Title page

TITLE: Age-related slowing of contractile properties differs between power-, endurance- and non-athletes; a tensiomyographic assessment

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Abstract

Although master athletes maintain high levels of physical activity, they also suffer from an age-related decline in skeletal muscle function. There are indications of disproportional age- and physical inactivity-induced muscle wasting between muscles. Tensiomyography is a non-invasive tool that has been used to study the effects of a variety of sports on the contraction time (Tc) in different skeletal muscles. The aim of this cross-sectional study was to assess age-related changes in the Tc of the vastus lateralis, gastrocnemius medialis and biceps femoris muscles with Tensiomyography in older non-athletes (age = 62.1±12.7 years; N_{MALES} = 133; $N_{FEMALES}$ = 246), and power (age = 56.9±13.5 years; N_{MALES} = 100; $N_{FEMALES}$ = 78) and endurance master athletes (age = 56.5±14.5 years; N_{MALES} = 76; $N_{FEMALES}$ = 73). We found an age-related slowing in all muscles, irrespective of discipline, where endurance master athletes had the longest and power master athletes had the shortest Tc. The longer Tc in endurance master athletes than in non-athletes suggests that regular endurance sport activity aggravates slowing of skeletal muscles during ageing.

Key words: Skeletal muscle, TMG, Contraction time, Master athletes, MHC

Introduction

In non-athletes muscle mass shows a progressive decline of as much as 1 to 1.5% per year after about the age of 50 [1]. The age-related loss of muscle mass is a consequence of loss and atrophy of muscle fibres [2–4]. Since many of the age-related changes in skeletal muscle are similar to those induced by disuse [5] it is likely that the decrease in physical activity in old age [6,7] is a major contributor to the muscle wasting during ageing. Master athletes, however, maintain high levels of physical activity [8–10] and suffer from fewer morbidities [11], thereby providing a unique human research model to disentangle the effects of disuse and co-morbidities from ageing *per se*.

Master athletes are athletes older than 35 years who train for and participate in athletic competitions. Their training regimes range from 6 to over 20 hours per week [12] and they may well work at the limits of physiology [10]. Even though their athletic performance exceeds that in age-matched non-athletes, also master athletes suffer from an age-related decline in muscle force and power [2,13,14], ventilatory function [15] and maximal oxygen consumption [16].

In line with these decreases in physiological parameters, jump, sprint and endurance performance decline with age [13,17], something seen even at the individual level [18], and after the age of 70 it even appears to be an exponential decline [19]. This age-related decline in performance is not only caused by a loss of muscle mass and force generating capacity, but also by slowing of movement as a consequence of slower muscle contractile properties and increased tendon compliance [13].

Part of the slowing of skeletal muscle is attributable to a preferential atrophy of fast type II fibres [3,4,20,21], which produce at least 5-times as much power as slow type I fibres [22]. Single-

fibre studies have also provided evidence of an age-related decrease in shortening velocity independent of fibre type switching in fibres expressing types I and IIa myosin heavy chain (MHC) isoforms [23,24]. Thus, the loss of muscle mass, changes in fibre type composition and slowing of muscle fibres may all contribute to the decline in anaerobic performance.

Tensiomyography (TMG) is a non-invasive tool to assess the contractile properties (e.g. contraction time – Tc) of a muscle and can be used to estimate the percentage of type I myosin heavy chain at least in the vastus lateralis (VL) muscle [25]. We have recently shown in a large cohort of adolescents that regular sport exercise decreased Tc in the non-postural biceps femoris (BF), but not in the postural VL [26]. In addition, we observed that 8 weeks of plyometric training in young athletes results in an 8-26% shorter Tc in five lower limb skeletal muscles that explained ~30% of the improvement in explosive power [27]. This suggests that Tc is indeed related to muscle contractile properties and secondly that regular exercise may modulate the effects of ageing on skeletal muscle properties.

