Skeletal Muscle Fatigability and Myosin Heavy Chain Fiber Type in Resistance Trained Men

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Abstract

Bagley, JR, McLeland, KA, Arevalo, JA, Brown, LE, Coburn, JW, and Galpin, AJ. Skeletal muscle fatigability and myosin heavy chain fiber type in resistance trained men. *J Strength Cond Res* 31(3): 602–607, 2017–Forty years ago, Thorstensson and Karlsson in 1976 described the link between muscle fatigability and fiber type, finding that more fast-twitch fibers were associated with a quicker onset of quadriceps fatigue. This provided the foundation for the Classic Thorstensson Test of fatigability and subsequent noninvasive fiber type prediction equation. This equation was developed with data from recreationally active (REC) men but has been implemented in participants with heterogeneous physical activity/exercise backgrounds. The accuracy of this approach in resistance trained (RET) men has not been established. Moreover, muscle fiber typing techniques have evolved considerably since this seminal work. Therefore, we reexamined this relationship using RET men and a more sensitive fiber typing method (single fiber myosin heavy chain [MHC] isoform classification). Fifteen RET men (age = 24.8 ± 1.3 years) performed maximal knee extensions (via isokinetic dynamometry) to determine peak torque (PT) and quadriceps fatigue percentage (FP) after 30 and 50 repetitions. Vastus lateralis (VL) single fiber MHC type was determined and fibers were grouped as %Fast (expressing MHC IIa, IIa/IIX, or IIx; *no* MHC I containing fibers). Resistance trained men exhibited 46% greater PT (RET = 207 ± 28 N·m vs. REC = 130 ± 8 N·m) and 28% more %Fast (RET = 61 ± 4% vs. REC = 44 ± 4%) than REC men. Additionally, RET men had a relatively homogeneous FP (64 ± 1%) ranging from 53 to 72%. No relationship was found between FP and MHC fiber type ($R^2 = 0.01$, $p > 0.05$). The Classic Thorstensson Test may not accurately estimate VL fiber type composition in RET men, highlighting the (a) unique phenotypical/functional adaptations induced by chronic RET and (b) the need for more sensitive cellular/molecular analyses in RET muscle.

Key Words single muscle fiber, vastus lateralis, fatigue, isokinetic dynamometer, muscle function, SDS-PAGE

Introduction

Forty years ago, Alf Thorstensson and Jan Karlsson of the Swedish School of Sports and Health Sciences (GIH) first described the link between muscle fatigability and fiber type in humans (33). They found a linear relationship ($r = 0.86$) between the decline in maximal force production of the quadriceps (i.e., fatigability) and vastus lateralis (VL) fast-twitch fiber type percentage in recreationally ("habitually") active men. This provided foundations for the still-popular Classic Thorstensson Test (CT) of quadriceps fatigability (10) which established a prediction equation to estimate VL fiber type composition (4).

Owing to technological limitations of the time, Thorstensson and Karlsson classified muscle into only 2 categories, "slow (type I)" or "fast (type II)" using adenosine triphosphatase (ATPase) histochemistry. A more sensitive approach was developed in the 1980s, wherein single human muscle fibers were categorized by myosin heavy chain (MHC) protein isoform (spectrum of slow to fast: MHC I, MHC IIa, MHC IIx) (6,30) via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). This led to the discovery of "hybrid" fibers which coexpress multiple isoforms (MHC I/IIa, MHC IIa/IIx, MHC I/IIa/IIx) (7). The simplification of this MHC continuum into 2 types (type 1 and type 2) leads to fiber type misclassifications (2,31), thus single muscle fiber MHC isoform identification is considered the "gold standard" for fiber typing in humans (26).

