Characteristics of Myosin Heavy Chain and Titin in Strength and Endurance Athletes

Ryan M. Donovan

Faculty Sponsor: Michael R. McGuigan, Department of Exercise and Sport Science

ABSTRACT

The purpose of this investigation was to identify differences in myosin heavy chain (MyHC) and titin (T) isoforms in different athletic populations with increased levels of muscular endurance or strength relative to non-athletes. Subjects consisted of three groups based on training status: (1) endurance trained athletes (ET; n=5), (2) strength trained athletes (ST; n=5); or (3) non-athletes (NA; n=5). From gel electrophoresis analysis of muscle samples, MyHC I, MyHC IIa, and MyHC IIb along with T1 and T2 protein bands were identified and quantified. The percentages [mean ± SD] of MyHC I isoforms in ET, ST, and NA were 40.2 ± 16.0, 38.0 ± 8.4, and 38.0 ± 6.6, respectively, while MyHC IIa isoform distribution among the samples was 51.8 ± 14.0, 53.6 ± 12.6, and 44.4 ± 13.2, and MyHC IIb isoform distribution was 7.8 ± 9.1, 8.4 ± 8.3, and 18.2 ± 17.1. Similarly, T1 distribution among ET, ST, and NA groups was found to be 95.4 ± 7.1, 91.1 ± 9.2, and 69.2 ± 20.2, respectively, and T2 distribution was 4.6 ± 7.1, 8.9 ± 9.2, and 30.8 ± 20.2. There was a significantly higher expression of T2 in the NA group. These results suggest that different modes of training may result in changes in MyHC and T isoform distribution among athletes.

Keywords: muscle proteins, SDS-PAGE, training adaptations

INTRODUCTION

Not all athletes are created equal. While an individual has the ability to develop his/her skills through practice and training, genetics play a crucial role in determining an athlete's overall level of success in any particular sport. One such genetic factor is the physiological composition of an athlete's muscle. An extensive amount of past research has shown the existence of three primary myosin heavy chain (MyHC) protein isoforms within skeletal muscle: MyHC I, MyHC IIa, and MyHC IIb (Baechle & Earle, 2000). Each isoform utilizes a different energy system and, therefore, possesses unique characteristics. MyHC I fibers, for example, generate energy aerobically and are capable of producing a low level of force for an extended period of time. MyHC IIb fibers, on the other hand, produce a much greater force for a brief period of time through the chemical reactions of ATP and creatine phosphate. MyHC IIa fibers exhibit some of the characteristics of both MyHC I and MyHC IIb fibers (Hochachka, 1994; McComas, 1996).

Similarly, two isoforms of the large structural protein titin, (T1) and (T2) have been identified within skeletal muscle (Fry et al., 1997). Titin is an integral component of the A-band thick filament within the sarcomere and is believed to play a crucial role in the storage and re-utilization of elastic energy (Spierts, 1997; Wang et al., 1991). The titin protein is elastic in nature and contributes to the production of passive and active force by skeletal muscle. Passive tension, which can be measured directly in relaxed muscle, increases during sarcomere elongation and is responsible for the spontaneous retraction to the rest length upon release. Each titin isoform is associated with unique force-producing capabilities that could influence the stored elastic energy of muscle (Fry et al., 2003). According to Labeit & Kolmerer (1995), the long slack length characteristics of elastic muscle necessitate longer I bands. As a result, more elastic muscle may express larger titin isoforms.

Despite the increased interest in the characteristics of titin in skeletal muscle, no studies have directly compared strength and endurance athletes. Therefore, the primary purpose of this study was to identify myosin heavy chain and titin isoform distribution differences in athletic populations with increased levels of endurance or strength relative to non-athletes.

METHODS

Subjects

Fifteen male subjects between the ages of 18 and 28 were recruited for this study. Subject characteristics appear in Table 1. Each subject was given a written questionnaire and asked to describe his training protocols and competitive status for a minimum of the past 3 years and a maximum of the past 5 years. Subjects were chosen that were not currently taking, and had not previously taken, anabolic steroids, growth hormone, or related performance enhancing drugs of any kind. Subjects were not eliminated if taking vitamins, minerals, or other related natural supplements. After being informed on the nature of the experiment, written informed consent was obtained from all subjects. Approval for this study was granted by the University of Wisconsin-La Crosse Institutional Review Board for the Protection of Human Subjects.

