Superior Aerobic Capacity and Indices of Skeletal Muscle Morphology in Chronically Trained Master Endurance Athletes Compared with Untrained Older Adults

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Running Title: Exercise across the life-course improves musculoskeletal ageing

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ABSTRACT

The study aim was to comprehensively assess physiological function and muscle morphology in chronically-trained older individuals against untrained young and older individuals. In a cross-sectional design 15 young untrained controls (YC) (20±2.7 y, 78.9±13.3kg), 12 untrained older controls (OC) (69.8±4.1 y, 77.5±14.2kg) and 14 endurance-trained master athletes (MA) (67.1±4.1 y, 68.7±6.5kg) underwent assessments of body composition, aerobic capacity, strength, muscle architecture and fibre-type morphology. Skeletal muscle index was lower and body fat greater in OC vs. MA. Estimated VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$) was similar between MA and YC, but lower in OC. Isometric leg strength normalized to fat-free mass was similar between groups, whereas normalized isometric arm strength was greater in YC than MA. Myosin heavy chain (MHC) I fibre area was greater in MA than OC, whilst MHC II fibre area was greater in YC than OC. MHC II fibre myonuclear domain size was greater in YC than OC and MA, whereas MA had greater MHC I and MHC II fibre capilliarisation than OC and YC. Satellite cell content was similar between groups. Chronic endurance training enhances indices of muscle morphology and improves body composition and aerobic capacity in older age, with potentially important implications for healthspan extension.

Keywords: Sarcopenia, Exercise, Muscle, Human Ageing

INTRODUCTION

The shift towards an ageing population presents a significant and overwhelming global demand on healthcare resources. The major trepidation is not that individuals are living longer (i.e. lifespan), but that they endure a larger portion of their later years with multiple age-associated co-morbidities (i.e. healthspan) [1]. Thus, strategies to close the gap between health- and life-span are of paramount importance.

Age-related reductions of skeletal muscle mass, strength and function (termed 'sarcopenia') and cardiorespiratory fitness may prematurely force individuals into a state of physical dependence and are independent predictors of all-cause mortality [2-4]. Skeletal muscle mass is indispensable for locomotion, basal metabolism, energy storage and nutrient deposition [5]. By the 8th decade of life skeletal muscle mass has reduced by ~18% in men and 27% in women [6] and is accompanied by a greater relative loss of muscle strength [7]. Sarcopenia is characterised by reductions in muscle fibre cross-sectional area (CSA) [8], satellite cell content [9], motor unit remodelling [10], infiltrations of fat and connective tissue [11], alterations to the microcirculation [9,12] and reduced oxidative capacity [13]. The extent to which this physiological deterioration is due to inherent ageing processes, free from artefacts of biological ageing that exacerbate the decline (i.e., physical inactivity), is unclear.

The study of individuals who have chronically undertaken structured exercise training and continue to compete, into their later years, referred to as Master Athletes (MA), provides a model to investigate the impact of inherent ageing on physiological function, without confounding aspects of biological ageing. In a recent meta-analysis, we reported that endurance-based MA displayed a 55% greater aerobic capacity compared with age-matched untrained individuals, whereas resistance trained MA demonstrated greater strength than age-matched untrained individuals [14]. Furthermore, others have shown that although a decline in physiological function remains apparent in MA, high physical activity may ultimately

improve the healthspan by shifting the 'set point' of age-related physiological deterioration to later in life [15,16]. Nevertheless, firm conclusions on the influence of ageing and chronic exercise on physiological deterioration cannot be drawn, as very few studies have included sufficient parameters to comprehensively characterise skeletal muscle morphology at macro- and microscopic level in MA [17-19], or have not included a young control group for comparison [16,20]. The few studies that have investigated aspects of MA physiology and muscle morphology report conflicting findings, which may relate to different analytical methods or the specific muscle studied. Specifically, some have demonstrated superior leg strength and muscle fibre diameter in senior sportsmen compared with untrained older individuals [18], whereas others have shown similar muscle size, strength and fibre CSA between MA and untrained older individuals [17,19].

Therefore, to better understand the role of inherent ageing processes in physiological function, and the extent to which chronic exercise training might improve the healthspan, the aim of the current study was to conduct the most comprehensive comparison, to date, of physical function, cardiorespiratory fitness, body composition, muscle strength, architecture and fibre-type morphology between MA and healthy untrained younger and age-matched older individuals. We hypothesised that untrained older individuals would exhibit an impairment in all of the above parameters compared with younger individuals, which would be partially or, in some cases, completely absent in MA.

METHODS

Participants

Fifteen untrained young men (YC) and 12 older untrained men (OC) were recruited alongside 14 Master endurance trained men (MA) through local advertisements, the British Masters Athletics Federation and the League of Veteran Racing Cyclists. Young (18-35 years) and

older untrained controls (60-80 years) were deemed eligible for study participation only if they maintained habitual activity and had not previously participated in any form of structured exercise training outside of recreational activities. MA (60-80 years) were included only if they had maintained continuous endurance training at least twice per week for ≥20 years preceding the study. Exclusion criteria included regular consumption of analgesic or anti-inflammatory drug(s), prescription or non-prescription, that may affect muscle metabolism (e.g. Beta-blockers, corticosteroids, non-steroidal anti-inflammatories). Participant anthropometric and training characteristics are detailed in Table 1. All participants were informed of the purpose and methodology of the study, were deemed healthy by completion of a general health questionnaire assessment, and provided their written informed consent. Ethical approval was obtained through the West Midlands - Solihull Research Ethics Committee (16/WM/0167). The study conformed to the standards set by the Declaration of Helsinki (7_{th} version).