The CT is still popular among researchers and athletes/coaches and in exercise physiology classrooms worldwide as a noninvasive method to estimate fiber type (4). The original fiber type prediction equation was derived from data on recreationally active (REC) men (33); however, individuals most interested in identifying their fiber type composition are presumably those with experience exercise training (e.g.,

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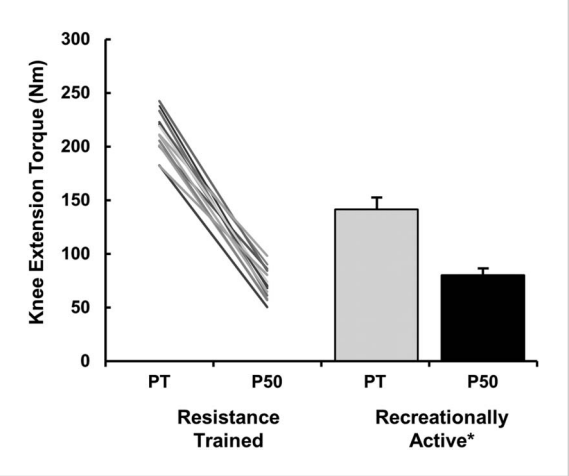

Figure 1. Peak torques (N·m) before (PT) and after 50 (P50) maximal knee extensions in resistance trained and recreationally active men (*data from Thorstensson and Karlsson (33)). A single line represents individual data from PT to P50. Mean ± *SD* are used for recreationally active data. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

involved in resistance training, sport, or other physical activities). Therefore, we reexamined the relationship between fatigability and fiber type using (a) the more sensitive fiber typing method of single muscle fiber MHC isoform classification and (b) a homogenous group of resistance trained (RET) men. We hypothesized that the CT prediction equation developed with data from REC men would not be valid when implemented in participants with homogeneous physical activity backgrounds (i.e., RET).

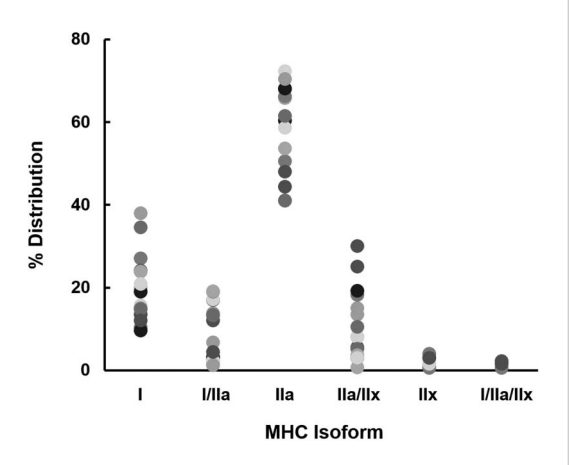

Figure 2. Single muscle fiber myosin heavy chain (MHC) fiber type profile of the vastus lateralis in resistance trained men ($n = 2,245$ muscle fibers analyzed).

Methods

Experimental Approach to the Problem

All subjects performed a maximal effort leg extension test and received a biopsy from the same leg. Subjects received written and oral information about experimental procedures and potential risks before giving written informed consent. The university institutional review board approved the study procedures and consent forms.

Subjects

Fifteen RET men (≥6 months of upper and lower body strength-based resistance exercise ≥3 $d \cdot wk^{-1}$; average years of training 5.3 ± 2.7, age = 24.8 ± 1.3 years, height = 1.79 ± 0.05 m, mass = 82.2 ± 8.0 kg) volunteered to participate in this study. Participants received written and oral information about experimental procedures and potential risks before giving written informed consent. The university institutional review board approved the study procedures and consent forms.

Performance and Fatigability Measures

Participants underwent a familiarization session on an isokinetic dynamometer (Biodex; System 3, Shirley, NY, USA) after which they performed maximal concentric knee-extension contractions (range of motion from 90 to 10° of flexion; 0° at full extension) at $180° \cdot s^{-1}$ to determine quadriceps peak torque (PT) and fatigue percentage (FP) after 30 and 50 repetitions as described previously (24). Subjects were instructed to give maximal effort during each concentric action and to relax their leg during flexion back to the starting position at 90°. Peak torque from the load range portion of each concentric action (8,9), velocity, and position were recorded via computer sampling at 1,000 Hz running custom LabVIEW collection and analysis software (version 2013; National Instruments, Austin, TX, USA). Three models for calculating FP were used as described previously (24). Model 1 was calculated using the same approach as the CT, where the average PT of repetitions 1–3 is termed R3 and average PT of repetitions 48–50 is termed R50. Models 2 and 3 were calculated by taking torque of the PT repetition and torque of the specific range final repetition (24,34):