Previously we found in older people that a lower performance in the timed-up-and-go and 6-minute walk tests was to a significant extent related to a lower maximal shortening velocity of the muscle [28]. A longer Tc in older people may likewise have similar implications for functions of daily life. Yet, despite numerous studies reporting TMG results in children, adults and athletes, there is only one study [29] that investigated the impact of ageing on the TMG-derived velocity of contraction. They reported that the oldest group had the lowest velocity of contraction, but it is unclear whether this was due to a lower Tc and/or higher Dm. Therefore, the aim of this study was to assess age-related changes in VL, BF and gastrocnemius medialis (GM) muscle contractile properties with TMG in male and female older non-athletes, and master endurance and power athletes in a cross-sectional study.

Methods

Participants

Altogether 706 participants (311 men; aged 35 to 90 years) were recruited. Master athletes were recruited during: (i) European Veteran Athletics Championships in Ljubljana 2008 (EVACS; N=253); (ii) European Master Games in Lignano 2011 (EMG; N=56); and (iii) Slovenian Championships in Master Athletics Ljubljana 2015 (VAD; N=18). From the 176 male master athletes 40 were sprinters (≤ 400 m), 38 were throwers, 22 were jumpers, 57 runners (≥ 800 m) and 19 competed in walking events. From the 151 female master athletes 29% were sprinters (≤ 400 m), 29% were throwers, 20% were jumpers, 51 runners (≥ 800 m) and 22% competed in walking events. All master athletes had competed for more than 3 years in master championships. Data of non-athletes were obtained from mass measurements during two international projects led by the Institute for Kinesiology Research at the Science and Research Centre Koper: (i) Physical Activity and Nutrition for Great Ageing 2013-15 (PANGEA; N=259); and (ii) Active and Quality Ageing in Home Environment 2015-16 (A-Qu-A; N=120). Non-athletes were not members of any sport clubs and did not compete in sport championships. Participants were excluded if they suffered from major skeletal, muscular or nervous disorders. Basic anthropometric data and age, sex and sport group distribution are presented in Table 1. The study was reviewed and approved by the Republic of Slovenia National Medical Ethics Committee (EVACS, PANGEA, and AQUA), Italian Ethics Committee at regional level (EMG), and Institutional Ethics Committee of the University of Primorska (VAD). Data collection conformed to the standards set by the Declaration of Helsinki (2002) and its amendments. All participants were fully informed of any risks and benefits associated with the study, and written informed consent was obtained from each participant prior to data collection.

<< Table 1 around here >>

Research design

The study was cross-sectional and participants were divided by sex, sport and age. We defined three sport groups: non-athletes (NA), power athletes (PowA: sprints ≤ 400m, jumps and throws) and endurance athletes (EndA: runs ≥ 800m; walking). We defined three age groups: 35-49 years, 50-64 years and ≥65 years, where the oldest participant was 90 years old.

Anthropometric measurements

The participants were asked to abstain from intense physical activity for 24 hrs (NA) or competition (PowA and EndA) before the assessment. After determination of body height and body mass (Seca Instruments Ltd., Hamburg, Germany) TMG was performed.

TMG

TMG was used to assess skeletal muscle contractile properties in the VL, BF and GM muscles. All measurements were performed during electrically-evoked isometric contractions. For VL TMG, participants were in a supine position with the knee angle at 30^0 flexion (where 0^0 represents a fully extended knee joint). For the BF they were in a prone position with the knee at 5^0 flexion and for the GM they were in a prone position with the ankle in neutral position as previously reported [25,30]. Foam pads were used to support the joints.

A single 1-ms maximal monophasic electrical impulse was used to elicit a twitch that caused the muscle belly to oscillate. These oscillations were recorded using a sensitive digital displacement sensor (TMG-BMC, Ljubljana, Slovenia) that was placed on the surface of the skin over the mid belly of the muscle of interest. If needed, the measuring point and electrode

positions were adjusted to obtain maximal Dm of the muscle belly. Initially, the stimulation amplitude was set just above the threshold and then gradually increased until the Dm of the radial twitch displacement increased no further. From two maximal twitch responses, Tc was calculated and the average used for further analysis. Tc was defined (Figure 1) as the time for the amplitude to increase from 10% to 90% of Dm [25].