Study design

In a parallel study design, YC, OC and MA were recruited to investigate the effect that ageing and continuous endurance exercise exert on indices of muscle mass, function and morphology. Following initial study screening and consenting, participants reported to the School of Sport, Exercise and Rehabilitation Sciences (SportExR) laboratory on two separate occasions with each visit separated by ~7 days. For each visit participants reported to SportExR in an overnight fasted-state, having refrained from strenuous physical activity and alcohol for at least 48 h previously, and from caffeine consumption on the day of the trial. During the initial visit, participants underwent assessments of body composition, aerobic capacity, maximal limb strength and a battery of functional tests. Approximately 1 week

later, participants underwent ultrasound scanning, a single venous blood sample and a single muscle biopsy.

Visit 1

Body mass, height and composition: Body mass was determined by weighing each participant in loose clothing, without shoes, to the nearest 0.1kg using a digital balance scale (Esca 813, Hamburg, Germany). Height measurements were made to the nearest 0.1cm using a stadiometer (Seca 217, Hamburg, Germany). Participants underwent a dual-energy x-ray absorptiometry (DXA) scan (Discovery DXA Systems, Hologic Inc., Bedford, MA) to determine whole-body and regional fat- and fat-free mass. Skeletal muscle index was calculated as fat-free mass as a percentage of whole-body mass.

Blood pressure: Blood pressure was measured using a standard fully automatic blood pressure monitor (OMRON M2, OMRON Healthcare UK Ltd., UK). Participants were asked to remove any clothing that obstructed the blood pressure cuff. Participants were seated with their legs uncrossed and back supported, encouraged to relax and refrain from talking during the assessment. This test was repeated 3 times and the lowest of 3 readings taken. Following blood pressure assessment, participants underwent the Ekblom-Bak submaximal cycle ergometry test for the estimation of $\dot{V}O_{2max}$ [21], the details of which are described in the supplemental materials.

Aerobic capacity: Participants underwent the Ekblom-Bak test [21]; a submaximal cycle ergometry test for the estimation of $\dot{V}O_{2max}$. Briefly, the test is based on the change in heart rate between a standardised low workload and a higher workload predetermined by sex and current habitual activity. Participants were instructed to maintain a constant cadence of 60 rpm throughout the test. First, the participant cycled for 4 min, while investigators ensured constant cadence and resistance. Heart rate was recorded every 15 s and averaged during the

final minute of the low workload. The Electrocardiogram (ECG) was monitored over 5 min prior-to and continuously throughout exercise by a cardiologist. The resistance was then increased to the predetermined level for the next 4 min, whilst cadence and resistance remained constant. During the second minute at the higher workload, individuals communicated their rate perceived exertion (RPE) using a standard scale [22]. If RPE was <10 the resistance was increased by 1kp and the second workload timings started again. If RPE was 10-11, the workload was increased by 0.5kp and the second workload timings started again. If RPE was 12-16 participants were instructed to maintain this workload. If RPE >17 the exercise bout was stopped. Heart rate was recorded every 15 s and averaged during the final minute of the higher workload. $\dot{V}O_{2max}$ was calculated using the following equation [21]:

$$\dot{V}O_{2max} = 4.98196 - 2.88618\left(\frac{\Delta HR}{\Delta PO}\right) + 0.65015(Sex) - 0.01712(Age)$$

Where ΔHR is the difference in the average heart rate between the two workloads, ΔPO is a constant reflecting the difference in power output between the two workloads, where sex is 0=woman and 1=man, and age is participants age in years as an integer.

Short physical performance battery (SPPB): Participants completed the SPPB of balance, gait speed and low limb power [23] The balance test comprised three increasingly difficult stances (side-by-side, semi-tandem, full tandem), which participants were required to hold for 10 sec. For the gait speed test, participants were instructed to walk a 3-metre-long course 'at your usual speed as if walking to the shops'. Finally, participants completed a repeated chair stand test, in which they were instructed to rise from a chair, with arms folded, five times as quickly as possible. The result from each of the three tests were scored out of 4 marks and compared against predetermined criteria for a score of physical function.

Handgrip and leg strength: Individuals stood, feet side-by-side, with their arms adducted, wrists neutrally rotated and the dynamometer (Jamar Hydraulic, Patterson Medical, UK) positioned comfortably in the participant's self-reported dominant hand. Participants were

instructed to squeeze the handle as hard as possible and three attempts were recorded, with the highest value recorded. Maximal knee extension and elbow flexion isometric strength were measured using a KinCom Dynamometer (KinCom 125AP, KinCom, USA) on the self-reported dominant limb. Participants were seated, with the tested limb, chest and hips stabilised and with the knee and elbow angle positioned at 70 degrees of flexion, respectively. After familiarisation, participants performed 3 maximal voluntary contractions, the greatest of which was recorded.