$$\text{Model 1: CT} = ([\text{R3} - \text{R50}]/\text{R3}) \times 100$$

$$\text{Model 2: P30} = ([\text{PT} - \text{rep30}]/\text{PT}) \times 100$$

$$\text{Model 3: P50} = ([\text{PT} - \text{rep50}]/\text{PT}) \times 100$$

Myosin Heavy Chain Fiber Type Identification

Muscle biopsies occurred 24–48 hours after the isokinetic test in a similar fashion as described previously (25). Participants arrived at the laboratory after a 10-hour fast and underwent a mid-muscle belly biopsy of the VL (by the same technician) using the Bergström technique (5) to determine skeletal

Table 1. Relationship between fiber type composition and fatigue percentage (FP) during a 50 repetition isokinetic leg extension test in resistance exercise trained men.*

	FP analysis method		
	CT (*r*)	P30 (*r*)	P50 (*r*)
Fiber Type Group			
%MHCI	0.18	0.28	0.15
%MHCIIa	−0.41	−0.60	−0.38
%Fast	−0.12	−0.17	−0.08
%FastHybrid	−0.20	−0.31	−0.18
%Hybrid	0.30	0.39	0.27
%SlowHybrid	0.10	0.14	0.07

*Pearson correlation coefficients (*r*) for fiber type groupings and FP. Single muscle fibers were categorized into 6 groups: MHC I = only pure myosin heavy chain (MHC) I fibers. MHC IIa = only pure MHC IIa fibers. Only Fast = all fibers expressing a fast-twitch isoform, but no slow-twitch (i.e., MHC IIa, IIa/IIx, and IIx). All Fast = all fibers expressing any fast-twitch isoform, including coexpression of slow twitch (i.e., MHC I/IIa, MHC IIa, IIa/IIX, IIx, and MHC I/IIa/IIx). All Hybrids = all fibers in hybrid state (i.e., MHC I/IIa, MHC IIa/IIX, and MHC I/IIa/IIx). All Slow = all fibers expressing any slow twitch (i.e., MHC I, MHC I/IIa, and MHC I/IIa/IIx). Fatigue percentage was analyzed using 3 methods: CT, P30, and P50. CT = fatigue percentage from the Classic Thorstensson Test, average of first 3 and last 3 repetitions of 50, P30 = fatigue percentage from peak torque repetition to repetition 30; P50 = fatigue percentage from peak torque repetition to repetition 50.

muscle fiber type. Briefly, tissue was obtained using a 6-mm Bergström needle with suction (11) through a small incision after administering local anesthetic (lidocaine hydrochloride 1%). The muscle sample was immediately cleansed of excess blood, and connective tissue was removed using a scalpel and fine-tipped tweezers; then, muscle samples were divided into ~15-mg strips. To facilitate smooth and verifiable fiber isolation from the muscle bundle, samples were placed directly in cold skinning solution ([in millimolar]: 125 K propionate, 2.0 ethylene glycol tetraacetic acid, 4.0 adenosine triphosphate, 1.0 magnesium chloride, 20.0 imidazole [pH 7.0], and 50% [vol/vol] glycerol) and stored at −18° C for at least 1 week before muscle fiber isolation.

Segments of approximately 150 randomly selected single fibers per sample were mechanically isolated using fine tweezers under a light microscope at room temperature and placed in 80 μl of sodium dodecyl sulfate (SDS) buffer (10% SDS, 6 mg·ml^{-1} EDTA, 0.06 m Tris [pH 6.8], 2 mg·ml^{-1} bromophenol blue, 15% glycerol, and 5% b-mercaptoethanol) for MHC isoform identification via SDS-PAGE as described previously (25). Briefly, 2 μl aliquots of SDS buffer were loaded into individual wells in a 3.5% loading gel and 5% separating gel and run at 5° C (SE 600 Series; Hoefer, San Francisco, CA, USA) for 15.5 hours. Gels were then silver stained, revealing MHC isoforms for each individual fiber based on known molecular weights and standards (14). Fiber types were identified as MHC I, I/IIa, IIa, IIa/IIx, IIx, or I/IIa/IIx.

