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C. R. Hall, E, Semenova, E, Bondareva, EA, Borisov, O, Andryushchenko, ON, Andryushchenko, L, Zmijewski, P, Generozov, E and Ahmetov, II (2021) Association of muscle fiber composition with health and exercise-related traits in athletes and untrained subjects. *Biology of Sport*. 38 (4). pp. 659-

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Association of muscle fiber composition with health and exercise-related traits in athletes and untrained subjects

AUTHORS: Elliott C. R. Hall¹, Ekaterina A. Semenova², Elvira A. Bondareva³, Oleg V. Borisov^{2,4}, Oleg N. Andryushchenko⁵, Liliya B. Andryushchenko⁶, Piotr Zmijewski⁷, Edward V. Generozov², Ildus I. Ahmetov^{6,8,9,10}

¹ Department of Sport and Exercise Sciences, Manchester Metropolitan University, Manchester, United Kingdom

² Department of Molecular Biology and Genetics, Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency, Moscow, Russia

³ Research Institute and Museum of Anthropology, Lomonosov Moscow State University, Moscow, Russia

⁴ Institute for Genomic Statistics and Bioinformatics, University Hospital Bonn, Bonn, Germany

⁵ Department of Physical Education, Financial University under the Government of the Russian Federation, Moscow, Russia

⁶ Department of Physical Education, Plekhanov Russian University of Economics, Moscow, Russia

⁷ Institute of Sport – National Research Institute, Warsaw, Poland

⁸ Laboratory of Molecular Genetics, Kazan State Medical University, Kazan, Russia

⁹ Sports Genetics Laboratory, St Petersburg Research Institute of Physical Culture, St Petersburg, Russia

¹⁰ Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, United Kingdom

ABSTRACT: Skeletal muscle is a heterogenous and metabolically active tissue, the composition of which is associated with multiple traits. The aim of the study was to determine whether there are additional health and exercise-related traits associated with muscle fiber composition in athletes and non-athletes. This study recruited 164 Russian participants (51 endurance and 48 power athletes; 65 controls). Vastus lateralis muscle fiber composition was assessed by immunohistochemistry. Slow-twitch muscle fiber percentage (STMF%) was significantly greater in endurance than power athletes and non-athletes, and in non-athlete females than males. STMF% was positively associated with athletes' training frequency, non-athletes' and endurance athletes' age, endurance athletes' competition level and chest depth, and power athletes' training age. STMF% was negatively associated with diastolic blood pressure in power athletes and with systolic blood pressure and reaction time in non-athletes. In all participants, STMF% was positively associated with age, tolerance to long distance exercise, chest depth and fracture incidence, and negatively with systolic blood pressure and resting heart rate. Age, sex and training frequency explained 10.6% and 13.2% of the variance in STMF% in endurance and power athletes, respectively. This is one of the most comprehensive studies involving athletes and untrained subjects and provides novel information concerning associations of increased STMF percentage with lower resting heart rate, better tolerance to long distances, faster reaction time and larger chest depth. On the other hand, the increased percentage of fast-twitch muscle fibers was associated with rare fracture incidence.

CITATION: Hall ECR, Semenova EA, Bondareva EA et al. Association of muscle fiber composition with health and exercise-related traits in athletes and untrained subjects. *Biol Sport*. 2021;38(4):659–666.

Received: 2020-11-14; Reviewed: 2020-11-27; Re-submitted: 2020-11-28; Accepted: 2020-12-20; Published: 2021-02-05.

Corresponding author:

Ildus I. Ahmetov
Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK.
E-mail: i.ahmetov@ljmu.ac.uk

ORCID:

Elliott Hall
0000-0003-0540-0735

Ekaterina A. Semenova
0000-0003-1248-2855

Elvira A. Bondareva
0000-0003-3321-7575

Oleg V. Borisov
0000-0001-8700-4335

PiotrZmijewski
0000-0002-5570-9573

Edward V. Generozov
0000-0002-6314-4883

Ildus I. Ahmetov
0000-0002-6335-4020

Key words:

Muscle fibers
Endurance
Heart rate
Blood pressure
Fractures

INTRODUCTION

Skeletal muscle is a heterogeneous and metabolically active tissue containing Type I (slow-twitch) and Type II (fast-twitch, subdivided into IIa and IIx) fibers [1]. Slow-twitch muscle fibers (STMF) have higher oxidative capacity than fast-twitch muscle fibers (FTMF), which have higher glycolytic enzyme activity [2] and faster maximal shortening velocities that contribute to superior maximal power [1]. Subjects with a greater proportion of STMF generally complete a greater number of repetitions at 80% 1 repetition maximum (1RM) during resistance training than individuals with increased proportion of FTMF [3].