Dietary analysis: Between visits 1 and 2, participants were provided with a 4-day weighed food diary designed to capture habitual food intake on 2 consecutive weekdays and 2 weekend days. Participants were instructed not to change their usual diet and to be as accurate as possible when describing the food (cooking method, brand, amount etc.). Dietary intake was analysed using MyFitnessPal software (MyFitnessPal Inc.).

Physical Activity: Between visits 1 and 2 (~7 days), participants were provided with a wrist-worn accelerometer (GENEActiv, ActivInsights Ltd., UK) designed to capture habitual activity in free-living conditions over 5 consecutive days (including both weekend days). Accelerometers were initialized to sample data at a 10 Hz. Data were converted into 60 second epochs and analysed using the GENEActiv software (version 2.2, ActivInsights). Activities were split into 4 categories based on metabolic equivalent (MET) values; i) sedentary activity (<1.5 METs) ii) light activity (1.5 – 3.99 METs) iii) moderate activity (4.0 – 6.99 METs) and iv) vigorous activity (>7 METs) [24].

Visit 2

Muscle architecture: B-mode ultrasonography with a linear array probe was used for all measurements of muscle architecture. Participants lay fully relaxed in a supine position with a small towel rolled and placed under the knee for anterior measurements. For posterior

measurements, participants were in a fully relaxed prone position with feet overhanging the end of the bed. Biceps Brachii architecture was measured with the participants seated on the edge of the bed with the arm freely hanging. The muscles measured included; *Vastus Lateralis, Vastus Intermedius, Rectus Femoris, Biceps Brachii, Gastrocnemius Medialis, and Tibialis Anterior*. Measurements of muscle thickness (MT), pennation angle (θ) and fascicle length (L_f) were made in triplicate. MT was considered to be the distance between deep and superficial aponeuroses. θ was calculated as the angle between the muscle fascicle and the deep aponeurosis. Fascicle length was measured as the length of the fascicular path between the 2 aponeuroses. Measurement sites were set at 60% of the upper arm length distal to the acromion process for Biceps Brachii, 50% of the muscle length for upper thigh muscles, and 30% of the leg length distal to the popliteal crease for lower leg muscles. The ultrasound probe was covered with water-soluble transmission gel, to provide acoustic contact without compressing the dermal surface, and an image was taken when a number of fascicles could clearly be identified.

Blood sample: A blood sample was obtained via venepuncture from an antecubital forearm vein. Blood was collected in separate ethylenediaminetetraacetic acid (EDTA) and serum-separating polymer gel containing BD vacutainers (BD, Oxford, UK). Blood samples were centrifuged at 3000g for 10 min at 4°C, and serum and plasma aliquots were frozen at −80°C for later analysis.

Muscle Biopsy: A muscle biopsy sample was obtained from the quadriceps *vastus lateralis* under local anaesthesia (1% lidocaine) using the Bergström needle technique [25]. Muscle biopsy tissue was quickly rinsed in ice-cold saline and blotted to remove any visible fat and connective tissue before being frozen in liquid nitrogen or placed in a pipette tip with optimum cutting temperature compound (Tissue-Tek® (O.C.T.) Compound, Sakura®

Finetek) and frozen in liquid nitrogen-cooled isopentane and stored at −80°C for later analysis.

Sample Analysis

Blood samples: Plasma glucose was analysed using a commercially available blood glucose analyser (HemoCue® Hb 201+ System, HemoCue AB, Sweden). Serum insulin and C-reactive protein (CRP) were analysed using commercially available enzyme-linked immunosorbent assay (ELISA) kits (IBL International GmbH, Hamburg, Germany) following the manufacturer instructions. Postabsorptive insulin sensitivity was also estimated using the homeostasis model of insulin resistance index (HOMA-IR) [26].

Immunohistochemistry:

Detailed information on primary and secondary antibodies for immunohistochemistry is presented in Supplemental Table 1. For analysis of myonuclear domain, SC content and myofibre capilliarisation muscle cross sections were stained as follows. Briefly, tissue sections were fixed in 4% paraformaldehyde (PFA) for 10 min, washed 3 x 5 min in PBST, blocked for 60 min at RT (in PBS containing 2% bovine serum albumin, 5% FBS, 0.2% Triton x-100, 0.1% NaAzide, and 2% goat serum) then incubated overnight in primary antibodies specific for Pax7 and CD31at 4°C. Following washes, sections were then incubated in appropriate secondary antibodies for 2 hours. Following washes sections were then re-blocked in 10% goat serum in PBS and incubated in a primary antibody cocktail consisting of MHC I (A.4.951; slow isoform) and laminin. Sections were washed then incubated in a secondary antibody cocktail, nuclei were labelled with DAPI (49,6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada) prior to cover slipping with DAKO fluorescent mounting media (Burlington, ON, Can). Slides were visualised with the Nikon Eclipse Ti Microscope (Nikon Instruments, Inc., Melville, NY, USA), equipped

with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments). Images were captures and analysed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments). All images were obtained on the x20 objective.

