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<td>Drey, Michael; University of Erlangen-Nürnberg, Institute for Biomedicine of Aging&lt;br&gt;Sieber, Cornel; University of Erlangen-Nürnberg, Institute for Biomedicine of Aging&lt;br&gt;Degens, Hans; Manchester Metropolitan University, School of Healthcare Science&lt;br&gt;McPhee, Jamie; Manchester Metropolitan University, School of Healthcare Science&lt;br&gt;Korhonen, Marko; University of Jyväskylä, Gerontology Research Center&lt;br&gt;Müller, Klaus; German Aerospace Center, Institute of Aerospace Medicine&lt;br&gt;Ganse, Bergita; German Aerospace Center, Institute of Aerospace Medicine&lt;br&gt;Rittweger, Joern; German Aerospace Center, Institute of Aerospace Medicine</td>
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<td>Master athlete, Sarcopenia, MUNIX, Aging, Muscle, EMG</td>
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Relation between muscle mass, motor units and type of training in master athletes

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Master athletes and motor units
Summary

Objective

The aim of the present study was to measure the number of motor units and muscle mass in power trained and endurance trained master athletes compared to community-dwelling older adults.

Methods

75 master athletes (52 power and 23 endurance trained athletes) were recruited at the 2012 European Veteran Athletics Championships in Zittau (Germany). 149 community-dwelling older adults served as controls. In all participants the Motor Unit Number Index (MUNIX) in the hypothenar muscle and whole body muscle mass was determined.

Results

In both male and female master athletes there were significant negative correlations between age and muscle mass (female: r=-0.510, p=0.002; male: r=-0.714, p<0.001). Master athletes showed a weak correlation (r=-0.295, p=0.010) between MUNIX and age. Master athletes exhibited significantly higher values than the control group with regard to both muscle mass (p=0.002) and motor units (p=0.004). Sub-analysis showed that only power trained master athletes had both a larger muscle mass (p<0.001) and a higher MUNIX (p=0.014) than the control group. Among the master athletes, power trained athletes had a larger (p<0.001) muscle mass than endurance trained athletes.
Conclusions

The present data of master athletes are compatible with the hypothesis of an age-related decline in whole body muscle mass and motor units. Nevertheless, the data suggest that the master athletes’ high level of physical activity may protect motoneurons. In addition, power training seems to have a positive effect on muscle mass, and could therefore be an effective method of training to prevent sarcopenia.

Keywords: Master athlete, Sarcopenia, MUNIX, Aging, Muscle, EMG
Introduction

Age-associated loss of muscle mass, also called sarcopenia, is becoming an ever-greater medical and economic problem in aging societies. Those affected exhibit greater morbidity and mortality (Thomas, 2010). One pathomechanism that can lead to sarcopenia is the loss of motoneurons (Walston, 2012). Motoneuron numbers are very difficult, or impossible, to count in humans, but the use of electromyographic techniques enables the number of motoneurons controlling individual muscles to be estimated (Doherty et al., 1995; McComas et al., 1971). MUNIX is a relatively new electromyographic method for assessing the number of motor units using the Compound Muscle Action Potential (CMAP) and the Surface electromyographic Interference Pattern (SIP) (Nandedkar et al., 2004). This technique is non-invasive, and easy and quick to perform. Using the MUNIX method in the hypothenar muscle, it was demonstrated that a small number of motoneurons was associated with sarcopenia (Drey et al., 2013) and muscle weakness in old age (Drey et al., 2014; Kaya et al., 2013).

Low physical activity is one further cause of sarcopenia and a high level of physical activity is recommended in order to counteract the age-related loss of muscle mass (Aagaard et al., 2010; Walston, 2012). Master athletes are characterized by a high level of physical activity beyond the age of 35 with a simultaneously low morbidity rate (Kettunen et al., 2006; Kujala et al., 2003). For this reason, this group is particularly suited for investigating the effects of physical training on the loss of muscle mass and the number of motoneurons. In addition, it is possible to investigate how the type of training (power versus endurance training) undertaken by master athletes influences the number of motoneurons and muscle mass. This investigation could lead to recommendations for optimum methods of training for the general public.
population in order to counteract sarcopenia. In the present study, the MUNIX
technique was used to estimate the number of motor units of the hypothenar muscle
in master athletes in comparison to controls. Further, a comparison of power versus
endurance trained master athletes should provide insight into the effect of the training
paradigms on muscle mass and motor units.
Methods