<< Figure 1 around here >>

Muscle biopsies

In a subsample of master athletes (N=17; 4 EndA + 13 PowA) we have taken VL muscle biopsies after TMG assessment and determined the myosin heavy chain (MHC) composition as described previously [25]. Briefly, VL muscle biopsies were obtained with a conchotome after skin sterilization and local anaesthesia (2% lidocaine) and sterile conditions. The sample (~100 mg) was taken at 40% from the distance of the knee joint (0%) and the spina iliaca anterior superior (100%). The samples were placed on cork perpendicular to the long axis of the fibres, frozen in liquid nitrogen with vigorous shaking and stored at -80 ºC until analysis. For MHC determination, 10-µm cross-sections were cut in a cryostat, collected in a centrifuge tube with Laemli sample buffer (3 sections in 100 µL) and boiled for 2 minutes to denature the proteins. Twelve-µL samples were loaded on SDS poly-acrylamide gels run at 15 ºC, 120 V for 27 hours. The stacking gel contained 4% acrylamide and the separating gel had 7% acrylamide with both containing 30% glycerol. After the run, the gels were stained with the Silver Stain Plus kit following the instructions of the manufacturer (BIORAD LABORATORIES, United Kingdom). Bands (Figure 2) were identified based on the migration distance, and relative quantities determined with Quantity One software (BIORAD LABORATORIES, United Kingdom).

<< Figure 2 around here >>

Statistics

SPSS (IBM, USA) software was used for all statistical analyses. All data in text and tables are presented as mean ± standard deviation, while in figures standard errors were used. Visual inspection and the Shapiro-Wilk test indicated that all data were normally distributed. Sphericity (homogeneity of covariance) was verified by the Mauchly's test. When the assumption of sphericity was not met, the significance of the F-ratios was adjusted according to the Greenhouse-Geisser procedure. Main effects were studied with a General Linear Model repeated-measures ANOVA with muscle (VL, GM, and BF) as within factor, and three between factors: sport group (three levels: NA, PowA, EndA), sex (man or woman) and age (three levels: 35-49 years, 50-64 years and ≥65 years). Where significant effects were found for sport or age effects or 2-way interactions (three-way interactions were excluded from the analysis), post-hoc analysis with Bonferroni corrections was used to locate the differences between sport and age groups. We used the Pearson correlation coefficient to assess associations between age and Tc in each muscle and sex. We also performed stepwise multiple regression analysis of Tc with age, sex, muscle and sport as predictors, with entry criteria at $p \leq .05$ and removal criteria at $p \geq .1$. In a subsample of 17 master athletes who also gave a VL muscle biopsy we performed Pearson correlation analysis to assess to what extent VL Tc is affected by the MHC-I content of the muscle, age and sport. Statistical significance was accepted at p-values <.05. Additionally, the effect size for dependent variables was assessed as partial eta-squared (η^2).

Results

Table 1 shows the anthropometric data. We found that PowA were the tallest (P=.005), and that they and NA had a higher body mass than EndA (P<.001). NA had a higher body mass index than PowA and EndA (P<.001). Women were smaller (P<.001), lighter (P<.001) and had a lower body mass index (P=.001) than men.

Tc was positively correlated with age, irrespective of muscle and sport group (Figure 3). This correlation was weakest in VL (even not significant for female NA), and strongest in BF for PowA and EndA, where EndA had the strongest correlation in GM.

<< Figure 3 around here >>

A multiple regression analysis (Table 2) confirmed an overall correlation of R=.690 (p<.001) with 47.6% of the variance in Tc explained by Age, BF, VL, and athletic discipline as predictors, but not sex. The largest contributors (part correlations) to the explained variance in Tc are BF and Age. The multiple regression prediction model is as follows:

$$Tc = 11.754 \times BF + .193 \times Age - 3.292 \times An - 3.185 \times VL + 3.142 \times Ae + 18.098$$

where Tc is a muscle contraction time (in ms), BF is the biceps femoris muscle (0 or 1), VL is the vastus lateralis muscle (0 or 1), Age is an age (in years), An is Power athletes (0 or 1), and Ae is Endurance athletes (0 or 1).

<< Table 2 around here >>

In a subsample of master athletes (N_{POW}=13 + N_{END}=4) we assessed the MHC composition of the VL (Table 3) and found a significant bi-variate correlation between Tc and MHC-I (r=.864; P<.001), while there was no significant correlation between age and Tc (P=.309) or MHC-I (P=0.719). A correlation was found also between VL Tc and MHC-IIa (r=-.760; P<.001) but not between VL Tc and MHC-IIx (r=-.326; P=.201).