Table 1. Subject characteristics. *ET* endurance trained, *ST* strength trained, *NA* non-athletes. Values are mean ± SD.

Variable	ET (n=5)	ST (n=5)	NA (n=5)
Age (years)	21.2 ± 2.3	21.0 ± 1.6	22.4 ± 3.4
Height (cm)	182.9 ± 11.2	185.8 ± 6.7	173.8 ± 8.4
Weight (kg)	76.6 ± 11.7	90.6 ± 14.1	86.4 ± 13.6
Training (hr/week)	[a] 10.4 ± 3.7	[b] 7.0 ± 2.0	0.6 ± 0.09

[a]Significant difference from the ST and NA groups.
[b]Significant difference from NA group ($P \leq 0.05$).

Study Design

This study consisted of three groups: (1) endurance trained athletes (ET; n=5), (2) strength trained athletes (ST; n=5); or (3) non-athletes (NA; n=5). Muscle biopsies were obtained from each subject.

Muscle Biopsies

All muscle biopsy specimens were obtained from the vastus lateralis muscle by a standard procedure involving a double-chop and suction method (Bergsrtöm 1962; Evans et al. 1982). Approximately 60 mg of skeletal muscle tissue was removed. Muscle fibers were then aligned, mounted on cork, frozen in isopentane pre-cooled in liquid nitrogen, and stored at -80°C for later analysis.

Gel Electrophoresis

Muscle samples were prepared using procedures previously described by Fry et al. (1997) and Granzier & Wang (1993). Samples were serially sectioned using a cryostat to a thickness of 40 μm and placed in a lysing buffer. This buffer consisted of 25% glycerol, 6.25% 2-mercaptoethanol, and 2% sodium dodecyl sulphate (SDS) in TRIS-HCL buffer (pH 6.8) and denatured at 60°C for ten minutes. For MyHC analysis, small amounts of the extracts (8 μm) were loaded on 8% SDS-polyacrylamide gels with 5% stacking gels and run using a Mini-Protean II electrophoresis system (Bio-Rad, Hercules, CA). The running voltage consisted of 70 V (constant voltage) for 30 minutes followed by 150 V (constant voltage) for 18 hours utilizing a Bio-Rad PowerPac power supply. Similarly, a 2% SDS-PAGE gel reinforced with agarose was used for the separation of titin bands as previously described by McGuigan et al. (2003). The electrophoretic run was carried out at 7 mA/two plates for 30 minutes, followed by 15 mA/two plates for 2-3 hours at room temperature (24 °C) (Granzier & Wang, 1993; Tatsumi & Hattori, 1995).

All gels were stained using a colloidal Coomassie blue staining protocol (ICN, Costa Mesa, USA). The gels were then scanned electronically, and the identification of protein bands was conducted using a gel plotting macro (NIH Image Program). The MyHC bands were identified as MyHC I, MyHC IIa, and MyHC IIb isoforms from a myosin molecular weight standard (Kaleidoscope Prestained Standards, Bio-Rad). A second macro was used to quantify T1 and T2 relative migration differences. Results were expressed as percentages of total MyHC or titin in each sample.

Statistical Analyses

Means and standard deviations were calculated for all variables. Comparisons of the study variables were performed between groups by one-way analysis of variance (ANOVA). A criterion alpha level of $P \leq 0.05$ was used for all statistical comparisons. All statistical analyses were performed through the use of a statistical software package (SPSS, Version 10.0, SPSS Inc., IL, USA).

RESULTS

No significant differences in MyHC distribution were found between the groups (Table 2, Figure 1). However, the trend towards a greater percentage of MyHC IIb in the NA group is consistent with previous research.

Table 2. Myosin heavy chain distribution (%) for athletes and non-athletes. Values are mean ± SD.