The proportion of each fiber type varies considerably according to muscle location [4] and also between individuals, with each related to the interaction of biology and environment [5]. Biological factors include age [6, 7], sex [8] and genetics [9], with exercise representing a strong environmental factor [10]. Muscle fiber composition is associated with athletic performance [11], with athletes who require superior aerobic and endurance capacity (such as middle- and long-distance runners) having as many as 90% STMF, and athletes reliant on strength, power and anaerobic capacity (such as

sprinters and weightlifters) having between 60 and 80% FTMF [12–14]. These differences reflect the suitability of oxidative, fatigue resistant STMF to endurance performance [12], and the favourable anaerobic properties of FTMF during short bursts of speed and power [15]. However, very few athletic activities rely solely on one fiber type, and there are several other traits related to muscle fiber composition.

The metabolic characteristics of skeletal muscle fibers can be modified through exercise, whereas the biological influence of an individual's age, sex and genetic code cannot [9]. In adults, a gradual increase in STMF and a gradual decrease in FTMF is observed with age [7, 16, 18], though this may differ between males and females [6]. In non-athletes, males exhibit greater cross-sectional area for all fiber types, with some suggesting males have a similar proportion of each fiber type to females [5] and others demonstrating that males have fewer STMF [17, 18]. Approximately 45% of muscle fiber composition is heritable and around 40% is attributable to the environment [9]. Heritable factors include common DNA sequence variants, such as single nucleotide polymorphisms (SNPs), indels and structural variations, which can alter gene expression and/or protein structure and are summarised elsewhere [16, 18, 19]. Nonetheless, it is suggested that the exclusive combination of environment and exercise determines up to 30% of the observed differences in muscle fiber composition between individuals [17]. In consideration of evidence that suggests the relationship of specific biological factors, such as genetic variation and age, is associated with sex [6, 7, 18], it is apparent that several biological factors interact with one another, as well as with external factors, to determine muscle fiber composition. It is also apparent that muscle fiber composition may be associated with measures of cardiovascular risk [20] and bone mineral density [21]. However, the association of these measures with muscle fiber composition in athletic populations is unknown.

Due to the effects of exercise [10, 22–24], the relationship of biological factors with muscle fiber composition is likely to differ between athletes and non-athletes. Whilst some investigations have recruited athletes [12, 22, 23, 25, 26], there is a need to explore

the combined association of multiple factors with muscle fiber composition in a sample containing athletes and non-athletes. With numerous factors associated with muscle fiber composition, investigating the combined relationship of these factors will explain a greater proportion of individual variability than one factor in isolation. It is also possible that there are additional factors associated with muscle fiber composition that are currently unknown. The aim of the present study, therefore, was to determine the independent and combined relationship of muscle fiber composition in the vastus lateralis with health- and exercise-related traits in athletes and non-athletes.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Ethics Committee of the Physiological Section of the Russian National Committee for Biological Ethics and Ethics Committee of the Federal Research and Clinical Center of Physical-chemical Medicine of the Federal Medical and Biological Agency of Russia. Written informed consent was obtained from each participant. The study complied with the guidelines set out in the Declaration of Helsinki and ethical standards in sport and exercise science research.

Study participants

A total of 164 Russian subjects (54 females, 110 males; 99 athletes, 65 untrained controls) participated in this study. Participants were divided into 6 subgroups: female endurance athletes ($n = 14$; age 18–42 years; 9 middle- and long-distance runners, 3 road cyclists, and 2 middle-distance speed skaters), female power athletes ($n = 15$; age 18–34 years; sprinters in cycling ($n = 5$), running ($n = 3$) and speed skating ($n = 3$), 3 powerlifters and one skeleton athlete), female controls ($n = 25$; age 20–51 years), male endurance athletes ($n = 37$; age 19–54 years; 21 middle- and long-distance runners, 5 cross-country skiers, 5 road cyclists, and 6 triathletes), male power athletes ($n = 33$; age 18–41 years; sprinters in cycling ($n = 6$), running ($n = 3$) and speed skating ($n = 3$), 4 weightlifters, 14 powerlifters, two skeleton athletes, one decathlete), and male controls ($n = 40$;