<< Table 3 around here >>

After dividing the participants into three age groups we found significant effects for muscle (P<.001; η^2=.395), sport (P<.001; η^2=.133), age (P<.001; η^2=.153), and sport*age (P<.001; η^2=.036), muscle*sex (P=.048; η^2=.005), muscle*sport (P<.001; η^2=.033), muscle*age (P<.001; η^2=.043) interactions. As there were interactions between muscle with sport, age and sex, we performed subsequent post-hoc ANOVAs first for the non-athletes only and then for each muscle separately (Figure 4).

<< Figure 4 around here >>

Looking at the NA only, we found the lowest Tc in VL (26.1 ± 4.2 ms), the longest in BF (43.1 ± 11.3 ms) and that of the GM (30.0 ± 7.7 ms) in between the two.

In the VL, there were effects of age (P<.001; η^2=.039), sex (P<.001; η^2=.017), sport (P<.001; η^2=.065), and sport*age (P=.003; η^2=.023) and sex*age (P=.072; η^2=.008) interactions. The interactions indicated that the effects of age differed between sports and sexes. The sex*age interaction was reflected by an age-related increase in Tc in men only (P=0.002). The sport*age interaction was reflected by the shorter Tc in PowA than NA at all ages in men only

(P=.004). In both male PowA and NA Tc increased from 35-49 to 50-64 years by 8.7% (P=.004) and 10.5% (P<.001), respectively. The Tc was higher in 65-90-year-old EndA than 50-64-year-old EndA (P=.015) and EndA had a longer Tc than NA (P=.008).

In the GM, there were effects of age (P<.001; η^2=.066) and sport (P<.001; η^2=.039), and a sport*age interaction (P=.009; η^2=.022). The latter indicated that the effect of age differed between the different sports. Although in all groups Tc was longer at 65-90 years in comparison to 35-49 years (10.2%; P=.017), PowA had shorter Tc than NA at 35-49 years (-9.7%; P=.014), and EndA had longer Tc at 65-90 years than NA (22.6%; P<.001).

In the BF there were effects of age (P<.001; η^2=.129) and sport (P<.001; η^2=.099), and sport*age (P=.086; η^2=.012) and sex*sport (P=.087; η^2=.007) interactions. The interactions indicated that the effects of sport differed between sports, and between men and women. Tc was consistently increased with increasing age in PowA, but PowA always had a shorter Tc than NA in both men and women. However, the relative difference of Tc between NA and PowA was decreasing with increasing age (in men: from 20.5% to 12.3%; in women from 27.4% to 11.3%). In EndA Tc was higher than in NA at 35-49 years (only in men: 22.5%; P=.001), then stabilised at 50-64 years and increased again at 65-90 years (in men: 33.2%; P<.001, in women: 26.5%; P<.001), when EndA had higher Tc than NA (only in men: 20.5%; P<.001). We found that body mass and body mass index explained at best 1% of the variance (data not shown).

Discussion

In the present cross-sectional study, we used TMG to non-invasively assess the contractile properties of leg skeletal muscles in master endurance and power athletes, and age-matched

non-athletes. The main observation of the present study is the age-related slowing of all muscles. Endurance athletes had slower muscles than age-matched non-athletes, while power athletes had the fastest contractile properties, irrespective of sex. For all muscles, an age*sport interaction was found, indicating that the effects of age differ between sports. Given that endurance athletes had slower contractile properties than non-athletes, this suggests that regular long-distance running accelerates the age-related slowing of skeletal muscle.

TMG

TMG was developed to non-invasively measure skeletal muscle contractile properties [31]. We have previously shown in a population of 20-83-year olds that Tc predicted 77% of the variance of the proportion of type I MHC in the VL [25], which was confirmed by our results obtained in a sub group of 17 master athletes (75%). Although this regression model has not been validated in other muscles, there are no obvious reasons to believe that it would not apply to other muscles, albeit with different regression coefficients. Here we show that Tc was lowest in the VL (26.1 ± 4.2 ms), then in GM (30.0 ± 7.7 ms) and longest in BF (43.1 ± 11.3 ms), corresponding with the reported differences in type I proportions between these muscles (50%, 54-63% and 67%, respectively) [32,33].

