., Osaka, Japan), and an eight-bit Matrox Meteor Framegrabber (Matrox Electronic Systems, Quebec, Canada), combined with image-analysis software (Tema, Scan Beam, Hadsund, Denmark). A fiber mask was automatically found by the computer along the cell borders of dystrophin immunostained sections. All incorrect borders were excluded, and only horizontally cut fibers were used in

the determination. A fiber number was assigned by the computer to each individual fiber. Images of ATPase stainings were fitted into the fiber mask. The staining of the each individual fiber was divided into three groups: light, intermediate or dark, and four different fiber types were defined (Types I, IIA, IIAB, and IIB). The relative proportion of the various fiber types, fiber type area, and fiber types sizes were determined from 225 ± 47 (mean ± SD, range 150–290 cells) representative cells.

From each biopsy, 10–20 serial cross-sections (10 μm) were cut and placed in 100–200 μL of lysing buffer and heated for 3 min at 90°C to analyze MHC composition using SDS-PAGE gel electrophoresis. Between 5 and 20 μL of the myosin-containing samples were loaded on a SDS-PAGE gel containing 6% polyacrylamide and 30% glycerol. Gels were run at 70 V for 42 h at 4°C (3). After fixation the gels were Coomassie stained. Three different MHC bands can be separated in normal adult human skeletal muscle with the present electrophoresis procedure. These bands correspond to the MHC isoforms I, IIA, and IIX (16). The percentage of different MHC isoforms were determined using a densitometric system (Cream 1D, Kem-En-Tec aps, Copenhagen, Denmark).

For titin analysis, 10–20 serial cross-sections (10 μm) were cut from each biopsy and placed in 700 μL of lysing buffer and heated for 10 min at 60°C. Between 5 and 10 μL of samples were loaded on a SDS-PAGE gel containing 3.3% to 12.0% linear gradient gel with Fairbanks buffer (14). Gels were run at 70 V for 24 h at room temperature and silver stained (Silver Stain Plus, Bio-Rad Laboratories, Hercules, CA). For identification of the titin bands, the gels were blotted to a PVDF membrane at 25 V overnight using a Mini-PROTEAN II Trans-Blot Cell system. The membranes were incubated with mouse monoclonal antititin (1:500, Sigma) overnight at 4°C. After washing with buffer, the bound primary antibodies were visualized by avidin-biotin peroxidase kits for mouse by using diaminobenzidine as a chromogen.

Statistical Analysis

Nonparametric statistical techniques were utilized in the present study. Friedman's two-way ANOVA (chi-square) tested the effects of experimental conditions, and the Wilcoxon matched-pairs signed-rank test emulated the *t*-test with repeated measures. For analyzing the importance of different variables on running economy, the method of seemingly unrelated regression was used. In addition, Spearman's rank order correlation coefficient was used to determine the relationship between the measured variables. All data are presented as mean ± SD.

RESULTS

Running economy. Oxygen consumption increased from 46.1 ± 2.3 to 60.8 ± 3.6 mL·kg⁻¹·min⁻¹ (*P* < 0.001) (from 65 to 82% $\dot{V}O_{2max}$, *N* = 7) and heart rate from 148 ± 10 to 183 ± 5 beats·min⁻¹ (*P* < 0.001) with increasing

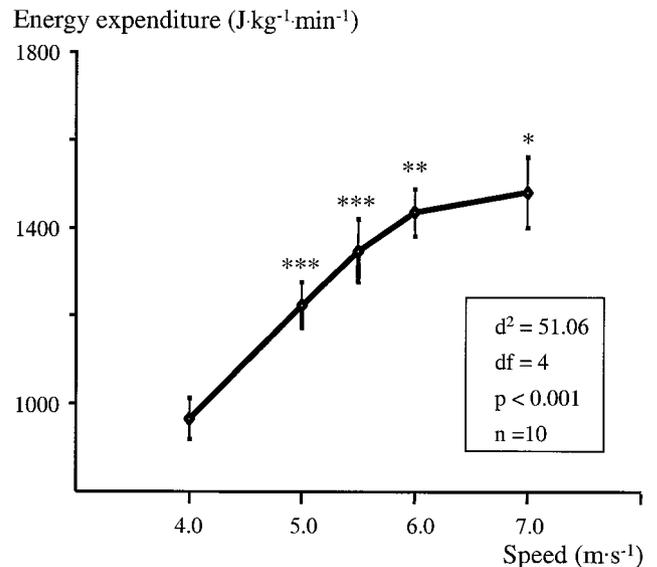


FIGURE 1—Mean (± SD) energy expenditure with increasing running speed. The values were corrected by the energetic values of blood lactate (22). Asterisks denote the statistical differences as compared with the previous condition. * *P* < 0.05, ** *P* < 0.01, and *** *P* < 0.001.