	ET	ST	NA
MyHC I	40.2 ± 16.0	38.0 ± 8.4	38.8 ± 6.6
MyHC IIa	51.1 ± 14.0	53.6 ± 12.6	44.4 ± 13.1
MyHC IIb	7.8 ± 9.1	8.4 ± 8.3	18.2 ± 17.1

The NA group had a significantly lower percentage of the T1 isoform and a higher percentage of the T2 isoform than the ET and ST groups (Table 3, Figure 2). No significant difference was found between the ET and ST groups.

Table 3. Titin distribution (%) for athletes and non-athletes. Values are mean ± SD.

	ET	ST	NA
T1	95.4 ± 7.1	91.1 ± 9.2	* 69.2 ± 20.2
T2	4.6 ± 7.1	8.9 ± 9.2	* 30.8 ± 20.2

*Significant difference from the ET and ST groups ($P \leq 0.05$).

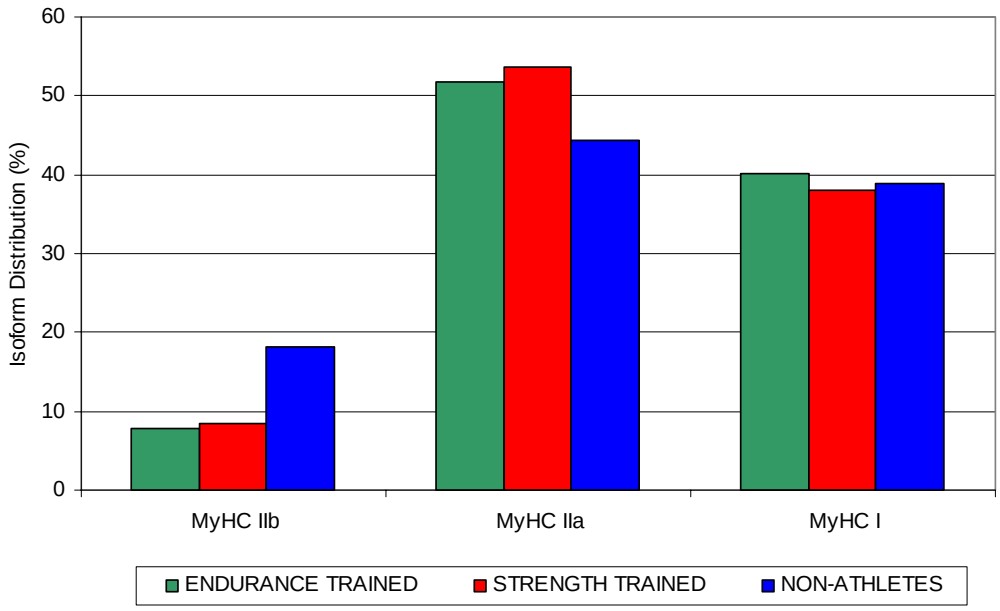

Figure 1. Myosin heavy chain distribution (%) for athletes and non-athletes.

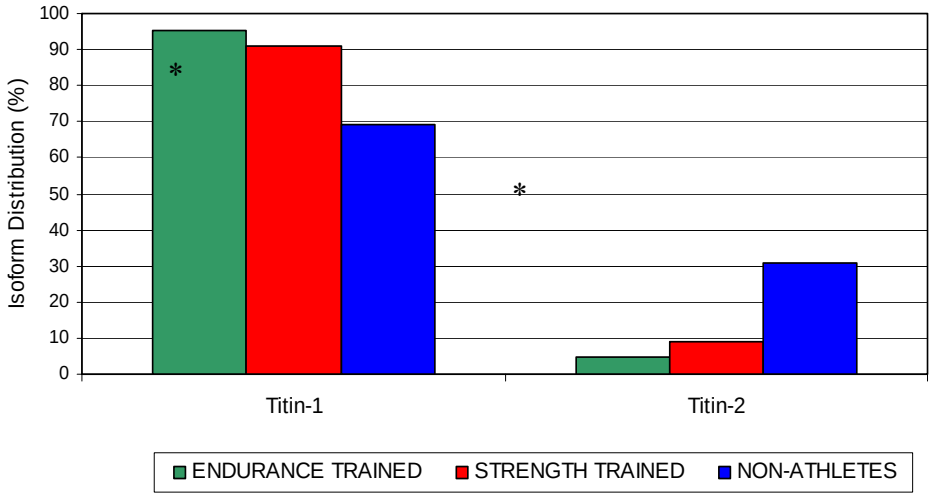

Figure 2. Titin isoform distribution (%) for athletes and non-athletes. *Indicates significant difference from ET and ST groups ($P \leq 0.05$).