i) Muscle Fibre Type and Cross-sectional area: This analysis was carried out on a subset of participants (YC; *n*=13: MA; *n*=14: OC; *n*=11). Serial 10µm sections of skeletal muscle biopsies were cut in the cryostat at -20°C. Briefly, slides were washed in triton x100 (0.02 %) to permeabilise the fibres. Slides were blocked for 90 mins in 5 % normal goat serum (Invitrogen, UK) and then incubated overnight in the primary antibody cocktail; myosin heavy chain (MHC) I, BAF8 (Developmental Studies Hybridoma Bank (DSHB, USA); MHC II, SC-71 (DSHB, USA); Laminin in phosphate buffered saline (PBS). Slides were washed in 3 x 5 min in PBST and incubated in the secondary antibody cocktail. Slides were then washed and mounted with coverslips in prolong gold anti-fade mountant (Thermo Fisher Scientific, Paisley, UK). Prepared slides were observed under a Nikon E600 microscope using a 20 × 0.75 numerical aperture objective. Images per area were captured under two colour filters achieved by a SPOT RT KE colour three-shot CCD camera (Diagnostic Instruments Inc., MI, USA), illuminated by a 170 W Xenon light source. Texas red (540–580 nm) excitation filter was used to capture MHC I images and FITC (465– 495 nm) excitation filter was used to capture MHC II and Laminin. Microscope slides were prepared such that each slide contained two serial sections from one individual from each of the included groups. Images were captured, and measured, such that ~ 600-1000 muscle fibres were included for analysis from each group. All viable muscle fibres in any particular image, excluding those displaying freeze fracture artefact and any longitudinal fibres (assessed as those with a circularity of <0.6), were included for analysis and were analysed using Image J Fiji [27].

Calculations and Measurements:

i) *Myonuclear domain*: This analysis was carried out on a subset of participants (YC; *n*=10: MA; *n*=12: OC; *n*=11). From acquired images specific channels were extracted so that only MHC I, laminin and DAPI were visualised. Myonuclear domain was determined as the fibre area of MCH I and MHC II fibres (μm^2) per nucleus. Only nuclei that were located within the basal lamina as identified by laminin were counted. Myonuclear content was determined by counting the number of nuclei residing within the basal lamina of either Type I (MHC I positive) or Type II (MHC I negative) fibres expressed in relation to total MHC I and MHC II fibres.

ii) *Myofibre capillary analysis*: This analysis was carried out on a subset of participants (YC; *n*=10: MA; *n*=11: OC; *n*=11). From acquired images specific channels were extracted so that only MHCI, laminin and CD31 were visualised. Quantification of (i) capillary contacts (CC, the number of capillaries around a fibre), (ii) the capillary-to-fibre ratio on an individual fibre basis (C/Fi), (iii) the number of fibres sharing each capillary (i.e., the sharing factor), and (iv) the capillary-to-fibre perimeter exchange index (CFPE index, calculated as the C:Fi/P, where P is the fibre perimeter) was based on previous published protocols [28,29].

iii) *Myofibre satellite cell analysis*: This analysis was carried out on a subset of participants (YC; *n*=10: MA; *n*=11: OC; *n*=10). From acquired images specific channels were extracted so that only MHCI, laminin, Pax7 and DAPI were visualised. Fibre type specific SC content was determined by identifying nuclei co-localised with Pax7 that resided within the basal lamina.

Statistical Analysis: Data were expressed as mean ± standard deviation (SD). Normality of data was assessed using the Shapiro-Wilk test. Between group differences in anthropometric characteristics, physical performance, dietary intake and habitual activity levels were identified via one-way ANOVA analysis with one within-group factor (e.g. fat-free mass)

and three groups for comparison. Muscle morphological data were analysed via mixed-design ANOVA with one between factor (three levels; group) and one within factor (two levels; fibre type). Specifically, the average of each participant's data was incorporated into the grouped analyses. For example, when analysing fibre-specific CSA ~100 fibres were measured for each individual, following which, the average fibre-CSA for each individual was incorporated into the grouped analysis. Significant main effects indicated group differences with fibre-types collapsed (i.e., MA vs. OC vs. YC for MHC I and II combined), or fibre-type differences with groups collapsed (i.e., MHC I vs. MHC II for MA, OC, and YC combined). Significant interaction effects indicated that age and/or training status (group; YC, MA, OC) influenced a given fibre-type parameter (i.e. MHC I CSA). Whenever a significant F ratio was found (i.e. variation between sample means), interaction or main effects were followed up with pair-wise comparisons using independent t-tests to locate specific differences, with the Bonferroni correction applied to account for multiple comparisons. Significance level was set at $P < 0.05$ for all analyses. All statistical analyses were performed using SPSS version 22.0 (Chicago, IL).

RESULTS

Participant Characteristics: Participant anthropometric and training characteristics are detailed in Table 1. YC were taller than MA (P=0.031). MA had lower whole-body fat mass (P=0.011), a lower body fat percentage (P=0.002) and a greater skeletal muscle index (P=0.001) than OC. YC had significantly greater appendicular lean mass than OC (P=0.03). YC and MA had significantly lower fasting glucose concentrations than OC (P=0.005 and P=0.009, respectively). MA had significantly lower serum insulin (P=0.048 and P=0.041) and HOMA-IR (P=0.03 and P=0.003) than YC and OC, respectively. There were no other significant differences in anthropometric characteristics between the groups.

Physical Function: Physical function characteristics are detailed in Table 2. There were no significant differences between groups for any of the SPPB component nor total scores or handgrip strength. YC produced significantly greater knee extension MVC than MA and OC (P=0.006 and P<0.001, respectively), although no difference was apparent when normalized to leg fat-free mass (FFM) indicating no between-group difference in relative force production. YC produced significantly greater elbow flexion than MA (P<0.001) that was apparent when normalized to arm FFM (P<0.05), indicating lower relative force production in MA vs. YC. YC and MA had a significantly greater estimated $\dot{V}O_{2max}$ than OC (P<0.001).