Myosin Heavy Chain Fiber Type Grouping

Comparing fiber type percentages between 2 different methods (single muscle fiber SDS-PAGE vs. ATPase histochemistry) is only possible by combining fiber types into similar categories. Thus, we grouped all fibers into 6 categories to allow the most direct comparison with the Thorstensson and Karlsson (33) study.

Group 1 (%MHCI) = only pure MHC I fibers ("MHC I")
Group 2 (%MHCIIa) = only pure MHC IIa fibers ("MHC IIa")
Group 3 (%Fast) = all fibers expressing a fast-twitch isoform but no slow-twitch (i.e., MHC IIa, IIa/IIx, and IIx).
Group 4 (%FastHybrid) = all fibers expressing any fast-twitch isoform, including coexpression of slow twitch (i.e., MHC I/IIa, MHC IIa, IIa/IIX, IIx, and MHC I/IIa/IIx).
Group 5 (%Hybrid) = all fibers in hybrid state (i.e., MHC I/IIa, MHC IIa/IIX, and MHC I/IIa/IIx).
Group 6 (%SlowHybrid) = all fibers expressing any slow twitch (i.e., MHC I, MHC I/IIa, and MHC I/IIa/IIx).

Statistical Analyses

A 3 × 6 repeated-measures analysis of variance compared FP (models 1–3) with fiber type percentage (groups 1–6). Separate linear regression models were fit to the FP vs. fiber type groups. Pearson product moment correlation coefficients (*r*) were used to determine relationships between PF model and fiber type group. Fisher (*r* to *z*) transformations were performed to examine potential significant differences between correlations. An a priori alpha of 0.05 was used to determine statistical significance. Data are presented as mean ± *SD* unless otherwise stated. Statistical analyses were conducted using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp, Armonk, NY, USA). Additionally, data from the original Thorstensson and Karlsson article (33) were highlighted and compared (although, not statistically) with the data in the current article.

Results

Performance and Fatigability

Peak torque (207.0 ± 28.2 N·m; range: 128.1–242.5 N·m) and torque after R50 (72.0 ± 13.4 N·m; range: 50.64–98.26 N·m) are shown in Figure 1 compared with data from REC from the Thorstensson and Karlsson (33) study. In our RET participants, FP was 62.3 ± 6.0% (CT), 44.7 ± 8.8% (P30), and 66.0 ± 6.8% (P50).

Myosin Heavy Chain Fiber Type

A total of 2,245 single muscle fibers ($n = 150 \pm 12$ per subject) were analyzed for MHC type (%MHCI [$n = 431$; 19.2%], %MHCIIa [$n = 1{,}302$; 58.0%], %Fast [$n = 1{,}602$, 71.4%], %FastHybrid [$n = 1{,}814$, 80.8%], %Hybrid [490,

