ORIGINAL ARTICLE

Mitochondrial respiratory chain deficiency in older men and its relationship with muscle mass and performance

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Abstract

Introduction Sarcopenia is the loss of muscle mass and physical performance with age, and recognition of its importance in clinical practice is growing. Age-related decline in muscle mitochondrial function has been described although less is known about the role of mitochondrial dysfunction in sarcopenia. The aim of this study was to investigate whether respiratory chain deficiency is associated with muscle mass and physical performance among a sample of healthy older men participating in the Hertfordshire Sarcopenia Study.

Methods We used immunofluorescence on biopsies of the vastus lateralis to measure levels of the NDUFB8 subunit of complex I and the COX-1 subunit of complex IV per fibre. We measured muscle mass using dual-energy x-ray absorptiometry. We assessed physical performance using grip strength, gait speed, chair rise time, timed up and go and standing balance time, and composed an aggregate performance score on the scale of 0 (worst) and 5 (best performance). We used linear regression with a cluster sandwich estimator to test relationships between complex I / IV and muscle mass / physical performance. Study approval was granted by the Hertfordshire Research Ethics Committee.

Results Samples were available from 77 participants of mean age 72.6 (2.5) years. The median number of fibres analysed per participant was 157 (104, 237). We expressed complex I and IV levels as Z-scores relative to that expected in young controls. The overall participant mean Z-scores were 0.3 (1.3) and -1.5 (0.9) for complex I and IV, respectively. We saw no relationship between complex I or IV and muscle mass. Each unit (SD) increase in complex I was associated with an increase in aggregate performance score of 0.06 (95% CI: 0.02, 0.09, P = 0.003), whilst the relationship for complex IV did not reach significance.

Conclusion We saw marked heterogeneity in complex I and IV levels, both between and within participants, as well as lower overall levels of complex IV. The finding of a small but statistically significant positive association between complex I levels and physical performance suggests that mitochondrial dysfunction may have a role in the development of sarcopenia. These findings will help inform the design of future studies across a wider range of ages and in both women and men.

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Abbreviations

ATP	Adenosine triphosphate
COX	Cytochrome c oxidase
HSS	Hertfordshire Sarcopenia Study
EWGSOP	European Working Group on Sarcopenia in Older People
SDH	Succinate dehydrogenase
SMI	Skeletal muscle index
VL	Vastus lateralis

Introduction

Simple tests of physical performance such as grip strength and gait speed show overall declines with age, as well as considerable variability between individuals [1]. These measures have graded associations with a range of ageing outcomes, the most striking being higher all-cause mortality in those with lower performance [2,3]. The linked clinical condition, sarcopenia, has been defined by the European Working Group on Sarcopenia in Older People (EWGSOP) as low physical performance (weak grip strength or slow gait speed) in the presence of low muscle mass [4] and there is increasing recognition of its importance in clinical practice [5,6]. However, much remains to be understood in terms of the cellular and molecular processes underlying the development of sarcopenia. A range of areas have been investigated including mitochondrial dysfunction, anabolic resistance, motor unit remodelling and oxidative stress [7].

Mitochondrial dysfunction is likely to play a role in ageing and long-term conditions, especially in high energy tissues such as muscle [8,9]. A range of changes in muscle mitochondrial function with increasing age have been demonstrated, including reduced activity of the complexes of the respiratory chain [10,11] and an overall lower rate of ATP production [12,13]. These may manifest as individual muscle fibres with respiratory chain deficiency, displaying a similar pattern of histochemical abnormality as seen in mitochondrial myopathies: loss of activity for cytochrome c oxidase (COX^-) and enhanced activity of succinate dehydrogenase (SDH^{++}). The prevalence of such fibres has been shown to increase with age. For example, Bua et al found this appearance in 0.2, 0.7 and 0.9% of fibres from three post-mortem samples of the vastus lateralis (VL) muscle at ages 49, 67 and 92, respectively [14]. Brierley et al similarly found a positive correlation between age and the presence of COX^- fibres in VL biopsies from a sample of 49 participants aged 21-95 years, including no evidence of negative fibres in those aged under 64 years [11]. The overall activity of SDH on histochemistry has also been found to decline across adulthood, although maintenance of regular physical activity may prevent this decline [15].There has been less investigation of whether respiratory chain deficiency is more common in muscle in people with sarcopenia, as recently defined by the EWGSOP. However, a previous case series of nine patients at mean age 71 demonstrated that mitochondrial myopathy can present later in life, with biopsy findings showing a characteristic COX^- / SDH^{++} pattern that was not seen in controls [16]. COX activity in VL biopsy samples has also been found to be lower in older people with low scores (≤ 7) on the Short Physical Performance Battery compared to young controls, a difference not seen in older people with high scores (≥ 11) [17]. A potential limitation of this approach is the clear potential for confounding by clinical and lifestyle factors which differ between the groups being compared.