running speed from 4 to 7 m·s⁻¹. At the same time, B-La increased gradually from 1.8 ± 0.5 to 8.1 ± 0.9 mmol·L⁻¹ (*P* < 0.001) and energy expenditure from 965 ± 44 to 1436 ± 77 J·kg⁻¹·min⁻¹ (*P* < 0.001) (Fig. 1).

Muscle structure. Muscle fiber distribution revealed that Type I fibers dominated in the subject group: 64 ± 16% Type I, 24 ± 8% Type IIA, 5 ± 4% Type IIAB, and 6 ± 10% Type IIB. The distribution of muscle fiber areas showed a similar trend: 63 ± 18%, 25 ± 10%, 5 ± 4%, and 6 ± 9%, respectively. The mean size of the Type I fibers were 5240 ± 1605 μm² and Type II fibers 5369 ± 1585 μm².

The mean distribution of MHC isoforms was 67.0 ± 14.2% (range, 39.4–88.6%) for MHC I, 31.5 ± 12.9% (range, 11.4–52.5%) for MHC IIA, and 1.5 ± 2.8% (range, 0–8.1%) for MHC IIX. The titin analysis revealed that one titin band was observed in nine subjects, whereas two titin bands were observed in only one subject (Fig. 2).

The mean muscle enzyme values of CS, LDH, PFK, and HAD were 53 ± 9, 1833 ± 1086, 204 ± 48, and 257 ± 18 μmol·g⁻¹ dry weight·min⁻¹, respectively.

Neuromuscular capacity of knee extensors. The mean maximal force value was 1426 ± 286 N, and the rate of force development was 5281 ± 1482 N·s⁻¹. The maximal force correlated positively with the area of the Type II fibers (*r* = 0.59, *P* < 0.05), whereas the area of the Type I fibers had a trend for negative correlation with MVC (*r* = -0.56, *P* = 0.08).

Muscle structure versus running economy. At the slowest running speeds, there were not any significant correlations between running economy (oxygen uptake or energy expenditure) and either muscle fiber distribution or MHC composition. However, at the running speed of 7 m·s⁻¹, the oxygen consumption and energy expenditure

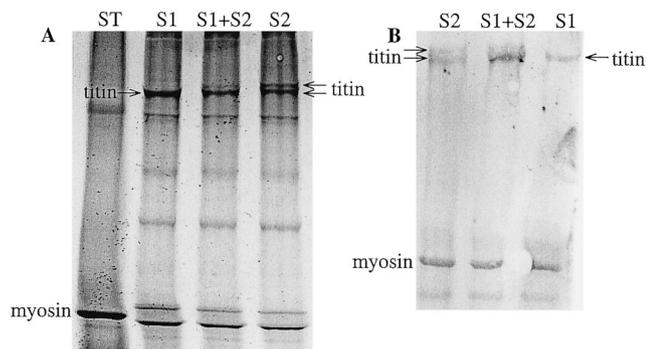


FIGURE 2—SDS-PAGE analysis of titin. **A**, The faster-mobility band (subject 1, S1) was observed in all 10 subjects, whereas only subject 2 (S2) showed also lower-mobility band. The samples from subjects 1 and 2 were mixed to ensure that subject 2 had lower-mobility band. A protein standard (ST) marked the location of myosin (Kaleidoscope Prestained Standard, Bio-Rad). **B**, Western blot of similar gels as in **A** with titin antibody.

both correlated with Type II fiber area ($r = -0.67$ and -0.67 , $P < 0.05$) and with Type IIB fiber distribution ($r = -0.63$ and -0.64 , $P < 0.05$, respectively). In addition, Figure 3 demonstrates that the higher the percentage of MHC II, the lower the energy expenditure at the speed of $7 \text{ m}\cdot\text{s}^{-1}$ ($r = -0.61$, $P < 0.05$).