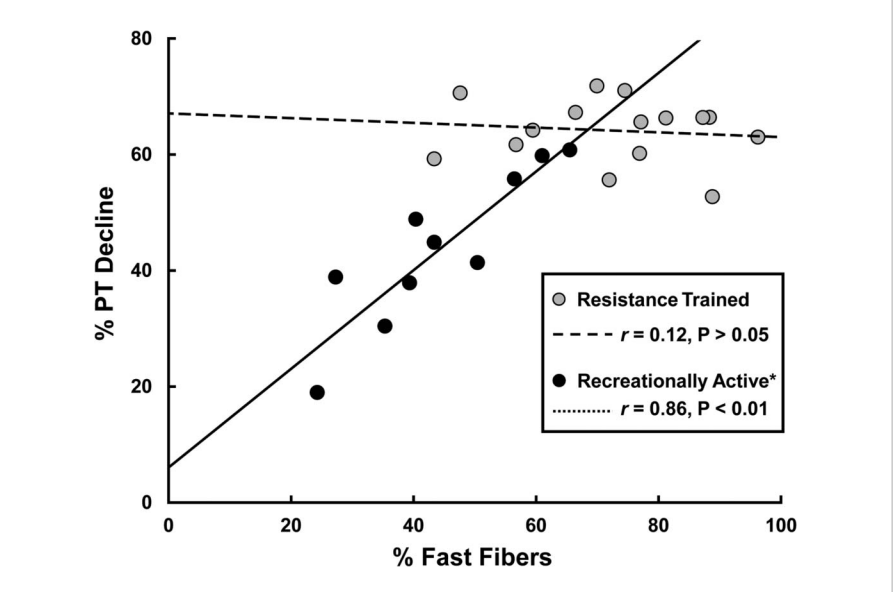

Figure 3. Decline in peak torque (PT) after 50 maximal knee extensions (fatigue percentage [FP]) *vs.* fast fiber composition (vastus lateralis). Fast fibers (%fast) in resistance trained men included myosin heavy chain (MHC) IIa, IIa/IIx, and IIx types (measured on single fibers via sodium dodecyl sulfate polyacrylamide gel electrophoresis) and fast fibers in recreationally active men were identified via adenosine triphosphatase histochemistry (*data from Thorstensson and Karlsson (33)). Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

21.8%], %SlowHybrid [643, 28.6%]). Total fiber type distribution or RET was 19.9 ± 8.4% MHC I, 9.7 ± 6.1% MHC I/IIa, 57.8 ± 10.5% MHC IIa, 11.2 ± 8.7% MHC IIa/IIx, 2.1 ± 1.3% MHC IIx, and 1.4 ± 0.9 MHC I/IIa/IIx (Figure 2 for individual participant fiber type distributions).

Fatigability and Fiber Type

No significant relationships were identified between any of the fiber type groups and any of the FP models ($p > 0.05$), as shown in Table 1. Additionally, No significant differences were identified between correlations following the Fishers r to z transformation.

Figure 3 shows decline in PT after 50 repetitions as in the CT model vs. fiber type (%Fast) comparing RET men from the current study with REC men from the study by Thorstensson and Karlsson (33).

Discussion

This investigation reexamined the relationship between fatigability and fiber type using (a) single muscle fiber MHC isoform classification and (b) a group of RET men with similar training backgrounds. We found that (a) RET men exhibited 46% greater PT and 28% more %Fast fibers than REC men in the original study by Thorstensson and Karlsson (33), (b) RET men had a relatively homogeneous FP (64 ± 1%), and (c) no relationship existed between FP and MHC fiber type in RET men ($R^2 = 0.01$, $p > 0.05$). As hypothesized, when using more sensitive fiber typing methods, these data suggest the popular CT prediction equation developed with data from REC men may not be valid when implemented in RET men.

ATPase histochemistry was the standard human skeletal muscle fiber typing procedure from the 1960s to the 1990s. However, over the past 2 decades, single muscle fiber MHC isoform identification has gained popularity and is now considered the "gold standard" for human fiber typing (26). Using this method, no strong relationship was found between muscle fiber phenotype and knee extension fatigue in a homogenous group of RET. Our findings likely differ from those of Thorstensson and Karlsson because we were able to differentiate fibers into 6 isoform "types" (as opposed to only 2, e.g., %Fast and %Slow). In the current investigation, RET had an average %Fast fiber composition of 61.4% (MHC IIa, IIa/x, IIx combined), whereas Thorstensson and Karlsson reported a wide range of 25–65% (33). This relatively high distribution of fast fibers is similar to those found in other investigations with strength/RET men (23,25). Typically, ~18–40% of fibers in sedentary/REC men are classified as "hybrids" (combining MHC I/IIa, IIa/IIx, and I/IIa/IIx fibers), whereas RET muscle expresses significantly less (20–22,25). The relatively low total hybrid count reported here (11.8%) supports previous research and highlights the trained status of our participants. A major drawback of ATPase fiber typing is the high potential for fiber misclassification (1,19,27,28,31), most often underestimating hybrid fiber quantities and, as a result, overestimating MHC IIx fiber frequency (2,31). Thus, the hybrids identified here would have likely been misclassified using ATPase histochemistry, making our choice of using single fiber SDS-PAGE for fiber typing analysis critical to the accuracy of our results.