An alternative approach is to examine whether levels of respiratory chain deficiency are related to components of sarcopenia in older people in a continuous fashion. We previously developed a quadruple immunofluorescence technique for assessment of subunits of mitochondrial complex I and complex IV from muscle cryosections [18]. The quadruple immunofluorescent assay produces continuous and objective measures of protein expression in individual muscle fibres, as compared to the categorical and subjective assessment of cytochrome oxidase using histochemistry. In addition, the quadruple immunofluorescent assay includes measurement of complex I expression. We have applied this technique to samples from a study of community-dwelling older men, the Hertfordshire Sarcopenia Study (HSS), which included assessment of a panel of sarcopenia measures as well as VL muscle biopsy [19,20]. Our aims were firstly to describe the levels of respiratory chain deficiency present in older men and secondly to test the hypothesis that higher levels of deficiency would be associated with lower muscle mass and physical performance.

Methods

Participants and muscle biopsy

The HSS participants were recruited from within the UK Hertfordshire Cohort Study [20] and comprise 105 community dwelling men (average age 72 years, range 68 to 77) who were seen for a further assessment including muscle biopsy and extensive characterisation of their body composition and physical performance in 2007/08 [19]. Participants completed the SF-36 health-related quality of life questionnaire which was used to derive self-reported general health and physical functioning scores [21]. All participants gave written informed

consent to participate in the study and ethical approval was granted by the Hertfordshire Research Ethics Committee, number 07/Q0204/68. Biopsies of the VL muscle were obtained from 99 participants using a Weil Blakesley conchotome using a standard aseptic technique [19,22]. A portion of the biopsy was snap frozen in liquid nitrogen and stored at -80°C until further analysis as per the HSS protocol. Samples were available for the present study in 77 participants.

Histological and immunohistochemical (quadruple immunofluorescence) analyses

In order to improve the morphology of snap frozen biopsies we thawed the tissue blocks at room temperature and refrozen in isopentane cooled in liquid nitrogen. We cut the blocks at 10 µm and collected the sections onto glass slides (Superfrost, Fisher Scientific). We used one section per participant to assess the tissue morphology by haematoxylin and eosin (H&E).

In the next section we performed quadruple immunofluorescence to detect laminin, NDUFB8 (subunit of complex I), COX-I (subunit of complex IV) and porin. We used a further section as a no primary antibody control as described previously [18]. Briefly, following the immunofluorescent protocol, we imaged the sections using Zeiss Axio Imager M1 and Zen 2011 (Blue Edition) software. We collected images of the entire biopsy and stitched them into a single mosaic image which we analysed using Imaris (Bitplane). In each myofibre the optical density of the fluorescent signal was measured by the software and then corrected by background fluorescence derived from the matching no primary antibody control. Previous work has demonstrated that the quadruple immunofluorescene technique is reliable, reproducible and correlates well with conventional COX/SDH histochemistry with respect to the complex IV measurements [18].

The results for the complex I and IV levels of each fibre were then expressed as Z-scores relative to the level that would be expected for the fibre's porin density, based on previous findings from healthy young controls [18]. We categorised Z-score values as follows: positive if $Z \geq -3$; intermediate positive if $-4.5 \leq Z < -3$; intermediate negative if $-6 \leq Z < -4.5$ and negative if $Z < -6$. We also performed sequential COX/SDH histochemistry on a single section from each participant using an established protocol [23], in order to confirm the lack of COX enzymatic activity in fibres deficient in the COX-1 subunit.