TABLE 1. Participant characteristics

Group	n	Age	Height (cm)	Weight (kg)	Training age (years)
Female endurance	14	27.1 (5.9)	168.8 (5.9)	58.3 (6.9)	12.9 (5.5)
Female power	15	24.8 (5.5)	168.3 (5.6)	61.1 (4.9)	11.0 (6.3)
Female controls	25	30.4 (7.8)	165.7 (4.6)	58.8 (5.1)	-
Male endurance	37	34.8 (8.6)	180.3 (6.4)	76.8 (9.3)	12.9 (9.1)
Male power	33	27.8 (6.1)	179.8 (5.4)	86.8 (11.8)	10.3 (5.6)
Male controls	40	29.7 (8)	180.4 (6.6)	79.6 (8.2)	-

Note: all data are presented as mean (SD).

age 18–53 years). There were 30 non-elite (amateur level), 32 sub-elite (regional competitor) and 37 elite athletes (international competitor). Basic characteristics of each subgroup are provided in Table 1.

Anthropometry

Anthropometric measurements were taken according to standard techniques [27] using the GPM anthropometric measurement kit (DKSH, Switzerland). Subjects were measured barefoot, wearing only underwear. Body mass was measured using a battery-operated digital scale (precision 100 g). Height was measured using an anthropometer (1 mm precision). Chest depth is defined as the sagittal diameter of the chest and was measured using a spreading caliper (1 mm precision).

Health status

Resting systolic and diastolic blood pressure (BP) and resting heart rate (RHR) were assessed in the morning before any type of activity and eating after 5 min of seated rest using an automatic blood pressure/heart rate monitor (Omron M2, Kyoto, Japan). Fracture incidence was evaluated using questionnaire, with participants classified as having suffered any type of fracture in their lifetime (score 1) or none (score 0). Health-related traits in each subgroup are presented in Table 2.

Evaluation of muscle fiber composition by immunohistochemistry

Vastus lateralis samples were obtained from the left leg using the modified Bergström needle procedure [28] with aspiration under local anaesthesia with 2% lidocaine solution. Prior to analysis, samples were frozen in liquid nitrogen and stored at -80°C. Serial cross-sections (7 µm) were obtained from frozen samples using an ultratom (Leica Microsystems, Germany). Sections were thaw-mounted on Polysine glass slides, maintained at room temperature (RT) for 15 min and incubated in PBS (3 x 5 min). The sections were then incubated at RT in primary antibodies against slow or fast isoforms of the myosin heavy chains (M8421, 1:5000; M4276; 1:600, respectively; Sigma-Aldrich, USA) for 1 h and incubated in PBS

(3 x 5 min). Next, the sections were incubated at RT in secondary antibodies conjugated with FITC (F0257; 1:100; Sigma-Aldrich) for 1 h. The antibodies were removed, and the sections washed in PBS (3 x 5 min), placed in mounting media and covered with a cover slip. Images were captured by fluorescent microscope (Eclipse Ti-U, Nikon, Japan). All analyzed images contained 330 ± 11 fibers. The ratio of the number of stained fibers to the total fiber number was calculated. Fibers stained in serial sections with antibodies against slow and fast isoforms were considered hybrid fibers.

Physical activity and training parameters

Training parameters were assessed by questionnaire. Athletes were classified according to their training frequency as mildly active (2 training sessions per week), moderately active (3–4 training sessions per week), highly active (5–7 training sessions per week) and extremely active (two training sessions per day). Training age was expressed as years of training. Participants (athletes and controls) scored their individual tolerance to long distances and sprinting ability as poor (0), fair (1), good (2) or excellent (3).

Reaction time measurement

Visual reaction time was evaluated using the previously described Traffic light test [29]. Briefly, subjects sat at a table with the palm of the dominant hand supported and their index finger on a computer mouse. The participants were consistently presented with light signals in the centre of the monitor screen and were instructed to click the button when a green signal appeared. The duration of the intervals between red and green signals ranged from 0.5 to 5 s. The first 5 signals were trial efforts and were not recorded. The best three attempts from the following 5 signals were recorded and the average reaction time used for analysis.