Skeletal muscle fiber type distribution is a major determinate of whole muscle performance in humans (15,18,34,35). The CT was previously established as a noninvasive method to estimate fiber type percentage (4), and over the past decade, new noninvasive methods to estimate fiber type have become increasingly popular (3,13,16,17,29). Studies show fiber type distribution correlates with muscle carnosine

content (measured via proton magnetic resonance spectroscopy) (3), surface mechanomyographic amplitude (16,35), and tensiomyographic radial twitch response (29) in human muscle. Although these studies found significant relationships between fiber type and performance/fatigue measures, they used immunohistochemistry (3) or homogenate SDS-PAGE (13,16,17,29,35) (which represents the total area that each fiber type occupies rather than single fiber % distribution). It should be noted that ATPase fiber typing and homogenate SDS-PAGE fiber typing show similar findings for identifying % fiber area (12,32); however, they both overestimate the number of MHC IIx fibers and underestimate hybrid fibers (2,31). Although all fiber type measurement techniques provide unique benefits and suffer limitations, we chose single fiber MHC analysis to more accurately represent the continuum of MHC types. Future studies should consider analyzing both % fiber area (i.e., homogenate fiber type) and % fiber distribution (i.e., single fiber type) to provide a more complete picture of skeletal muscle structure related to the MHC type.

Studies investigating the complex relationship between skeletal muscle fiber type and performance have found significant correlations between MHC fiber type and fatigue (16,17,35), in contrast to the present study. These divergent findings were likely due to our more sensitive fiber typing methodology and homogeneous RET population. Individuals most interested in identifying their fiber type composition are presumably those involved in physical activity and/or sport. The CT and fiber type prediction equation may therefore encompass limitations, as the original equation was derived from a cohort of "habitually active" (REC) men (33). Average RET PT in the current study was $\times$1.6 greater than REC PT (33), although both groups finished repetition 50 at a nearly identical $\sim$72 N·m (Figure 1). This resulted in an average FP of 64 $\pm$ 1% (range 50–70%) in RET men, which was larger than the highest single score in REC men (range of 20–60%) (33). This collectively suggests our participants were stronger and fatigued more rapidly than REC men, indicating that the CT fiber type prediction equation is not valid for relatively strong RET men with a large proportion of fast fibers. The results of the present study, which used recreationally RET men, may not translate to elite bodybuilding, weightlifting, powerlifting, or otherwise highly strength trained individuals. Future research should investigate the efficacy of the CT fiber type prediction equation in specific populations based on training status, sex, age, and other demographic variables.

The CT developed in 1976 (33) and subsequent fiber type prediction equation (4) are still valuable tools for studying the relationship between muscle fiber type and performance, especially in untrained and/or heterogeneous populations. However, our findings show that more research is needed in this area, specifically using (a) more advanced/precise technologies and (b) individuals with various exercise training backgrounds. Although MHC fiber types have been studied in humans for decades (18,25,32), we still lack a complete understanding of the practical significance of fiber type in relation to whole-body performance. This highlights the need for more robust examinations of the fiber type that includes not only % distribution (as in this study) but also % fiber area (16,35), as well as other structural, metabolic, and functional parameters associated with specific MHC fiber types in humans.

Practical Applications

The CT is a popular noninvasive method to estimate quadriceps fiber type. Our data suggest that this method may not be accurate in RET men. Exercise trained individuals express unique phenotypical/functional adaptations and the best way to accurately identify their fiber type distribution is still through muscle biopsies with single fiber MHC identification. The ability to noninvasively predict a complex variable such as muscle fiber type probably requires separate equations (or tests) for populations with different training backgrounds. This highlights the necessity for future research in this area, and the need to reexamine past studies using new technologies.

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