Assessment of muscle mass and physical performance

Muscle mass was measured in HSS using dual-energy x-ray absorptiometry (Hologic Discovery, auto whole body software version 12.5). We used overall lean mass (kg) and also skeletal muscle index (SMI), calculated as appendicular lean mass divided by height squared (kg / m^2). Physical performance was assessed using five tests: grip strength (kg) measured with a Jamar handheld hydraulic dynamometer (Promedics, UK) following a standard protocol [24], customary gait speed over 3 metres, the time taken to complete five chair rises [25], a 6 metre timed up and go test [26], and time able to stand on one leg with eyes open (up to a maximum of 30 seconds) [27]. We produced an aggregate performance score similar to that used by Guralnik et al [28] where performance in each of the five tests were rescaled to between 0 (worst) and 1 (best performance in the sample). Further details on the derivation of the aggregate score are included in Appendix 1. We also implemented the EWGSOP sarcopenia algorithm [4] using cut-points for overall lean mass based on the lowest third of the distribution (< 53.2 kg), gait speed of ≤ 0.8 m/s and grip strength of ≤ 30 kg as previously applied in the HSS [29].

Statistical analyses

We summarised variables using means and standard deviations (SD), medians and percentiles, and frequency and percentage distributions as appropriate. We applied a logarithmic transformation to participants' standing balance times. We used intraclass correlation coefficients to estimate the proportion of variation in complex I and IV Z-scores at the within-participant level. We used linear and logistic regression models to examine the associations between the levels of complex I and IV, and muscle mass, physical performance (both the individual tests and the aggregate performance score) and the presence of EWGSOP sarcopenia. As these data consist of multiple muscle fibres per participant, we used a clustered sandwich estimator in models to account for the correlation of values within participants. The following sets of adjustments were used in regression models: unadjusted and adjusted for age. We set our level for statistical significance as $P < 0.05$. We performed all analyses using Stata version 14.0 [30].

Results

Seventy-seven participants of mean age 72.6 (2.5) years had data on respiratory chain deficiency, muscle mass and grip strength. As shown in Table 1, participants had high levels of self-reported physical functioning and general health compared to previously published normative data for the same age group [31]. Their mean levels of lean mass, gait speed and grip strength were also above the sarcopenia cut-points that we used and as such the prevalence of sarcopenia in our sample was low at 8% (n=6). The median number of fibres analysed per participant was 157 (104, 237). An example of the section used from one participant for quadruple immunofluorescence immunohistochemistry is shown in Figure 1, along with the adjacent COX/SDH section used to validate the complex IV immunofluorescence findings.

Table 1 Participant characteristics of the HSS men with data on respiratory chain deficiency. * n = 77 unless shown otherwise, † Median (IQR), DXA: dual-energy x-ray absorptiometry, EWGSOP: European Working Group on Sarcopenia in Older People, HSS: Hertfordshire Sarcopenia Study, SMI: skeletal muscle index.

Characteristic Mean (SD) unless shown otherwise	n *	
Age (years)		72.6 (2.5)
Height (cm)		173.9 (6.2)
Weight (kg)		81.6 (13.1)
BMI (kg / m^2)		26.9 (3.6)
Self-reported health (SF-36)		
General health (0-100)		78.0 (13.2)
Physical functioning (0-100)		93.4 (10.5)
Number of muscle fibres analysed †		157 (104, 237)
Muscle mass		
DXA lean mass (kg)		56.1 (6.9)
SMI (kg / m^2)		7.9 (0.9)
Physical performance tests		
Grip strength (kg)	77	38.2 (7.7)
Gait speed (m / s)	75	1.11 (0.19)
Chair rise time (s)	74	16.9 (3.9)
Timed up and go (s)	75	10.7 (2.2)
One-legged stand time (s) (n=76) †	76	3.7 (2.2, 9.9)
Aggregate performance score	74	2.6 (0.6)
Any EWGSOP sarcopenia (n (%))	75	6 (8%)