DISCUSSION

The major findings of the present study can be summarized as follows: 1) Energy expenditure corrected by B-La energy equivalent increased linearly up to a speed of $6 \text{ m}\cdot\text{s}^{-1}$. 2) At a speed of $7 \text{ m}\cdot\text{s}^{-1}$, energy expenditure was inversely correlated with the amount of MHC II and with % Type II fibers. In addition, the percentage of Type II fibers and fast MHC isoforms correlated positively with the maximal force and with the rate of force production.

The maximal values of oxygen consumption and heart rate do not seem to explain running economy at the submaximal running speeds, especially, at the speed of $7 \text{ m}\cdot\text{s}^{-1}$,

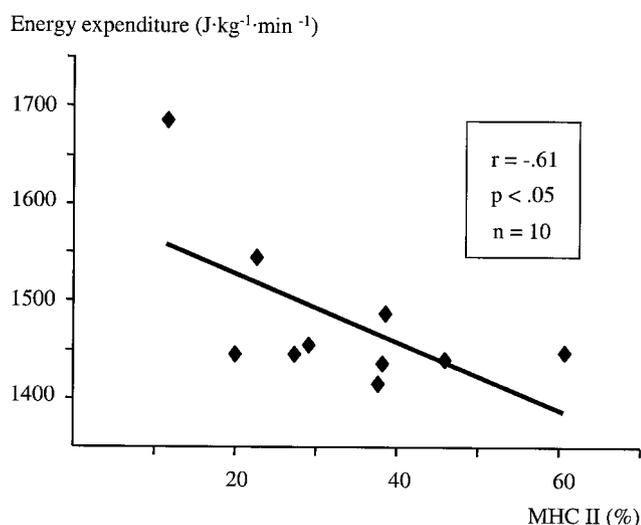


FIGURE 3—The relationship between the energy expenditure and MHC II.

neither does the dominating role of Type I fibers (64%) with higher oxidative capacity explain oxygen utilization. On the other hand, the more MHC II/Type II fibers, the less oxygen they used when running close to the competition speeds. These results are in agreement with earlier findings of Kaneko et al. (15), who found that endurance-trained runners were more economical than sprinters at lower speeds, whereas sprinters were more economical at faster speeds. The results of the present study are, however, in contrast with results of Bosco et al. (6). They found a positive correlation between Type II fibers and energy expenditure but the running speed they used was very slow, only $3.3 \text{ m}\cdot\text{s}^{-1}$. Thus, the relative amount of fast MHC seems to affect running economy, as well as it affects maximal force production and rate of force development (1). Additionally, the present data confirm earlier findings of very low MHC IIX expression in the muscles of endurance runners, suggesting that this MHC isoform is down regulated by the type of activity (2).

In the present study, due to a methodological limitation, oxygen consumption and energy expenditure values at the higher running speeds should, however, be interpreted with caution. The submaximal running tests of 1-min duration were obviously not enough to reach the highest possible oxygen consumption. Thus, the equivalent oxygen consumption may have been underestimated. As the lactate turnover versus clearance can be studied only in laboratory conditions, the method of di Prampero (22) was used to estimate the equivalent energy expenditure.

In a review article, Bassett and Howley (5) stated that the speed corresponding to lactate threshold is the best physiological predictor of distance running performance. They also recognized the importance of skeletal muscle factors such as capillary density and oxidative enzyme levels in determining the lactate threshold. The present findings among middle-distance runners did not show a relationship between muscle enzyme activities and the variable of running economy. On the other hand, middle-distance runners are adapted to different energy metabolism than long-distance runners. Thus, the present results in middle-distance running further emphasize the role of Type II fibers and ability to achieve a fast rate of force production. This was true, especially, at the speeds of 6 and $7 \text{ m}\cdot\text{s}^{-1}$, when the oxygen requirement was well above the $\dot{V}O_{2\text{max}}$. The measured oxygen uptake during running at those speeds was about 83% of their $\dot{V}O_{2\text{max}}$, when it could be expected that most of the motor units were active in working muscles. Therefore, the difference in oxygen uptake can be expected in runners having different muscle structure. The higher the number and area of Type II fibers, the lower the oxygen consumption but higher B-La production. Thus, when more and more motor units are recruited at the higher speeds, the importance of Type II fibers increases together with anaerobic energy metabolism.