Statistical analyses

Statistical analyses were conducted using GraphPad InStat (GraphPad Software, Inc., USA) software. Differences in slow-twitch muscle fiber percentage (STMF%) between athlete and controls and between

TABLE 2. Health-related characteristics in participants

Group	n	SBP, mmHg	DBP, mmHg	Heart rate, bpm	Fracture incidence, %
Female endurance	14	115.5 (8.1)	70.4 (8.3)	52.0 (7.8)	50.0
Female power	15	111.6 (9.5)	72.0 (9.8)	58.4 (7.1)	20.0
Female controls	25	111.6 (6.7)	73.2 (6.3)	68.1 (7.4)	16.0
Male endurance	37	120.5 (7.4)	73.1 (8.0)	47.4 (6.4)	32.4
Male power	33	121.1 (8.1)	74.3 (11.3)	62.9 (9.2)	57.6
Male controls	40	122.2 (6.0)	78.9 (9.1)	68.6 (9.3)	37.5

Note: Data are presented as mean (SD).

sexes were analysed using unpaired t-tests. Multiple regression was used to assess the relationships between STMF% and all other continuous variables (adjusted for sex, age, training frequency, type of training, BMI where appropriate) and to determine the combined association (R^2 , the percentage of variance in STMF percentage) of individual factors adjusted for covariates. All data are presented as mean (SD). P values < 0.05 were considered statistically significant.

RESULTS

Muscle fiber distribution in athletes and controls

There were no significant differences in STMF% or FTMF% between subgroups of athletes for both sexes (e.g. strength athletes vs sprinters for power athletes or road cyclists vs middle- or long-distance

runners for endurance athletes), therefore we felt justified in combining these subgroups of athletes into one major group (i.e. endurance and power athletes only). As expected, STMF% was significantly greater in endurance athletes than power athletes and controls for both sexes (Table 3). However, there was no difference in STMF% between power athletes and controls. In untrained controls, STMF% was higher in females than males (52.7 (14.7) vs. 44.2 (14.0)%, $P = 0.018$) (Table 3).

Relationship between muscle fiber composition and health-related traits

Slow-twitch muscle fiber percentage was positively related to age in endurance athletes ($P = 0.019$) and controls ($P = 0.0002$), with

TABLE 3. Muscle fiber distribution according to sex and training type

Group	n	Percentage of STMF	Percentage of FTMF	P values for STMF	
				Endurance vs power	Athletes vs controls (same sex)
Female endurance	14	61.8 (9.9)	41.2 (10.0)	0.014*	0.047*
Female power	15	51.1 (11.8)	51.6 (10.4)	-	0.724
Female controls	25	52.7 (14.7)	50.4 (13.4)	-	-
Male endurance	37	60.8 (16.2)	42.6 (17.1)	< 0.0001*	< 0.0001*
Male power	33	46.3 (10.7)	56.7 (10.7)	-	0.486
Male controls	40	44.2 (14.0)	58.7 (13.6)	-	-

* $P < 0.05$, statistically significant differences. All data are presented as mean (SD).

TABLE 4. Relationship between slow-twitch muscle fiber percentage and multiple traits

Factor	P value			
	All participants (n = 164)	Endurance athletes (n = 51)	All power athletes (n = 48)	All controls (n = 65)
Age	0.0001*	0.019*	0.529	0.0002*
Sex	0.0033 [#]	0.515	0.245	0.018 [#]
Training frequency	-	0.042*	0.038*	-
Training age	-	0.538	0.045*	-
Level of competition	-	0.034*	0.555	-
Reaction time	0.094	0.786	0.235	0.026 [#]
Self-reported tolerance to LD	0.0002*	0.219	0.629	0.421
Self-reported sprinting ability	0.30	0.324	0.853	0.756
Height	0.099	0.118	0.939	0.993
Weight	0.754	0.862	0.948	0.766
BMI	0.657	0.994	0.973	0.877
Chest depth	0.023*	0.03*	0.145	0.609
Systolic BP	0.042 [#]	0.825	0.358	0.005 [#]
Diastolic BP	0.141	0.896	0.045 [#]	0.406
Resting HR	0.0067 [#]	0.163	0.118	0.284
Fracture incidence	0.021*	0.074	0.09	0.443

* $P < 0.05$, positive relationship between STMF% and a variable adjusted for covariates (where appropriate: sex, age, training frequency, type of training, BMI); [#] $P < 0.05$, negative relationship between STMF% and a variable adjusted for covariates (where appropriate: sex, age, training frequency, type of training, BMI). LD, long distances

no relationship in power athletes ($P = 0.529$) (Table 4). Systolic ($P = 0.005$) and diastolic ($P = 0.045$) BP were negatively related to STMF% in controls and power athletes, respectively. In all participants, STMF% was positively related to fracture incidence ($P = 0.021$), and negatively related to systolic BP ($P = 0.042$) and RHR ($P = 0.007$). In endurance athletes ($P = 0.03$) and all participants ($P = 0.023$), chest depth was positively related to STMF%. There was no association between muscle fiber composition and other anthropometric traits (i.e. height, weight, BMI etc.).