DISCUSSION

The data from this study confirms that the muscle physiology of athletes is different from that of non-athletes. Although not statistically significant, due in part to the study's small sample size and large variability, the apparent trend of athletes, both ET and ST, possessing a lower percentage of MyHC IIb fibers, relative to non-athletes, is consistent with previous findings. Because IIb muscle fibers have a high activation threshold, they are rarely, if ever, recruited during sub-maximal activities (Baechle & Earle, 2000). It, therefore, is advantageous for a shift in muscle fiber type and/or isoform expression to take place (MyHC IIb to MyHC IIa). This adaptation follows an extended period of training, and is believed to be a contributing factor in athletic performance improvement (Starton et al., 1990).

Data from this study also indicates a trend toward a greater level of the MyHC I isoform in ET athletes. This observation, too, is consistent with a substantial amount of past research (Kraemer et al., 1995). Because endurance sports, such as distance running or cycling, require an individual to produce a relatively low level of force for an extended period of time, it is advantageous for ET athletes to possess primarily MyHC I muscle fibers. As previously mentioned, MyHC I fibers generate energy aerobically and are resistant to fatigue. In the presence of a sufficient quantity of oxygen, active muscles are able to not only prevent pyruvate, the end product of glycolysis, from being converted into lactic acid but also transport the pyruvate to the mitochondria where it enters the Krebs cycle. Here, the pyruvate is further oxidized and more ATP is produced (Baelche & Earle, 2000). In addition to the added energy produced by the complete metabolism of each glucose molecule, the use of the oxidative energy system by MyHC I fibers allows fat to be utilized as an energy source. Although fat does not metabolize as rapidly as glucose, the total amount of energy produced from one triglyceride molecule is substantially greater than that of one glucose molecule. For example, one (18-carbon) triglyceride molecule yields 463 ATP while one glucose molecule yields only 40 ATP (Baechle & Earle, 2000). This extreme difference supports the hypothesis that it would be advantageous for an ET athlete to possess a greater percentage of MyHC I fibers in order to sustain force production over an extended period of time.

Along with the MyHC isoform distribution differences noted, the current study supports previous research that human skeletal muscle contains at least two isoforms of titin: T1 and T2 (Fry et al., 1997). Results indicate that trained strength or endurance athletes have a tendency to possess a greater percentage of T1 and a lower percentage of T2 than non-athletes. Several ideas have been presented as to the true nature of the existence of two titin isoforms within human skeletal muscle. Labeit & Kolmerer (1995) associated a higher molecular weight titin with the longer slack length of a more elastic muscle, yet a study by McBride et al. (1999) found no significant correlations between T1 and T2 and the percentage of fast twitch or slow twitch muscle fibers. It has also been hypothesized that sarcomere strain could be a stimulus for changes in titin isoforms (Spierts et al., 1997). Because of the increased physical demands

placed on athletes, and titin's proposed role in contributing to muscle elasticity, the results of this study seem to support this hypothesis.

PRACTICAL APPLICATIONS

Further research is needed to investigate specific protein isoform changes, and the implications associated with these changes, following various forms of exercise. In order to develop more effective athletic training programs, the physiological changes induced by certain modes of exercise, within the broad categories of endurance training and strength training, should be examined. Also, specific training variables, such as frequency, intensity, and duration of exercise, should be compared. Because titin's role in physical performance is not as well understood as that of myosin, future studies on titin are warranted.

REFERENCES

Baechle, T.R., & R.W. Earle (Ed.). (2000). *Essential of Strength Training and Conditioning*. Champaign, IL: Human Kinetics.

Bamman, M.M., M.S.F. Clarke, R.J. Talmadge, & D.L. Feeback. (1999). Enhanced Protein electrophoresis technique for separating human skeletal muscle myosin heavy chain isoforms. *Electrophoresis* 20:466-468.