Titin, a large elastic protein acting as anchor between the Z-lines and myosin within the sarcomere, may play a significant role in running economy. Whereas myosin is directly involved in the force production processes of muscle

contraction, titin is thought to have effects on the elastic characteristics of the muscle fibers (25). In the present study, all 10 subjects expressed the faster-mobility titin band. Only one subject expressed the lower-mobility titin band as well. This raises the question of whether the faster-mobility titin is more beneficial to middle-distance runners. Rabbit psoas muscle expresses the faster-mobility isoform (3.35 MDa), whereas rat soleus muscle expresses the largest titin isoform observed so far (3.7 MDa, rat) (12). The size difference between these two isoforms results from a shorter proximal tandem Ig and PEVK segments in psoas muscle. In a passive model, these segments behave as serially linked entropic springs in skeletal muscle (18). In short sarcomere extension, these springs are in a contracted state with high entropy, whereas on sarcomere extension the springs straighten, lowering their conformational entropy and resulting in a force. Thus, passive tension increase is steeper with increasing sarcomere length in psoas than in SOL muscle (12), suggesting better utilization of elastic energy. In the present study, the subject who expressed two titin isoforms was surprisingly the most economical (5% as compared with the mean of other runners). This suggests the

possible importance of the lower-mobility titin band as for an element increasing elasticity. However, Fry et al. (13) showed that each studied subject expressed the same titin isoforms in VL, GA, and SOL, and exercise did not appear to effect titin isoform composition. Further studies are needed to determine whether different titin isoform compositions are beneficial to specific athletes and whether exercise is a stimulus for composition changes.

In conclusion, muscle structure seems to play an important role in explaining differences of running economy among a group of homogeneous middle-distance runners. This may partly be explained by differences in muscle fiber distribution, MHC composition, and titin isoforms.

The authors would like to express their gratitude to Mr. Jarkko Finni, Mr. Markku Ruuskanen, Mr. Juha Kuosa, Mr. Erkki Helkala, Ms. Sirpa Roivas, Ms. Marja-Liisa Romppanen, and Ms. Pirkko Puttonen for their assistance during the measurements, Ms. Aila Ollikainen and Ms. Ursula Salonen for their assistance in the analysis, and Mr. Esko Levälähti for his assistance in the statistical analysis.

This study was supported by grant nos. 170/722/95, 104/722/96, and 96/722/97 from the Ministry of Education, Finland.