Participants

75 master athletes who participated in the 2012 European Veteran Athletics Championships (EVACS) in Zittau, Germany (www.evacs2012.com) were recruited for investigation. Of these, 23 were endurance trained (800 m, 1500 m, 5000 m, 10,000 m, 10 km road walk, 2000/3000 m steeplechase, marathon) and 52 were power trained (100 m, 200 m, 400 m, 80/400 m hurdles, javelin, long/high/triple jump, pole vault, hammer, discus, heptathlon, decathlon). A total of 149 untrained, community-dwelling elderly over the age of 65 from another investigation served as a control group (Drey et al., 2014). All participants gave written informed consent. Ethical approval was obtained from the ethics committee of North Rhine Medical Association, number 2012157. The study was registered in the DRKS clinical trial registry, number DRKS00004209.

Muscle mass determination

Muscle mass was calculated using Janssen’s regression formula by applying Bioelectrical Impedance Analysis (BIA) (Janssen et al., 2000). BIA resistance was obtained using an Akern BIA 101 (SMT Medical GmbH & Co. KG, Würzburg, Germany) with an operating frequency of 50 kHz at 400 µA. Whole-body BIA measurements were taken between the right wrist and ankle with the subject in a supine position. Method comparisons between BIA and Dual-energy X-ray Absorptiometry (DXA) to determine muscle mass showed a good correlation (Bosaeus et al., 2014; Moon et al., 2013). Based on this, muscle mass calculated using Janssen’s regression formula exhibited a strong correlation (r=0.952) with the muscle mass determined by DXA (Bosaeus et al., 2014).
The MUNIX technique

MUNIX is based on a mathematical model, which was described for the first time by Nandedkar using the CMAP and SIP of the hypothenar muscle (Nandedkar et al., 2004). For a detailed description of the method see also previous work (Drey et al., 2013). MUNIX is a three-step procedure. First, the CMAP is recorded by supramaximal stimulation of the ulnar nerve. The active electrode is placed over the motor point of the right hypothenar muscle. The reference electrode is placed on the distal phalanx of the little finger. The ground electrode is fixed to the wrist. Three consecutive supramaximal stimulations are performed to obtain the highest possible CMAP amplitude. The negative phase of the CMAP is used to compute its amplitude, area and power. The SIPs are recorded in the second step. Each SIP epoch is 300 ms long. The patient is then instructed to exert and maintain an isometric contraction at varying levels of effort. The progressive series of resistance is repeated to obtain 9 different SIP epochs. We recorded the CMAP and SIP using a bandpass filter setting of 3–3000 Hz. In the final step, area and power of CMAP and SIP signals were calculated offline. The "ideal case motor unit count" (ICMUC) is computed using

\[ ICMUC = \frac{\text{Area(SIP)}}{\text{Power(SIP)}} \cdot \frac{\text{Power(CMAP)}}{\text{Area(CMAP)}}. \]

The relationship between ICMUC and SIP area (\( ICMUC = A \cdot (\text{Area(SIP)})^\alpha \)) is modeled by a power function in SPSS (IBM-SPSS Inc., v 21.0, Chicago, USA). The values of \( A \) and \( \alpha \) are derived from a regression analysis fitting the power function. The regression curve characterizes the tested muscle. Finally, MUNIX is calculated using: \( MUNIX = A \cdot (20\, \text{mVms})^\alpha \).

The SIP area value of 20 mVms is chosen arbitrarily, based on the observation that very slight activity, produced by a few motor units, has an SIP area of around 20
mVms. The assumptions of the model are adequately satisfied for an SIP area of 20 mVms.

Prior to the analysis, the operator views the SIP epochs to identify all artifacts, such as high-frequency noise, power line frequency interference, baseline shifts and tremor. If artifacts are identified, the epoch is rejected from the analysis. If there is no apparent voluntary EMG activity, the SIP will have a low but finite area and power value. This results in a very high false ICMUC value. To exclude this artifact, we used the following three criteria to accept an SIP epoch, as proposed by Nandedkar (Nandedkar et al., 2010): 1) SIP area >20 mVms, 2) ICMUC <100, 3) SIP area/CMAP area >1.