Level of respiratory chain deficiency

We saw overall mean fibre Z-scores (ignoring within-participant clustering) for complex I of -0.1 (1.9) and for complex IV of -1.6 (1.6). The equivalent means of participants' mean Z-scores were 0.3 (1.3) and -1.5 (0.9), respectively. The complexes had similarly high within-participant variance (1.59 and 1.51). The between-participant variance was higher for complex I (1.66) than complex IV (0.81), leading to intraclass correlation coefficients of 0.51 and 0.35, respectively. We saw no evidence of correlation between participants' mean complex I and IV Z-scores (r=0.04, *P* = 0.7). There was evidence from clustered models of a negative correlation between age and participants' complex I Z-scores (r = -0.18, *P* = 0.047) and complex IV Z-scores (r = -0.19, *P* = 0.03). When we applied the deficiency categories to participants' Z-scores, we found that participants typically had a majority of positive fibres, with mean percentages of 97.1% for complex I and 89.6% for complex IV (Table 2). There were five participants with greater than 20% overall fibre deficiency (Z < - 3) for complex I and 15 participants for complex IV; in the case of two participants this exceeded 50% for complex IV.

Associations with muscle mass and physical performance

As shown in Table 3, we found evidence of positive relationships between complex I and both gait speed and one-legged stand time, with a 1 SD increase in complex I Z-score associated with a 0.03 m/s (95% CI: 0.01, 0.05, *P* = 0.007) increase in gait speed and a 0.14 (0.04, 0.24, *P* = 0.005) increase in one-legged stand time (ln secs), both after adjustment for age. The associations for between complex I and other measures of physical performance, and all associations with complex IV, did not reach statistical significance.
We also found a relationship between complex I and the aggregate performance score, with each SD increase in complex I associated with an increase in aggregate performance of 0.06 (95% CI: 0.02, 0.09, *P* = 0.003), again after adjustment for age. The equivalent relationship for complex IV did not reach significance. In sensitivity analyses (results not shown) we confirmed that the relationships seen between complex I Z-score and physical performance remained after the exclusion of the five participants with overall complex I deficiency levels above 20% (see previous section).

We saw no evidence of relationships between complex I / IV and SMI (kg/m^2). There was an apparent reduced odds of EWGSOP sarcopenia with increasing complex I and IV, although this did not reach statistical significance.

Figure 1 Sample sections showing quadruple immunofluorescence and COX/SDH staining. Two serial sections are shown: section one reacted for COX/SDH and section two labelled with quadruple immunofluorescence. Myofibres marked with white numbers present with COX enzymatic deficiency and lack COX-I protein. The myofibre marked in yellow (8) has preserved COX activity but is partially negative for NDUFB8 protein.