REFERENCES

1. AAGAARD, P., and J. L. ANDERSEN. Correlation between contractile strength and myosin heavy chain isoform composition in human skeletal muscle. *Med. Sci. Sports Exerc.* 8:1217–1222, 1998.
2. AAGAARD, P., and J. L. ANDERSEN. Muscle characteristics of the runner. In: *Running & Sciences, Proceedings to the 2nd ECSS Congress*, J. Bangsbo & H. Larsen (Eds.). Copenhagen: Munksgaard, 2001, pp. 49–66.
3. ANDERSEN, J. L., and P. AAGAARD. Myosin heavy chain IIX overshoot in human skeletal muscle. *Muscle Nerve* 23:1095–1104, 2000.
4. ÅSTRAND, P.-O. New records in human power. *Nature* 176:922–923, 1955.
5. BASSETT, D. R., JR., and E. T. HOWLEY. Maximal oxygen uptake: “classical” versus “contemporary” viewpoints. *Med. Sci. Sports Exerc.* 29:591–603, 1997.
6. BOSCO, C., G. MONTANARI, R. RIBACCHI, et al. Relationship between the efficiency of muscular work during jumping and energetics of running. *Eur. J. Appl. Physiol.* 56:138–143, 1987.
7. BRANDON, L. J. Physiological factors associated with middle distance running performance. *Sports Med.* 19:268–277, 1995.
8. BRANSFORD, D. R., and E. T. HOWLEY. Oxygen cost of running in trained and untrained men and women. *Med. Sci. Sports Exerc.* 9:41–44, 1977.
9. BROOKE, M. H., and K. K. KAISER. Three “myosin adenosine triphosphatase” systems: the nature of their pH lability and sulfhydryl dependence. *J. Histochem. Cytochem.* 18:670–672, 1970.
10. CAVAGNA, G. A., N. C. HEGLUND, J. D. HARRY, and M. MONTOVANI. Storage and release of mechanical energy by contracting frog muscle fibers. *J. Physiol.* 481:689–708, 1994.
11. COSTILL, D. L., W. J. FINK, and M. L. POLLOCK. Muscle fiber composition and enzyme activities of elite distance runners. *Med. Sci. Sports Exerc.* 8:96–100, 1976.
12. FREIBURG, A., K. TROMBITAS, W. HELL, et al. Series of exon-skipping events in the elastic spring region of titin as the structural basis for myofibrillar elastic diversity. *Circ. Res.* 86:1114–1121, 2000.
13. FRY, A. C., R. S. STARON, C. B. L. JAMES, R. S. HIKIDA, and F. C. HAGERMAN. Differential titin isoform expression in human skeletal muscle. *Acta Physiol. Scand.* 161:473–479, 1997.
14. GRANZIER, H. L. M., and K. WANG. Gel electrophoresis of giant proteins: solubilization and silver-staining of titin and nebulin from single muscle fiber segments. *Electrophoresis* 14:56–64, 1993.
15. KANEKO, M., A. ITO, T. FUCHIMOTO, Y. SHISHIKURA, and J. TOYOOKA. Influence of running speed of the mechanical efficiency of sprinters and distance runners. In: *Biomechanics IX-B*, D. A. Winter, R. W. Norman, R. P. Wells, K. C. Heyes, and A. E. Patla (Eds.). Champaign, IL: Human Kinetics, 1985, pp. 307–312.
16. KLITGAARD, H., M. ZHOU, S. SCHIAFFINO, R. BETTO, G. SALVIATI, and B. SALTIN. Ageing alters the myosin heavy chain composition of single fibres from human skeletal muscle. *Acta Physiol. Scand.* 140:55–62, 1990.
17. KYRÖLÄINEN, H., A. BELLI, and P. V. KOMI. Biomechanical factors affecting running economy. *Med. Sci. Sports Exerc.* 33:1330–1337, 2001.
18. LINKE W. A., D. E. RUDY, T. CENTNER, M. GAUTEL, C. WITT, S. LABEIT, C. C. GREGORIO. I-band titin in cardiac muscle is a three-element molecular spring and is critical for maintaining thin filament structure. *J. Cell Biol.* 146:631–644, 1999.
19. MCARDLE, W. D., F. I. KATCH, and V. L. KATCH. *Exercise Physiology*. Baltimore: Williams & Wilkins, 1991, p. 147.
20. MORGAN, D. W., P. E. MARTIN, and G. S. KRAHENBUHL. Factors affecting running economy. *Sports Med.* 7:310–330, 1989.
21. NOAKES, T. *Lore of Running*, 3rd Ed. Cape Town: Oxford University Press, 1991, 1–734.
22. PRAMPERO DI, P. E., C. CAPELLI, P. PAGLIARO, et al. Energetics of best performances in middle-distance running. *J. Appl. Physiol.* 74:2318–2324, 1993.
23. SALTIN, B., C. K. KIM, N. TERRADOS, H. LARSEN, J. SVEDENHAG, and C. J. ROLF. Morphology, enzyme activities and buffer capacity in leg muscles of Kenyan and Scandinavian runners. *Scand. J. Med. Sci. Sports* 5:222–230, 1995.
24. STARON, R. S., E. S. MALICKY, M. J. LEONARDI, J. E. FALKEL, F. C. HAGERMAN, and G. A. DUDLEY. Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur. J. Appl. Physiol.* 60:71–79, 1990.
25. WANG, K., R. MCCARTER, J. WRIGHT, J. BEVERLY, and R. RAMIREZ-MITCHELL. Viscoelasticity of the sarcomere matrix of skeletal muscles. *Biophys. J.* 64:1161–1177, 1993.