Bergsrtöm, J. (1962). Muscle electrolyter in man. *Scand. J. Clin. Lab. Med.* 14, 511-513.

Evans, W.J., S.D. Phinney, & V.R. Young. (1982). Suction applied to a muscle biopsy maximizes sample size. *Med. Sci. Sports Exerc.* 14: 101-102.

Fry, A.C., R.S. Staron, C.B. James, R.S. Hikida, & F.C. Hagerman. (1997). Differential titin isoforms expression in human skeletal muscle. *Eur. J. Appl. Physiol.* 161: 473-479.

Fry, A.C., B.K. Schilling, R.S. Staron, F.C. Hagerman, R.S. Hikida, & J.T. Thrush. (2003). Muscle fiber characteristics and performance correlates of male Olympic-style weightlifters. *J. Str. Cond. Res.* 17: 746-754.

Granzier, H., & K. Wang. (1993). Gel electrophoresis of giant proteins: solubilization and silver-staining of titin and nebulin from single muscle fiber segments. *Electrophoresis* 14: 56-64.

Hochachka, P.W. (1994). *Muscles as Molecular and Metabolic Machines.* Ann Arbor, MI: CRC Press.

Kraemer, W.J., J.F. Patton, S.E. Gordon, E.A. Harman, M.R. Deschenes, K. Reynolds, R.U. Newton, N. Travis, & J.E. Dziados. (1995). Compatibility of high-intensity strength and endurance training on hormonal and skeletal muscle adaptations. *J. Appl. Physiol.* 78: 976-989.

Labeit, S., & B. Kolmerer. (1995). Titins: giant proteins in charge of muscular ultrastructure and elasticity. *Science* 270: 293-296.

McBride, J., H. Kyröläinen, P.V. Komi, S. Koskinen, & T. Takala. (1999). Characteristics of titin in relation to running economy in elite distance runners. In: Parisi, P., Pigozzi, F., and Prinzi, G. (1999) *Sport Science '99 in Europe*, 309.

McBride, J., T. Triplett-McBride, A.J. Davis, P.J. Abernethy, & R.U. Newton. (2002). Characteristics of titin in strength and power athletes. *Eur. J. Appl. Physiol.* Published online: 22 Nov.

McComas, A.J. (1996). *Skeletal Muscle: Form and Function*. Champaigh, IL: Human Kinetics.

McGuigan, M.R., M.J. Sharman, R.U. Newton, A.J. Davie, A.J. Murphy, & J.M. McBride. (2003). The effect of explosive resistance training on titin and myosin heavy chain isoforms in trained subjects. *J. Str. Cond. Res.* 17: 645-651.

Potteiger, J.A., R.H. Lockwood, M.D. Haub, B.A. Dolezal, K.S. Almuzaini, J.M. Schroder, & C.J. Zebas. (1999). Muscle power and fiver characteristics following 8 weeks of plyometric training. *J. Str. Cond. Res.* 13: 275-279.

Spierts, I.L., H.A. Akster, & H.L Granzier. (1997). Expression of titin isoforms in red and white muscle fibers of carp exposed to different sarcomere strains during swimming. *J. Comp. Physiol. B.* 167: 543-551.

Starton, R.S., E.S. Malicky, M.J. Leonardi, J.E. Falkel, F.C. Hagerman, & G.A. Dudley. (1990) Muscle hypertrophy and fast fiber type conversions in heavy resistance-trained women. *Eur. J. Appl. Physiol.* 60: 71-79.

Tatsumi, R., & A. Hattori. (1995). Detection of giant myofibrillar proteins connectin and nebulin by electrophoresis in 2% polyacrylamide slab gels strengthened with agarose. *Anal. Biochem*. 224: 28-31.

Wang, K., R. McCarter, J. Wright, J. Beverly, & R. Ramirez-Mitchell. (1991). Regulation of skeletal muscle stiffness and elasticity by titin isoforms: A test of the segmental extension model of resisting tension. *Proc. Natl. Acad. Sci. USA.* 88: 7101-7105.