Muscle architecture: Muscle architecture results are detailed in Supplementary Table 2. YC had significantly greater MT than OC for *Vastus lateralis* (P<0.001), *Vastus intermedius* (P=0.002), *Rectus femoris* (P<0.001) and greater MT than MA for *Rectus femoris* (P<0.001). YC had significantly greater θ than OC for *Vastus lateralis* (P=0.007), *Vastus intermedius* (P=0.014), *Rectus femoris* (P=0.002) and significantly greater θ than MA for *Rectus femoris* (P=0.04). MA had significantly greater L_f than OC for *Gastrocnemius medialis* (P=0.032) and YC had significantly greater L_f than OC (P=0.024) and MA (P=0.041) for *Gastrocnemius lateralis*.

Habitual Dietary Intake and Physical Activity: Dietary intake results are detailed in Supplementary Table 3 (upper). YC total energy (P=0.014), fat (P=0.019), protein (P<0.001), relative fat (P=0.044) and relative protein intake (P=0.005) were greater than OC. Whereas, MA total energy (P=0.003), carbohydrate (P=0.014), relative carbohydrate (P=0.008), relative fat (P<0.007) and relative protein intake (P=0.029) were greater than OC. No differences in dietary intake were observed between YC and MA. Habitual activity can be found in Supplementary Table 3 (lower). MA carried out significantly more vigorous activity (49 ± 29 mins·day^{-1} or 5.6 ± 3.2%·day^{-1}) than both YC (13 ± 14 mins·day^{-1}, 1.7 ± 1.5%·day^{-1};

P<0.001) and OC (4 ± 6 mins·day^{-1}, 1.1 ± 2.6% day^{-1}; P<0.001). There were no other differences in physical activity between groups.

Muscle fibre properties: Muscle fibre data are detailed in Figure 1 A-E. A significant interaction effect of group*fibre type CSA was observed (P<0.001). MA displayed larger MHC I fibre CSA than OC (P=0.031), whereas YC had significantly larger MHC II fibre CSA than OC (P=0.008). A significant interaction effect of group*fibre type distribution was observed (P<0.001). MA (57 ± 15 %) had a significantly greater proportion of MHC I muscle fibres compared with YC (35 ± 10 %, P<0.001) and OC (41 ±10 %, P=0.008). MA (43 ± 15 %) had a significantly lower proportion of MHC II fibres compared with YC (65 ± 10 %, P<0.001) and OC (58 ± 10%, P=0.012).

Myonuclear domain: Muscle fibre data are detailed in Figure 2 A-E. A significant interaction effect of group*fibre type myonuclear domain was observed (P=0.04). No significant difference was observed between any of the groups for MHC I fibre myonuclear domain. YC had significantly greater MHC II fibre specific myonuclear domain than MA (P=0.018) and OC (P=0.006). No significant interaction effect for group*fibre type myonuclei content was observed.

Capillarisation and satellite cells: Capillarisation and SC data are detailed in Figure 1 A-G and Supplementary Figure 1 A-C, respectively. A significant interaction effect of group*fibre type capillary contacts (CC) was observed (P=0.007). MA had significantly more CC per MHC I myofibre compared with YC and OC (P=0.002 and P<0.001, respectively). MA had more CC per MHC II fibre compared with OC (P=0.006). A significant interaction effect of group*fibre type capillary-fibre ratio (C/Fi) was observed (P=0.007). MHC I C/Fi was greater in MA compared with YC and OC (P=0.001 and P=0.001, respectively). MA had a greater MHC II C/Fi than OC (P=0.004). No significant interaction effect of group*fibre type capillary to fibre perimeter exchange index (CFPE) was observed. However, CFPE showed a

significant main effect for group, such that MA had a significantly greater CFPE compared with YC (P=0.001) an OC (P=0.001). No significant interaction (group*fibre type) or main effects (group and fibre type alone) were observed for SC content.

DISCUSSION

Ageing has a deleterious effect on physiological function, muscle mass, and strength (sarcopenia) and muscle oxidative capacity that may be exacerbated by low physical activity or adverse change in body composition. Individuals who have chronically undertaken structured exercise training, or Master Athletes (MA), provide an opportunity to study mechanisms of inherent physiological ageing dissociated from aspects of biological ageing [15]. Previously, we have highlighted the dearth of studies providing a comprehensive characterisation of the MA phenotype against untrained young and older individuals [14]. Herein, we comprehensively address this issue by firstly demonstrating that endurance-trained MA display superior body composition (lower body fat and higher skeletal muscle mass index) and aerobic fitness compared with untrained age-matched older individuals (OC), to a level comparable with untrained healthy young individuals (YC). Secondly, we provide novel morphological insights demonstrating that MA display larger MHC I muscle fibre area than OC. Furthermore, MA display greater capilliarisation of MHC I and II fibres compared with OC and YC. MHC II fibre myonuclear domain size was greater in YC compared with OC and MA, whereas SC content was similar between groups. Collectively, these data suggest that chronic endurance training promotes superior aerobic capacity, body composition and fibre capilliarisation, with potential implications for healthspan extension.