Statistical analysis

SPSS (IBM-SPSS Inc., v 21.0, Chicago, USA) was used for statistical analysis. Linear regression analysis was applied to describe the association between muscle mass and age, and between MUNIX and age in master athletes. To describe group differences in muscle mass and MUNIX, adjusted for age and gender, multiple linear regression analysis was used (Muscle mass ~ Group x Gender x Age, MUNIX ~ Group x Gender x Age). The level of significance was set at 5%.
Results

Table 1 shows characteristics, muscle mass and MUNIX of participants. Figure 1 demonstrates whole body muscle mass of master athletes as a function of age, separated for gender. Linear regression coefficient is $r=-0.510$ ($p=0.002$) for female and $r=-0.714$ ($p<0.001$) for male, respectively. Figure 2 illustrates the association between MUNIX and age for the master athletes. No difference in MUNIX for gender was found ($p=0.574$). For linear regression the coefficient is $r=-0.295$ ($p=0.010$).

Table 2 shows the difference in MUNIX and muscle mass between master athletes and controls and between power and endurance trained master athletes and controls, respectively. Adjusted for age and gender, master athletes exhibit a significantly higher MUNIX than controls ($p=0.004$, Figure 3). This also holds for the comparison between power trained master athletes and controls ($p=0.014$), but not for the endurance athletes where MUNIX did not significantly differ from either controls or power athletes. Regarding muscle mass, master athletes had a higher muscle mass than controls ($p=0.002$, adjusted for age and gender). Sub-analysis of master athletes in comparison to controls revealed that only power, but not endurance trained master athletes had higher muscle mass ($p<0.001$, adjusted for age and gender). The inter-group comparison of master athletes revealed a significantly higher muscle mass in power trained master athletes ($p<0.001$, Figure 4). As mean age of master athletes and controls obviously differs (Table 1), multiple regression analysis was adjusted for age. Nevertheless, an additional multiple regression analysis was performed with age-matched master athletes over the age 65 only (Table 2). This analysis revealed no difference to the age-adjusted regression analysis.
Discussion

In the present study, it was demonstrated using the MUNIX technique that sustained physical activity, as performed by master athletes is associated with a larger number of motoneurons and greater muscle mass, as compared to community-dwelling older adults. Regarding muscle mass, this effect was particularly pronounced in power-specialized master athletes, but not in endurance trained master athletes. These results therefore are compatible with the view that power oriented physical activity may constitute an effective training concept to counteract sarcopenia. However, selection bias cannot be excluded.

Master athlete as a model of successful aging

Physical exercise plays an important role in the treatment and prevention of sarcopenia (Walston, 2012). Numerous intervention studies have demonstrated the positive effects of physical exercise on the muscular system, even in old age (Chodzko-Zajko et al., 2009). In this connection, not only an increase in muscle mass has been demonstrated, but also improvements in muscle strength and muscle power (Peterson et al., 2011, 2010; Steib et al., 2010). Most of the studies showing these benefits of exercise on muscle are intervention studies, which do not reveal to what extent maintained high levels of physical activity prevent loss of muscle mass in old age. Master athletes, by contrast, have been training for large parts of their life. Their commitment to training is likely to be much greater and sustained than in the published intervention studies. In this sense, they constitute a suitable model for investigating successful aging (Hawkins et al., 2003).

MUNIX and muscle mass: master athletes versus community-dwelling older adults
In the present cross-sectional study it was possible to show that old master athletes exhibit a lower muscle mass and a lower number of motor units than younger master athletes, suggesting an age-relation in both measures (Figures 1 and 2). Using the decomposition-enhanced spike-triggered averaging technique from the tibialis anterior muscle, Power and colleagues showed a larger number of motor units in master endurance runners than in an age-matched control group (Power et al., 2010), whereas the number of motor units in the biceps brachii muscle did not differ between master endurance runners and a control group of the same age (Power et al., 2012). These findings suggest that a protective effect was achieved only on the motor units of the muscle specifically trained by master runners. Due to the small number of master athletes investigated (n=9), however, the authors could not rule out a systemic protective effect on motoneurons by the high level of physical activity. By applying the MUNIX technique to investigate the hypothenar muscle in our own laboratory, it was possible to identify a subgroup of sarcopenic patients with pathological low MUNIX values that suggest that the low muscle mass is due to a low number of motoneurons (Drey et al., 2013). A further investigation was carried out using the same technique involving the hypothenar muscle of participants with pathological low MUNIX values; in this case, an odds ratio of 3.09 was determined for the existence of sarcopenia (Drey et al., 2014). Based on these results in a sample of n=149, determining MUNIX in the hypothenar muscle appears to lend itself as a measure of the systemic loss of muscle mass through loss of motoneurons. In spite of the age dependency of MUNIX and muscle mass among the master athletes (Figures 1 and 2), the present study revealed significantly higher values in both parameters than in the control group (Table 2, Figure 3). Thus the higher degree of physical activity undertaken by master athletes seems to counteract the loss of
motoneurons and the consecutive loss of muscle mass (Kanda and Hashizume, 1998).