Sex and sport related differences in contractile properties

We did not observe significant differences between men and women in the contractile properties of the VL, BF and GM. This corresponds with the similar fibre type distribution in men and women [21,34,35].

Power athletes had a lower Tc than endurance and non-athletes, particularly in the non-postural BF, and to a lesser extent in the postural VL and GM. The shorter Tc in power

athletes is most likely attributable to a higher shortening velocity in both type I (75%) and type IIa (45%) fibres, as reported after 12 weeks of resistance exercise in older men [36]. Interestingly, the same authors [37] demonstrated that the same programme in women induced a decrease in the shortening velocity of type I fibres, with no change in that of type IIa fibres. We did not observe a significant sex*sport interaction for VL, but such an interaction for the BF was reflected by a longer Tc in male, rather than female, endurance than non-athletes, while female endurance and non-athletes had a similar Tc. We have no explanation for these differences, but the effects of resistance exercise on single fibre contractile properties are not unequivocal, with at least one study showing no change in single fibre contractile properties in young men [38].

Age-related slowing

In the present study, we observed an age-related slowing of the muscle contractile properties, confirming the general observation of an age-related slowing of muscle in both non-athletes and master athletes [13,39]. This may be attributable to a preferential loss of type II fibres [40]. That, however, is not an unequivocal observation as other studies did not see such a change [4,21,41] and one longitudinal study even reported an increased proportion of type II fibres over a 12-year period [24]. Even if type II fibres are preserved, their contribution to the contractile properties of the muscle may decrease if they undergo a larger age-related atrophy than type I fibres. Indeed, some studies reported a protection of type I fibres as compared with type II fibres [4,21,40,42], but again, others have reported that type I and II fibres atrophy similarly with age [41,43]. The discrepancy between studies could be attributed to several factors: (i) pooling of muscle fibre phenotypes; (ii) different muscles investigated; (iii) small study samples; and (iv) by necessity when using biopsies, a relatively small amount of muscle tissue being analysed.

Age-related slowing may not only be due to fibre type shifts, but also slowing of fibres. In fact, it has been reported in both rodents [44] and humans [43,45] that particularly type I and IIa fibres are exhibiting an age-related slowing independent of shifts in myosin heavy and light chain isoform composition. Such an age-related slowing maybe caused by glycation of the myosin molecule [46] that may have a mitochondrial origin [47]. Given that endurance athletes have a larger proportion of type I and IIa fibres than power and non-athletes [14], we expected the slowing to be more pronounced in the endurance than power and non-athletes, something we did indeed observe. While this may to some extent, as in ageing, be related to glycation of myosin in these fibres, such an effect will be attenuated, but probably not entirely abolished, in endurance athletes, who have a higher insulin sensitivity than non-athletes (Goodpaster, 2001). The role of myosin glycation is admittedly speculative, but is an interesting avenue of investigation. If this pathway is indeed involved then consumption of anti-glycating agents [48] may alleviate some of the age-related slowing, particularly in endurance athletes.

Conclusion and Perspective

TMG revealed that the age-related slowing of muscle contractile properties occurs particularly in endurance athletes. Here we suggest that this may be related to their high proportion of type I and IIa fibres that have been reported to exhibit an age-related slowing independent of shifts in myosin heavy and light chain composition.

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Tables

Table 1: Anthropometric data of participants, grouped by age, sex and sport.