Relationship between muscle fiber composition, performance and exercise-related traits

Endurance ($P = 0.042$) and power athletes' ($P = 0.038$) STMF% was positively related to exercise frequency, with power athletes STMF% positively related to training age ($P = 0.045$) (Table 4). Endurance athletes' STMF% was positively related to level of competition ($P = 0.034$) and self-reported tolerance to long distances was positively related to STMF% for all participants ($P = 0.0002$). Non-athletes' STMF% was negatively related to reaction time ($P = 0.026$).

Contribution to the variability in STMF percentage

When combined, age, sex and exercise frequency explained 10.6% ($P < 0.0001$) and 13.2% ($P < 0.0001$) of the variance in STMF% for endurance and power athletes, respectively.

DISCUSSION

This comprehensive study aimed to determine the relationship between muscle fiber composition and health- and exercise-related traits in athletes and non-athletes. The novel findings of this study were the associations of RHR, bone fracture incidence and chest depth with STMF%, whilst confirming the relationship between STMF% and BP. As anticipated, endurance athletes had a greater STMF% than power athletes and non-athletes, female non-athletes had a greater STMF% than male non-athletes, and chronological age was associated with STMF%, though not for power athletes. Interestingly, STMF% was positively related to training age in power athletes, and to endurance athletes' level of competition, suggesting a greater STMF% is advantageous to elite endurance performance. Training frequency was positively related to STMF% in all athletes', with the combination of age, sex and training frequency explaining 10.6% and 13.2% of the variance in STMF% amongst endurance athletes and power athletes, respectively.

The present study found that BP and RHR were associated with STMF%. Systolic BP was negatively associated with STMF% in all participants and in non-athletes, with diastolic BP negatively associated with STMF% in power athletes. Thus, a greater STMF% was associated with lower BP and is in concordance with previous literature [20]. A novel finding was the negative relationship between STMF% and RHR. To our knowledge, this is the first association of STMF% with RHR and is in line with ours and previous observations

relating to BP, with elevated RHR previously associated with increased systolic and diastolic BP in healthy individuals [30]. Due to the physiological relationship between RHR and BP, it is logical that the association of both measures with STMF% follows a consistent pattern. We therefore speculate that an increased proportion of STMF may protect against the development of cardiovascular diseases.

Bone fracture incidence was associated with STMF%, with a positive relationship between STMF% and fracture risk. Low bone mineral density contributes to increased fracture risk [31] and there is a correlation between FTMF and bone mineral density [21]. Furthermore, the adaptive responses of FTMF strength [32] and bone mineral density [33] to resistance training indicates interplay between bone and skeletal muscle. Indeed, it is suggested that mechanical force primes both tissues to regulate and release specific factors to induce an adaptive response in the opposing tissue [34]. The recent discovery of pleiotropic genes that regulate muscle and bone [35] follows evidence that muscle and bone share a common mesenchymal precursor during development, which tightly orchestrates organogenesis to ensure the synchronous development of both tissues [36]. We suggest the association with fracture risk is due to individuals with greater STMF% having reduced protection against fracture risk, and as a consequence of the relationship between bone mineral density and FTMF percentage.

Chest depth is a measure of the chest's saggital diameter and this trait was positively related to STMF% in all participants and in endurance athletes alone. At present, little is known regarding the role of chest depth in exercise physiology, meaning it is unclear how this phenotype affects exercise capacity. Despite a lack of evidence relating to exercise, the "barrel-shaped chest" phenotype typical of high-altitude natives as an adaptive response to hypobaric hypoxia [37] suggests the positive association of STMF% with chest depth could benefit aerobic activity. We speculate that greater chest depth may aid maximal lung expansion during pulmonary ventilation and may favour aerobic performance when combined with a high percentage of oxidative muscle fibers. Therefore, given that chest depth is heritable [38], it is possible that individuals with inherently greater STMF% and chest depth may be suited to endurance performance. Indeed, chest depth and level of competition were both positively associated with endurance athletes' STMF%, suggesting there may be a benefit of this phenotype to endurance capacity. In non-athletes, we also observed that STMF% was related to faster reaction times, a finding which is currently unexplained. The lack of association between STMF% and reaction time in athletes may reflect the positive effect of long-term training on athletes' visuomotor reaction capacity [39], though further research is required to investigate the relationship of STMF% with reaction time.