Sarcopenia is often accompanied, and in some cases masked, by increased adiposity [30] and ectopic and visceral fat deposition [11,31]. Reduced muscle mass and increased body fat are independently and concomitantly associated with increased risk of metabolic disease [32],

frailty [33], and mortality [34]. Thus, the absence of sufficient physical activity in the face of surplus energy intake in older age, increases the likelihood of unfavourable changes in body composition (i.e., reduced muscle mass and increased body fat). Furthermore, periods of inactivity and disuse impair muscle protein anabolism and accelerate the progression of sarcopenia [35]. Our data demonstrate that highly active MA had a greater skeletal muscle index, lower body fat and lower whole-body insulin resistance than OC. Thus, continuation of structured endurance exercise training promotes a favourable body composition and could confer important metabolic health benefits.

Age-related muscle mass and strength loss involves alterations in architectural properties and fibre-type morphology. With ageing, muscle fibre fascicle length and pennation angle decrease, indicating a loss of sarcomeres (in series and parallel)[36]. To the best of our knowledge, we are the first to measure muscle architecture in an endurance trained MA cohort, for comparison against YC and OC. Our novel findings demonstrate that muscle fibre fascicle length and pennation angle properties were generally lower in OC and MA, compared with YC, with no differences between MA and OC. Similarly, others have reported equivalent *vastus lateralis* fascicle length between young and old sprint athletes [37]. Important to note however, was that fibre pennation angle and fascicle length of the *vastus intermedius* and *gastrocnemius medialis,* respectively, were statistically greater in YC compared with OC only, with no difference between OC and MA. These data indicate that the alterations to muscle architectural properties with advancing age are not preserved with chronic endurance exercise training. The observed architectural modifications in MA and OC may attenuate the decreased force production with age-related muscle loss. Indeed, isometric knee extensor strength was similar between groups when normalized to leg fat-free mass. Although normalized isometric biceps strength was lower in MA compared with YC, technical limitations prevented us from understanding whether alterations to the architectural

properties of this muscle could explain this deficit. Nonetheless, it is apparent that contractile loading in the form of resistance training may be required to generate sufficient mechanical tension to enhance muscle architectural properties and mitigate strength declines in older individuals [14,38].

Reductions in muscle fibre number and area, particularly MHC II fibres, is a characteristic of sarcopenia [8]. In older individuals, resistance training increases MHC II fibre area, whereas endurance exercise training primarily augments MHC I fibre area [14]. Skeletal muscle morphology data in MA are scarce, and available studies have yielded conflicting findings [14,17,18], making it difficult to fully elucidate the mechanisms through which chronic exercise enhances physiological function. In line with previous findings [18,39], we demonstrate that endurance trained MA have larger MHC I fibres than OC, and a greater proportion of MHC I fibres compared with YC and OC. Compared with OC, the superior MHC I fibre properties of MA suggests that their greater maximal aerobic capacity may be partly underpinned by a greater muscle oxidative potential, although cardiorespiratory adaptations undoubtedly also play a key role in VO_{2max}. Unsurprisingly, MHC II fibre size was lower in OC compared with YC. However, chronic endurance exercise did not preserve MHC II fibre size, which was similar between MA and OC. Due to technical limitations, we were only able to analyse two fibre types. Given evidence that ageing leads to a reduction in MHC IIA fibres, an increased proportion of fibres co-expressing different MHC isoforms and that physical activity can offset these alterations [40], future investigations should seek to determine more specific and hybrid fibre-types. Nevertheless, the current data suggest that chronic endurance exercise elicits a mode-specific remodelling of muscle fibres, such that MHC I fibre area and/or abundance is greater than OC and YC.

Skeletal muscle SC, as the main source of new myonuclei, play an important role in the repair and regeneration of skeletal muscle [41]. Given that age-related MHC II fibre atrophy

is accompanied by a reduction in MHC II SC, and that SC content is a strong predictor of muscle fibre size in older individuals [42,43], reductions in SC may impair the capacity for muscle maintenance in old age. As such, we were surprised to find no age-related difference in fibre-specific SC content between YC and OC. However, the absence of any effect of chronic endurance exercise on SC content (per fibre) is consistent with the work of Mackey and colleagues [17] and aligned to evidence that the SC pool remains constant in response to endurance-based training programmes in humans [44]. The absence of any difference in fibre-specific SC within or between groups, could be due to a large within-group variability in the sub-set of samples available for analysis. In addition to SC content, SC activity is critical for muscle repair and regeneration with exercise-induced damage. SC activity is reportedly delayed in older individuals [45], increases with endurance training [44] and could therefore be maintained in MA and warrants further investigation. The area of the cell governed by each myonucleus (myonuclear domain) has been reported to diminish in older age [42]. Our findings provide support for an age-related reduction in myonuclear domain in MHC II, but not MHC I, fibres. Interestingly, the age-related reduction in fibre-type myonuclear domain size was not offset in MA, analogous to findings from the only other investigation of myonuclei in chronically endurance trained MA [17]. Taken together, these data indicate that larger MHC I fibre area in MA vs. OC, occurs independently of alterations in SC content or myonuclear domain, which appear relatively constant with ageing and chronic endurance training. Further to this, the age-related diminution of MHC II fibre myonuclear domain in MA and OC appears to precede any change in SC content.