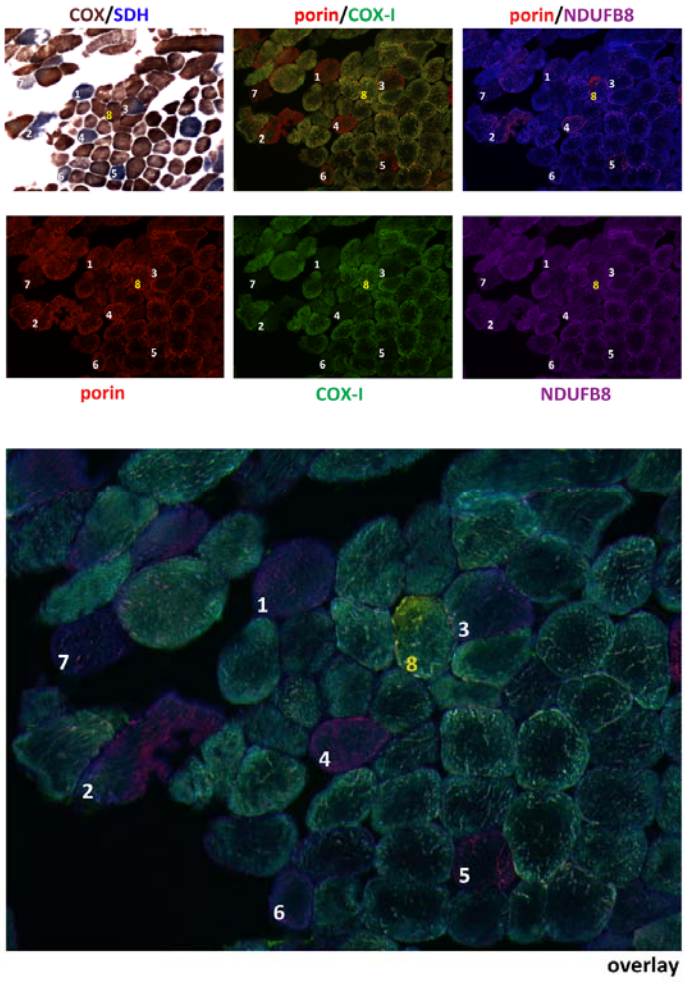

Table 2 Levels of respiratory chain deficiency among participants. Results shown are from the 77 HSS participants with data on respiratory chain deficiency. The summary statistics are for the within-participant percentages of each level of fibre deficiency.

	Measure of respiratory chain deficiency					
Level of deficiency (percentage of each participant's fibres in the relevant Z-score range)	**Complex I Z-score**			**Complex IV Z-score**		
	Mean	SD	Range	Mean	SD	Range
Positive (Z ≥ -3)	97.1	(8.5)	(55.1 – 100)	89.6	(14.0)	(43.0 – 100)
Intermediate positive (-4.5 ≤ Z < -3)	1.8	(5.5)	(0.0 – 30.9)	8.9	(11.5)	(0.0 – 45.7)
Intermediate negative (-6 ≤ Z < -4.5)	0.5	(2.1)	(0.0 – 14.8)	1.1	(2.5)	(0.0 – 14.2)
Negative (Z < -6)	0.6	(1.6)	(0.0 – 11.7)	0.5	(1.9)	(0.0 – 16.1)

Table 3 Age-adjusted associations between respiratory chain deficiency and muscle mass / physical performance
Results are shown from 74 HSS participants with complete data on respiratory chain deficiency, muscle mass and physical performance. Coefficients shown are change in outcome per unit (SD) increase in complex I / IV Z-score, after adjustment for age.
[*] In the chair rise and timed up and go tests, shorter times indicate better performance and hence negative coefficients are compatible with higher levels of complex I / IV being associated with better performance in these tests.
[†] We applied a logarithmic transformation to the one-legged stand times prior to analysis.
[‡] Values shown are the odds ratio of having EWGSOP sarcopenia per unit (SD) increase in complex I / IV Z-score, after adjustment for age.
EWGSOP, European Working Group on Sarcopenia in Older People. SMI, skeletal muscle index.

Outcome	Measure of respiratory chain deficiency					
	Complex I Z-score			Complex IV Z-score		
	Coefficient	(95% CI)	*P*-value	Coefficient	(95% CI)	*P*-value
Muscle mass						
SMI (kg / m^2)	0.00	(-0.05, 0.04)	0.9	0.05	(-0.01, 0.10)	0.09
Physical performance tests						
Grip strength (kg)	-0.57	(-1.19, 0.05)	0.07	0.05	(-0.71, 0.81)	0.9
Gait speed (m / s)	0.03	(0.01, 0.05)	0.007	0.02	(-0.01, 0.04)	0.14
Chair rise time (s) [*]	0.01	(-0.25, 0.26)	0.95	-0.14	(-0.44, 0.16)	0.4
Timed up and go (s) [*]	-0.04	(-0.24, 0.15)	0.7	-0.14	(-0.38, 0.10)	0.2
One-legged stand time (ln s) [†]	0.14	(0.04, 0.24)	0.005	-0.01	(-0.11, 0.10)	0.9
Aggregate performance score	0.06	(0.02, 0.09)	0.003	0.03	(-0.03, 0.10)	0.3
Any EWGSOP sarcopenia [‡]	0.74	(0.49, 1.13)	0.2	0.89	(0.67, 1.17)	0.4