Since the loss of motoneurons in old age is an irreversible process, it is important to understand the mechanisms that bring about the preservation of motoneurons through physical activity (Anziska and Sternberg, 2013). In this context, the protective effect of neurotrophic factors on motoneurons, the release of which is to be determined by neuromuscular activity, shall be discussed (Ogborn and Gardiner, 2010). This mechanism would help to explain the aforementioned local muscle-specific effects on the motoneurons determined by Power and colleagues (Power et al., 2012, 2010). Reduced oxidative stress under physical exercise could be responsible for a systemic effect on motoneurons, as demonstrated in the present investigation (Bouzid et al., 2013; Contestabile, 2011; Miyazaki et al., n.d.). Another systemic tropic factor like the Brain-Derived Neurotrophic Factor (BDNF), one of the most important neurotrophic factors in adult neurogenesis (Knaepen et al., 2010), is raised during physical exercise. This therefore counteracts a loss of motoneurons with consecutive muscle mass loss (Coelho et al., 2013). Another systemic effect of physical activity is its immunomodulating effect (Walsh et al., 2011). The reduced level of proinflammatory mediators such as IL-6 and TNFα caused by physical activity prevent the activation of microglia in the mouse model of Amyotrophic Lateral Sclerosis (ALS), thus preventing the destruction of motoneurons (Weydt and Möller, 2005).

Finally, a biological and genetical selection bias could explain the difference in MUNIX and muscle mass of the master athletes compared to the controls.
Influence of the training type on MUNIX and muscle mass

Physical exercise is recommended for sarcopenic patients (Aagaard et al., 2010; Walston, 2012). Especially strength training seems to counteract the loss of muscle mass and muscle strength (Hanson et al., 2009). Numerous studies have shown that although traditional strength training using slow velocity movements has considerable benefits on muscle strength in older adults, it only has a small to moderate effect on physical performance (Latham et al., 2004). In recent years, a variety of studies have shown that muscle power (generation of muscular work per unit of time or, in other words, the product of force and velocity of the muscle contraction) is more closely related to physical performance than muscle strength (Bean et al., 2010; Steib et al., 2010). The core element of the power training concept is that the concentric part (lifting or pushing) of strength training has to be completed as quickly as possible, whereas the eccentric part (lowering) has to be completed in approximately 2 to 3 seconds (Reid and Fielding, 2012). There is growing evidence that power training for older adults is a good method of improving physical performance (Steib et al., 2010; Tschopp et al., 2011; Webber and Porter, 2010). Depending on their disciplines, master athletes train in a more power oriented (jumpers, sprinters, throwers) or endurance-oriented manner (long-distance runners). For this reason, the master athletes investigated were divided according to their respective disciplines into power or endurance trained athletes, as described in the methods section. It was then possible to investigate the issue of how the type of training undertaken by master athletes affects muscle mass and motor units. It was shown that power trained master athletes have significantly greater muscle mass than endurance trained master athletes (Figure 4) and the control group (Table 2). No difference in muscle mass was demonstrated between endurance trained master athletes and the control
group. Since the two groups of master athletes do not differ with regard to the number of motor units (Table 2), the significantly larger muscle mass appears to be due to the hypertrophy of the muscle fibers. These findings suggest that a large muscle mass is required to meet the biomechanical function in power-oriented disciplines. Conversely, muscle mass in endurance athletes appears to play a subordinate role, particularly because their values do not differ to those of the control group. In endurance disciplines, the focus consequently appears to lie on muscle metabolic profile and cardiovascular endurance rather than on muscle mass per se. It has been suggested that decreased fiber size in endurance runners is a favorable adaptation that improves diffusion of oxygen and substrates in the cell during exercise (Trappe, 2001). According to a definition of sarcopenia (Chien et al., 2008), even two male endurance athletes in our cohort would be classified as sarcopenic, but this is misleading because the athletes clearly had exceptional mobility and running performance. In a mouse model of ALS, the effect of two training paradigms (i.e. running and swimming) on motoneuron survival was examined (Deforges et al., 2009). Incomprehensible for the authors, in that study swimming was considered as high-frequency and -amplitude exercise that preferentially recruits fast motor units, whereas running was considered to be a moderate training that triggers preferentially slow motor units. Mice in the swimming ALS group survived longer and had a higher total motoneuron number than the sedentary ALS group. This effect that power training, compared to endurance training, leads to a preservation of motoneurons in master athletes could not be shown in the present cross-sectional investigation.
Study limitations