Despite considerably different training practices between endurance and power athletes, training frequency and STMF% were positively associated in all athletes. For endurance athletes, this is probably due to heritable factors plus frequent exposure to aerobic training [23, 32]. However, whilst power-orientated sports rely

predominantly on FTMF [13, 14], the positive relationship of STMF% with power athletes' training frequency and training age may be because experienced power athletes are exposed to frequent training stimuli for longer than less experienced athletes, augmenting the documented improvements in oxidative capacity from resistance training [40]. It is also possible that power athletes with greater STMF% are able to recover faster between intense training sessions [41] and, therefore, can maintain a higher training frequency.

Endurance athletes had greater STMF% than power athletes and non-athletes, which was expected due to previous evidence [12, 13]. The positive association of STMF% with the level of competition in endurance athletes suggests that athletes with greater STMF% are more likely to reach the elite level of endurance sport. In contrast, similar STMF% between controls and power athletes supports previous work [11, 12] and suggests power performance is more reliant on exposure to environmental factors, with strength adaptations typically achieved through the hypertrophy of existing fibers [23, 24]. Together, it appears that the selection of endurance and power athletes into their respective sports depends heavily on muscle fiber composition, with the heritability of muscle fiber composition [9] likely to differentiate between successful athletes in each discipline. Evidence that genetic factors are associated with athletes' muscle fiber composition [42–45] and athletic performance [46–48] provide further support for this concept.

The positive association of chronological age with STMF% in endurance athletes and controls is likely to reflect the concomitant increase and decrease in STMF and FTMF proportions, respectively, that occurs with age [7, 18]. This contributes to the age-related loss of muscle mass termed sarcopenia, characterised by the decreased number and size of FTMF that leads to reduced muscle mass and strength [49]. The mechanical stimuli and anabolic signalling elicited by resistance exercise can offset this process [50] and may explain why STMF% was unrelated to power athletes' age. We also observed a greater STMF% in female controls than male controls, in support of previous work [8, 17, 18]. The combination of age, sex and training frequency explained 10.6% and 13.2% of the variance in STMF% amongst endurance athletes and power athletes, respectively. Considering that ~45% of the variability in muscle fiber composition is heritable [9], our findings suggest heritable factors are stronger determinants of athletes' muscle fiber composition than their age, sex and training frequency combined, providing further support to the concept that the heritability of muscle fiber composition is a key determinant of elite athletes' success.

This study included a sizeable number of athletes and non-athletes, providing novel information on the relationship of muscle fiber composition with health- and exercise-related traits. However, this study also has limitations. Firstly, the present data are associational and do not describe the mechanistic cause of each relationship. Second,

the assessment of athletes' training parameters and fracture incidence by questionnaire could be subject to self-report bias or recall error. We also acknowledge our findings are limited to Russian participants, and we welcome replicative studies in other nationalities. Finally, other factors potentially related to muscle fiber composition were not included in the present study. Future studies that combine the factors associated with muscle fiber composition in the present study with additional measures, such as genetic screening and quantification of physiological capacity, will help to improve the understanding of muscle fiber composition.

Practical applications

We report novel associations of STMF% and with heart rate, chest depth and fracture risk and support previous relationships of this trait with age, endurance ability and blood pressure. Once replicated in independent cohorts, measures of resting heart rate, chest depth and self-reported tolerance to long distances could be combined with additional variables, such as consecutive back squat repetitions at 80% 1RM [3], to investigate whether the indirect estimation of STMF% can be improved using multiple non-invasive measures. An ability to indirectly estimate muscle fiber composition with confidence would be particularly useful to practitioners working in health, exercise and professional sport, where there is limited access to direct assessment of muscle fiber composition.

CONCLUSIONS

The present study demonstrates that the combination of age, sex and training frequency account for 10.6% and 13.2% of the variance in STMF% in endurance and power athletes, respectively, with age and sex also associated with non-athletes' STMF%. This study also provides novel information concerning the positive association of STMF% with resting heart rate, tolerance to long distance exercise and reaction time, and larger chest depth. On the other hand, a greater FTMF percentage was associated with rare fracture incidence, and we confirm the negative relationship of STMF% with blood pressure. However, the contribution of age, sex and training frequency explained less of the variance in muscle fiber composition than that previously attributed to heritable factors, suggesting that the heritability of muscle fiber composition is among the strongest determinants of elite athletic performance.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Evgeny A. Lysenko, Tatiana F. Vepkhvadze, Egor M. Lednev and Daniil V. Popov for their help with determination of muscle fiber composition and valuable comments.

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