Discussion

Summary of findings

We applied a novel quadruple immunofluorescence technique to assess levels of respiratory chain deficiency in biopsies of the vastus lateralis muscle in a sample of 77 community-dwelling older men. We saw overall levels of the complex I subunit NDUFB8 that were similar to those measured in young controls, whilst mean levels of the complex IV subunit COX-1 were 1.5 SD lower. We found considerable intra- and inter-individual variation in the levels of both complexes, although frank deficiency as described in patients with mitochondrial disease [18] was uncommon.

We explored relationships between respiratory chain deficiency and the two components of sarcopenia, low muscle mass and poor physical performance. We saw no relationship with muscle mass although we did find that higher levels of complex I were associated with a small increase in gait speed, standing balance and an aggregate performance score.

Interpretation of findings

Levels of respiratory chain deficiency

The reason for the difference in the overall levels of complex I and IV in our sample compared to young controls is not clear. We are aware of two previous studies which have examined similar age-related changes and their findings were consistent with ours. Firstly, Gouspillou et al. compared complex I levels between healthy young (mean age 23.7) and older (mean age 71.2) men and found no difference [32]. Secondly, Boffoli et al. reported reduced content of several complex IV subunits in VL biopsy from an 87 year-old individual compared to those from a 31 year-old [10]. Such age-related abnormalities of complex IV can also be seen in the form of COX deficient fibres using histochemistry and these have been suggested to result from point mutations and deletions in mitochondrial DNA [14,33].

A further possible explanation for the complex I to IV discrepancy is that it relates to healthy lifestyle, since previous work has shown that regular endurance training has particularly beneficial effects for complex I activity [34] and this could potentially attenuate age-related decline. We did not measure physical activity although based on the high self-rated physical function in our sample, it is reasonable to assume that the participants were regularly active. Our findings require replication in future studies.

We saw considerable variance in both subunits, both between and within participants. This variance appears to be in excess of that seen from the young

controls, given that the standard deviations of our complex I and IV Z-scores are greater than 1. We have used a novel technique to measure the two subunits and as such there are no other data against which we can compare our findings. The participants in our sample are all a similar chronological age although it is likely that they have undergone different rates of biological ageing and this could contribute to the within-participant variability [35]. The heterogeneity of an individual person's mitochondria is also thought to increase with age [36] and this effect could have contributed to the within-participant variance that we observed.

Associations with components of sarcopenia

We saw associations between complex I and physical performance which were low in magnitude: for example, a one SD increase in complex I level was associated with only a 0.06 SD increase in aggregate performance score. This may be because tests such as gait speed require the contribution of multiple systems in addition to muscle mitochondrial function, including the central nervous system, sensory systems and joint function [37]. It has also been suggested that age-related mitochondrial dysfunction may need to reach high levels within a tissue in order to produce meaningful effects on phenotype [38]. In this regard the levels of complex I and IV in a single biopsy sample may only provide a limited indication of the proportion of mitochondria which are below a critical threshold throughout the entire muscle. Again our findings require replication in further studies.

We are not aware of other studies that have examined associations between complex I and IV levels and muscle mass or physical performance, although two studies have looked at complex IV activity in relation to muscle function. Brierley et al showed a positive correlation between complex IV activity and hand grip strength that was robust to adjustment for age in a sample of 44 individuals aged 21-95 [11]. Joseph et al. showed lower complex IV activity in older people with low as compared to high performance on the short physical performance battery [17]. Other related work in this area includes the findings that gait speed in older people is positively associated with respiratory control ratio [39] and a measure of mitochondrial efficiency [40].