This cross-sectional study is not suited for answering causal relationships between training type and number of motor units. It just gives hints regarding the relation and has to be investigated in longitudinal studies. This constitutes the main limitation of the study. Further it should be stressed that MUNIX is an index of the number of motor units, and is not an absolute value. Nevertheless, several investigations have demonstrated the good reliability and validity of MUNIX (Boekestein et al., 2012; Neuwirth et al., 2011). In addition, MUNIX determinations from another muscle of the lower extremity would have been desirable in order to demonstrate a systemic or training-specific effect. Since mainly motoneurons that innervate fast-twitch muscle fibers degenerate in old age, future investigations will have to include examinations of the muscle tissue (Brisswalter and Nosaka, 2013).
Conclusions

Both master athletes and community-dwelling older adults are subject to the age-associated loss of muscle mass and motor units. Nevertheless, the high degree of physical activity undertaken by master athletes appears to have a protective effect on motoneurons and muscle mass. In addition, power trained master athletes demonstrated significantly greater muscle mass. Therefore power oriented physical activity could be the best training concept for the elderly to counteract sarcopenia.

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Conflict of interest

The authors have no conflicts of interest.
References


Kanda K, Hashizume K. Effects of long-term physical exercise on age-related changes of spinal motoneurons and peripheral nerves in rats. *Neurosci Res* (1998); **31**: 69–75.


Figure 1: Muscle mass of master athletes as a function of age, separated for gender.
Figure 2: MUNIX of master athletes as a function of age
Figure 3: MUNIX of master athletes and controls

The p-value is adjusted for age and gender (see Table 2). Whiskers indicate a 95% confidence interval.
Figure 4: Muscle mass of power and endurance trained master athletes

The p-value is adjusted for age and gender (see Table 2). Whiskers indicate a 95% confidence interval.
Table 1: Characteristics of master athletes (MA) and controls

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<th>Measurement</th>
<th>Endurance trained MA</th>
<th>Power trained MA</th>
<th>Controls</th>
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<td>n_{total}=23, n_{female}=13</td>
<td>n_{total}=52, n_{female}=22</td>
<td>n_{total}=149, n_{female}=84</td>
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<td>Age (y)</td>
<td>58 (12), 36-77</td>
<td>57 (14), 36-89</td>
<td>77 (6.0), 65-94</td>
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<td>Muscle mass (kg)</td>
<td>24 (6.5), 16-39</td>
<td>29 (7.2), 18-42</td>
<td>22 (6.4), 12-36</td>
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<td>MMI (kg/m²)</td>
<td>8.4 (1.2), 6.7-11.4</td>
<td>9.7 (1.7), 7.0-13.0</td>
<td>7.7 (1.6), 5.2-12.1</td>
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<td>MUNIX</td>
<td>156 (43), 91-243</td>
<td>164 (47), 78-259</td>
<td>115 (40), 37-220</td>
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†) n=147 because two participants were unable to tolerate MUNIX measurement. 
MMI: Muscle mass index.
Table 2: Multiple linear regression for comparison of groups, adjusted for age and gender

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<td>222^c</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>PO vs. CON</td>
<td>199^c</td>
<td>0.014</td>
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<td>EN vs. CON</td>
<td>170^c</td>
<td>0.122</td>
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<tr>
<td></td>
<td>PO vs. EN</td>
<td>75</td>
<td>0.057</td>
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<tr>
<td>Muscle mass</td>
<td>MA vs. CON</td>
<td>224</td>
<td>-0.126</td>
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<tr>
<td></td>
<td>PO vs. CON</td>
<td>201</td>
<td>-0.189</td>
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<tr>
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<td>EN vs. CON</td>
<td>172</td>
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<tr>
<td></td>
<td>PO vs. EN</td>
<td>75</td>
<td>0.172</td>
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</table>

MA: Master Athletes, PO: Power trained MA, EN: Endurance trained MA, CON: Controls, ^) Number of participants, ‡) Standardised regression coefficient for group-variable, adjusted for age and gender §) Two participants from CON were unable to tolerate MUNIX measurement.