Impairments in muscle perfusion and the delivery of oxygen and nutrients to skeletal muscle from nearby capillaries, may impair muscle oxidative capacity and contribute to the development of impaired muscle anabolism and sarcopenia. Indeed, capillary density is reduced in older age, particularly in MHC II fibres [46], thereby impairing muscle fibre

perfusion. In older muscle, the capillary-to-fibre ratio and the capillary-to-fibre perimeter exchange index is reduced, and the distance between satellite cells and the nearest capillary is greater in MHC II fibres compared with younger individuals [9]. This may have consequences for the responsiveness of SC to facilitate skeletal muscle repair and remodelling processes in response to contraction-induced muscle damage. In contrast to the findings of Nederveen et al. [9], we did not observe any age-related reduction of indices of capilliarisation, which may be reflective of the greater aerobic capacity of our OC cohort (~10.0 $mL \cdot kg^{-1} \cdot min^{-1}$ greater than Nederveen et al). Nevertheless, it is clear from our results that chronic endurance exercise training leads to greater MHC I and II fibre capilliarisation in MA compared with OC and, in some cases, YC. These findings are consistent with recent evidence that the continuation of endurance training into older age maintains skeletal muscle capilliarisation [47]. However, recent evidence from a cross-sectional analysis of highly trained MA (55-79 y) suggests that chronic endurance training does not completely prevent the decline in capillary density [12]. Nevertheless, the greater muscle fibre capilliarisation and MHC I fibre area observed in our cohort of endurance-trained MA may, at least partly, explain their superior aerobic fitness levels.

Whilst our findings clearly demonstrate superior indices of physiological function and muscle morphology in MA compared with OC, we are unable to determine whether chronic endurance exercise offsets the trajectory of age-related physiological deterioration. The cross-sectional nature of this study, and absence of a young endurance trained cohort precludes us from determining how chronic exercise influences the rate of physiological decline. However, evidence suggests that MA experience a similar, or greater rate of physiological and functional deterioration compared with their untrained age-matched counterparts [15,16], but the 'set-point' from which this deterioration begins is delayed (or postponed) in MA [15]. Although chronic exercise training may lower the risk of disease and

disability in later life [48], the apparent shift to a 'slow' muscle phenotype (and reduction in MHC II fibre proportion) that we and others have observed in endurance-trained MA could, paradoxically, be viewed as a hallmark of aggravated sarcopenia [49]. However, others have recently reported that long-term athletic training (strength or endurance) facilitates more successful reinnervation of denervated muscle fibres [50], which would strongly influence final MHC expression. Thus, it would be prudent to examine whether the observed differences in physiological function, strength and muscle morphology between endurance-trained MA and OC, are further modified in very old age (i.e. >80 y). Ultimately, the inclusion of resistance-type exercise, may help to preserve strength and physical function in endurance-trained MA [39]. To date, studies of physiological function and muscle morphology in strength/power trained MA are scarce.

In conclusion, our findings demonstrate that endurance-trained MA with an average of ~37 years of training experience display superior aerobic capacity, body composition and indices of muscle fibre morphology compared with age-matched untrained older individuals. Irrespective of whether chronic endurance exercise offsets the rate of age-related physiological deterioration, or simply delays the point from which this decline begins, the novel insights shown here shed light on the extent to which chronic endurance training supports physical function and skeletal muscle health in older age, with implications for healthspan extension. Thus, the promotion of regular physical activity across the life-course, that incorporates endurance and strength training, may offer the greatest level of protection against the decline in physiological health and function with advancing age.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to disclose.

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AUTHOR CONTRIBUTIONS

All authors gave their final approval of the version of the article to be published. JM, CG and LB designed the study. JM and LB organized and carried out the experiments with the assistance of BL and SB. JM, SJ, GP and LB performed the data analyses. JM, SJ and LB performed the statistical analysis of the data. JM, CG and LB wrote the manuscript. JM, CG and LB are the guarantors of this work and take responsibility for the integrity and accuracy of the data analysis.

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TABLES

Table 1: Participant anthropometric and training characteristics

	YC (N=15)	MA (N=14)	OC (N=12)
Age (years)	20.0 ± 2.7**##	67.1 ± 6.4	69.8 ± 4.1
Height (m)	1.80 ± 0.04*	1.70 ± 0.06	1.80 ± 0.07
Body mass (kg)	78.9 ± 13.3	68.7 ± 6.6	77.5 ± 14.2
BMI ($kg \cdot m^{-2}$)	24.6 ± 3.6	23.0 ± 2.0	24.5 ± 3.8
Whole-body FFM (kg)	56.9 ± 6.6	52.2 ± 3.5	52.9 ± 7.8
Whole-body FM (kg)	17.6 ± 7.4	13.3 ± 3.9#	20.9 ± 7.1
Body fat (%)	22.0 ± 5.5	19.2 ± 4.1##	26.8 ± 5.4
Skeletal Muscle Index (%)	74.0 ± 5.2	77.0 ± 4.0##	69.8 ± 5.1
ALM/$Height^2$ ($kg.m^{-2}$)	8.18 ± 0.79#	7.71 ± 0.54	7.39 ± 0.95
Systolic Blood Pressure (mmHg)	126 ± 9	125 ± 7	137 ± 18
Diastolic Blood Pressure (mmHg)	64 ± 8	76 ± 7	83 ± 11
Fasting Plasma Glucose ($mmol \cdot L^{-1}$)	5.33 ± 0.39##	5.35 ± 0.63##	5.96 ± 0.35
Fasting Serum Insulin (μIU/mL)	10.95 ± 3.24*	7.21 ± 3.10	11.42 ± 4.38*
HOMA-IR	2.59 ± 0.75**	1.65 ± 0.61	3.05 ± 1.16**
CRP ($mg \cdot L^{-1}$)	1.71 ± 1.89	0.77 ± 0.43	1.10 ± 0.53
Training Experience (years)	-	36.5 ± 8.1	-
Training Frequency ($sessions \cdot week^{-1}$)	-	4.5 ± 1.4	-
Training Duration ($hrs \cdot week^{-1}$)	-	7.6 ± 4.7	-
Training Distance ($km \cdot week^{-1}$)	-	210±12 / 50 ±15	-