	Non-athletes		Power athletes		Endurance athletes	
	Men	Women	Men	Women	Men	Women
N	133	246	100	78	76	73
N_{35-44}	31	28	32	26	20	27
N_{45-64}	45	68	33	28	25	22
N_{65-90}	57	150	35	24	31	24
Age/years	57.8±14.0	63.4±11.4	57.5±14.1	55.5±12.2	56.8±14.7	56.2±14.6
Age_{35-49}/years	42.0±7.4	41.3±5.2	40.8±4.5	41.4±5.1	40.0±4.1	41.4±3.7
Age_{50-64}/years	57.2±4.6	60.3±4.8	58.8±5.3	56.4±4.5	56.5±5.3	56.7±4.9
Age_{65-90}/years	72.4±4.7	71.8±5.1	71.6±8.0	70.6±2.5	71.8±5.1	73.9±4.8
Body height/m	1.78±.07	1.62±.07	1.78±.08	1.69±.07	1.78±.07	1.66±.07
Body $height_{35-49}$/m	1.81±.07	1.68±.08	1.81±.07	1.73±.06	1.81±.07	1.70±.09
Body $height_{50-64}$/m	1.79±.07	1.63±.06	1.78±.05	1.67±.08	1.75±.08	1.66±.05
Body $height_{65-90}$/m	1.74±.06	1.61±.06	1.73±.08	1.67±.06	1.73±.06	1.59±.04
Body mass/kg	85±12	70±12	79±13	64±6	72±12	60±9
Body $mass_{35-49}$/ kg	86±12	66±10	83±16	64±6	75±9	63±8
Body $mass_{50-64}$/kg	87±13	70±13	79±13	63±8	72±16	59±10
Body $mass_{65-90}$/kg	83±11	70±11	72±12	65±11	64±6	55±8
BMI/kg/m^2	26.6±3.4	26.2±4.4	24.9±2.9	22.4±3.0	22.9±3.7	21.7±2.8
$BMI_{35-49}/kg/m^2$	26.1±3.4	23.6±3.3	25.5±3.6	21.3±2.5	23.1±2.9	21.9±2.9
$BMI_{50-64}/kg/m^2$	26.3±3.3	25.9±4.6	24.9±2.6	22.6±3.1	23.4±4.2	21.5±3.0
$BMI_{65-90}/kg/m^2$	27.2±3.1	27.0±4.3	24.1±2.1	23.1±3.2	21.6±1.7	21.9±2.5

BMI: body mass index; indexes $_{35-49}$, $_{50-64}$, $_{65-90}$ represent the age range in an age group. For statistical analysis see Results.

Table 2: Multiple linear regression analysis of age, sex (removed by regression), sport (Aerobic and Anaerobic), and muscle (biceps femoris and vastus lateralis) as predictors of tensiomyographic contraction time.

Predictors	Unstandardized coefficients	Standardized coefficients	P	Part r	VIF
Constant	18.098 ms	.907	<.001		
Biceps femoris	11.754 ms	.421	<.001	.453	1.371
Age	.193 ms/years	.013	<.001	.236	1.050
Anaerobic	-3.292 ms	.426	<.001	-.125	1.088
Vastus lateralis	-3.185 ms	.419	<.001	-.123	1.372
Aerobic	3.142 ms	.509	<.001	.100	1.069

Part r: Part correlation of each predictor to contraction time when controlling for effects of other predictors; VIF: variance inflation factor.

Table 3: Myosin heavy chain (MHC) composition of the vastus lateralis muscle for a subsample of master athletes (master athlete's data from Šimunič et al., 2011).

	Total N=17	Power athletes N=13	Endurance athletes N=4
MHC-I / %	35.6±18.4	31.7±16.3	48.1±21.5
MHC-II / %	64.5±18.4	68.3±16.3	52.0±21.5
MHC-IIa / %	44.7±14.4	45.2±12.4	43.3±22.0
MHC-IIx / %	19.7±15.1	23.1±15.0	8.7±10.3

Figure captions:

Figure 1: Typical tensiomyographic responses of the biceps femoris of a non-athlete, power athlete and endurance athlete (left) and of a non-athlete in different age groups (right). Tc: contraction time defined as the time from 10% to 90% of the maximal displacement amplitude (Dm).

Figure 2: Representative SDS-PAGE gel to determine the myosin heavy chain composition of the vastus lateralis muscle from master athletes.

Legend: Lane 1-3, 5, 7-11 contain all three myosin heavy chain isoforms (from top to bottom IIx, IIa, I) and lane 4 and 6 only contain type I and IIa myosin heavy chain isoforms.

Figure 3: Pearson correlation (r) between age and contraction time (Tc) for men (left) and women (right) in each muscle and sport group.

Figure 4: Contraction time (Tc) differences in three skeletal muscles between athletes and non-athletes in different age groups for men (left column graphs) and women (right column graphs).