Clinical relevance of findings

The prevalence of sarcopenia in our sample was low however those participants with lower muscle mass and physical performance are at higher risk of going on to develop sarcopenia. Our findings suggest a role of mitochondria in the development of sarcopenia, particularly in terms of loss of physical performance. It is possible that this process may be causal, for example with innate changes in mitochondria such as clonal expansion of mtDNA mutations leading to reduced muscle function [41]. It is also likely that mitochondria reflect prior exposures such as lifetime physical activity [42] which is recognised to be important for muscle function on entering old age [43]. Another possibility is that the mitochondrial respiratory deficiency occurs secondary to a separate pathology such as denervation, as recently demonstrated by Spendiff et al. among a sample of the very old [44].

Our findings may also provide normative data for complex I and IV deficiency from a sample of healthy older men as shown in Table 2. These can be contrasted with previous findings from young adults and patients with inherited mitochondrial disease [18]. Importantly they may be of use in the assessment of older patients with suspected mitochondrial pathology. Further studies will be needed to establish normative data in older women and in the very old.

Strengths and limitations

Our study has several limitations. Firstly, although our sample size was large in the context of a study involving muscle biopsy, we may have lacked power to detect associations. We have attempted to minimise this possibility by using an aggregate performance score. However, as stated above physical performance tests reflect the function of several systems as well as different groups of muscles which may harbour varying levels of mitochondrial deficiencies. Secondly, it was necessary to thaw and re-freeze the muscle samples prior to analysis; this may have caused disruption to muscle structure. We would not expect any resulting disruption to bias our findings, but rather to again reduce our power to detect associations. Finally, our sample had a narrow age-range and all of the participants of the HSS were men.

Strengths of our study include the use of an existing cohort with rigorous collection of sarcopenia measures including multiple tests of physical performance. We also used a novel quadruple immunofluorescence technique to assess the extent of respiratory deficiency in our biopsy samples. In contrast to conventional COX / SDH histochemistry, this technique allowed us to assess levels of complex I which is frequently involved in mitochondrial pathology, and also to assess levels of both complex I and IV in a continuous manner.

Conclusions

We have described the prevalence of respiratory chain deficiency and the relationships with muscle mass and physical performance in a sample of healthy older men. The finding of small but statistically significant positive associations between complex I levels and measures of physical performance suggests that mitochondrial dysfunction may have a role in the development of sarcopenia. These findings will help inform the design of future studies to investigate the mechanisms underlying these relationships across a wider range of ages and in both women and men.

Declaration of sources of funding

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Conflicts of interest

Karolina Rygiel, Richard Dodds, Harnish Patel, Holly Syddall, Leo Westbury, Antoneta Granic, Cyrus Cooper, Joshua Cliff, Mariana Rocha, Doug Turnbull and Avan Sayer declare that they have no conflict of interest.

Ethical standards

The ethical approval for the study was granted by the Hertfordshire Research Ethics Committee (07/Q0204/68) and informed written consent was obtained from all participants.

The authors certify that they comply with the ethical guidelines for authorship and publishing of the Journal of Cachexia, Sarcopenia and Muscle - Clinical Reports (von Haehling S, Ebner N, Morley JE, Coats AJS, Anker SD. Ethical guidelines for authorship and publishing in the Journal of Cachexia, Sarcopenia and Muscle - Clinical Reports. J Cachexia Sarcopenia Muscle Clinical Reports 2016; 1;e28:1-2.[45])

Appendix 1 Derivation of aggregate performance score

We derived a single variable to summarise participants' performance across the five tests used in our study. This was similar to the approach taken by Guralnik et al. [28]. We divided maximum grip strength (kg) by height (cm) prior to inclusion in the score. We then expressed each of grip strength (divided by height), gait speed, time to complete five chair rises and timed-up-and-go using the following formula:

Score value = (participant's value – minimum value in sample) / (maximum value in sample – minimum value in sample)

As such each score value could take the range between 0 and 1. In the case of timed up and go and chair rise time, we subtracted the score value away from 1 so that for all tests, higher values indicated better performance.

For one-legged stand (with eyes open) time, we produced the score value by dividing each participant's time by 30, the maximum possible time in the test.

We then added up the score values for each of the five tests to produce an aggregate score which could theoretically take a value between 0 and 5.

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