Data presented as mean ± SD. FFM; fat-free mass, FM; fat mass, ALM; appendicular lean mass, HOMA-IR; Homeostatic Model Assessment of Insulin Resistance, CRP; C-reactive protein. Training Distance is separated into cyclists (*n*=8, left) and runners (*n*=6, right). * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Significantly different from OC, P<0.05. ## Significantly different from OC, P<0.01.

Table 2: Physical function and strength

	YC (N=15)	MA (N=14)	OC (N=12)
SPPB Standing Balance (s)	10.0 ± 0	10.0 ± 0	10.0 ± 0
SPPB Semi-Tandem (s)	10.0 ± 0	10.0 ± 0	10.0 ± 0
SPPB Full Tandem (s)	10.0 ± 0	9.7 ± 1.0	9.7 ± 1.1
SPPB 3m Walk (s)	2.37 ± 0.34	2.43 ± 0.28	2.45 ± 0.44
SPPB 5x Sit-to-stand (s)	7.27 ± 1.34	7.97 ± 1.88	7.88 ± 2.03
Handgrip Strength (kg)	50.9 ± 7.6	47.0 ± 5.9	46.3 ± 8.4
Knee Extension MVC (Nm)	638 ± 82**##	520 ± 105	471 ± 118
Knee Extension MVC (Nm·kg^{-1} leg FFM)	68 ± 9	62 ± 13	57 ± 13
Elbow Flexion MVC (Nm)	293 ± 49**	223 ± 43	257 ± 49
Elbow Flexion MVC (Nm·kg^{-1} arm FFM)	79 ± 9*	68 ± 12	77 ± 10
Estimated $\dot{V}O_{2max}$ (ml·kg^{-1}·min^{-1})	53.2 ± 7.3##	49.3 ± 3.6##	36.7 ± 6.5
Estimated $\dot{V}O_{2max}$ (ml·kg^{-1} FFM^{-1}·min^{-1})	72.7 ± 5.8##**	65.2 ± 3.4##	53.3 ± 8.7

Data presented as mean ± SD. * Indicates significantly different from MA, P<0.05. ** Indicates significantly different from MA, P<0.01. # Indicates significantly different from OC, P<0.05. ## Indicates significantly different from OC, P<0.01. SPPB; short physical performance battery. MVC; maximal voluntary contraction. Nm; Newton metres. FFM; fat-free mass.

Figure 1: Cross-sectional area (CSA) of MHC I (A) and MHC II muscle fibres (B) and relative abundance of MHC I (C) and MHC II muscle fibres (D) in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistochemical image of muscle fibre CSA from a biopsy sample in YC (left), MA (centre) and OC (right) with MHC I fibre MHC in red, MHC II fibre MHC in green and laminin stained cell membrane in green (E). Significance was set at $P < 0.05$. # indicates significantly different from OC ($P < 0.05$) and * indicates significantly different from MA ($P < 0.05$). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).

Figure 2: Myonuclear domain in MHC I (A) and MHC II muscle fibres (B) and number of nuclei per MHC I (C) and MHC II muscle fibre (D) in young untrained individuals (YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistochemical image of fibre-type myonuclei from a biopsy sample in YC (left), MA (centre) and OC (right) with DAPI stained nuclei in blue, MHC I fibre MHC in green and laminin stained cell membrane in green (E). Significance was set at $P < 0.05$. # indicates significantly different from OC (# $P < 0.05$ and ## $P < 0.01$), * indicates significantly different from MA ($P < 0.05$). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).

Figure 3: Capillary contacts per fibre (CC), individual capillary to fibre ratio (Cfi) and capillary to fibre perimeter exchange index (CFPE) in MHC I fibres (A, C and E, respectively) and MHC II fibres (B, D and F, respectively), in young untrained individuals

(YC), old endurance trained Master Athletes (MA) old untrained individuals (OC). Representative immunohistochemical image of fibre-type capilliarization from a biopsy sample in YC (top), MA (middle) and OC (bottom) with CD31 stained capillaries in purple (denoted by white arrows), MHC I fibre stained in green and laminin stained cell membrane in green (G). Significance was set at $P < 0.05$. ## indicates significantly different from OC ($P < 0.01$), †† indicates significantly different from YC ($P < 0.01$). Values are presented as the median (central horizontal line), 25th and 75th percentiles (box), minimum and maximum values (vertical lines) and mean (cross).