Legend: a, b, c…significantly different from non-athletes at P<.05; P<.01, and P<.001, respectively; i, j, k… significantly different from previous age group at P<.05; P<.01, and P<.001, respectively; G. … Gastrocnemius.

Figure 1.

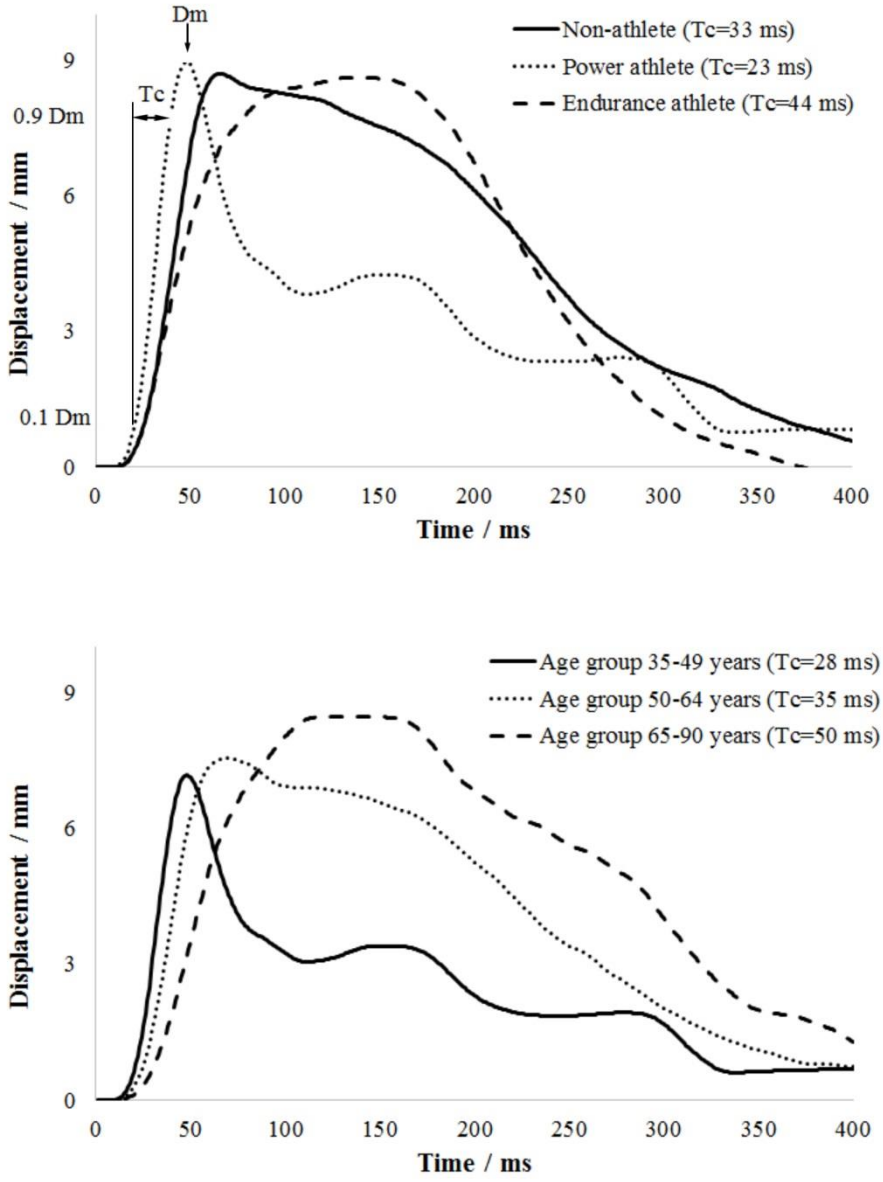

Figure 2.

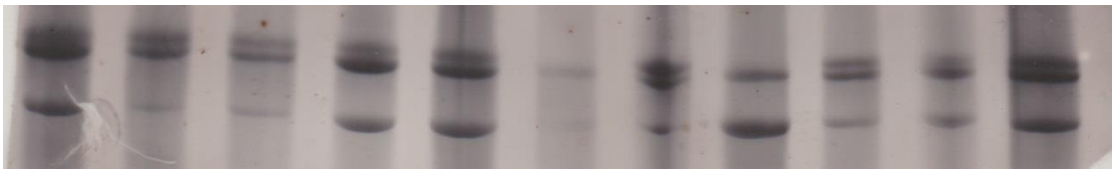

Figure 3.

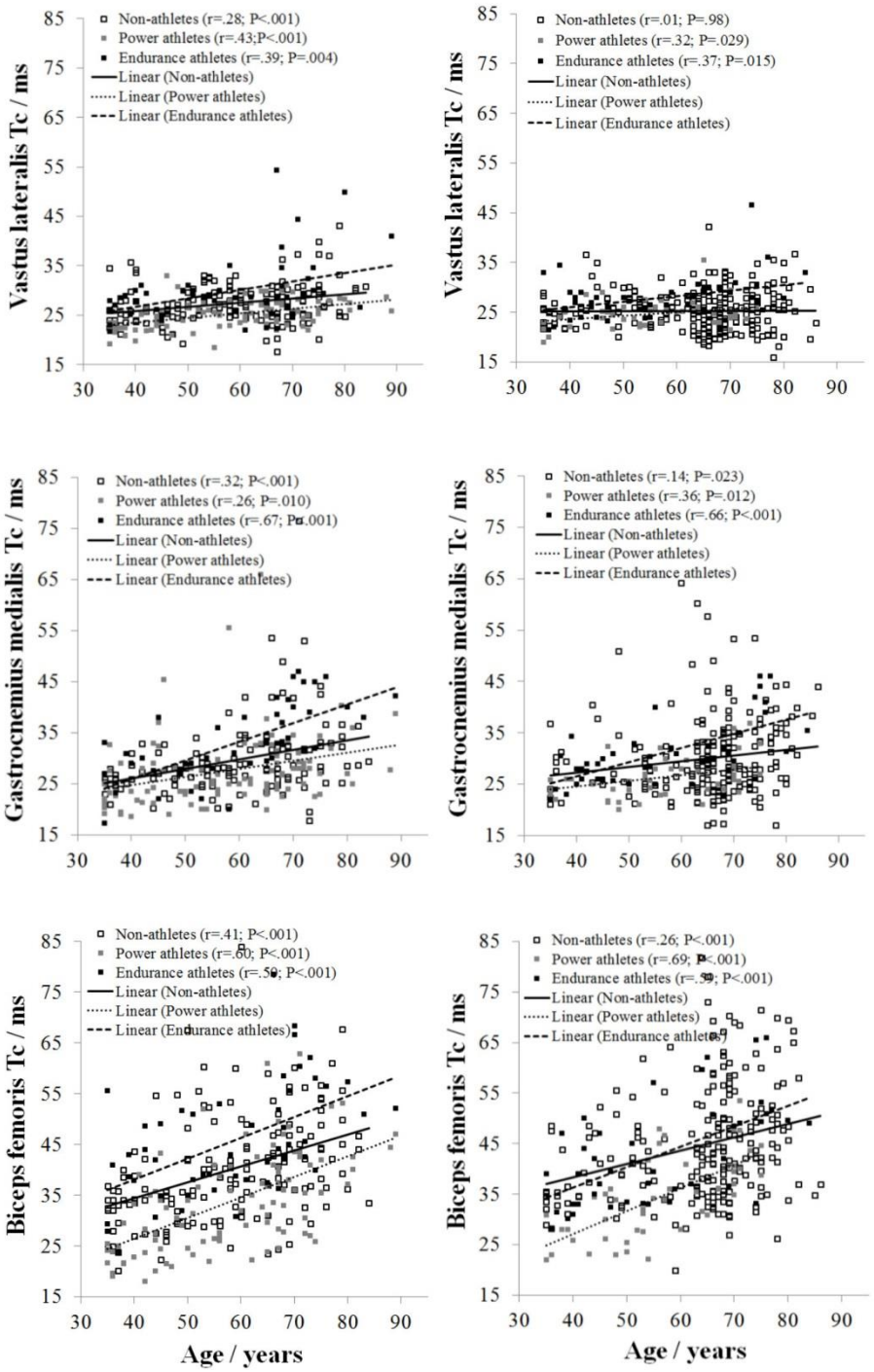

Figure 4.