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Association between mitochondrial function measured by 31P magnetic resonance spectroscopy and physical performance in older people with functional impairment

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Abstract

Background Mitochondrial dysfunction is a potential therapeutic target to improve skeletal muscle function, but the contribution of mitochondrial dysfunction to impaired skeletal muscle performance in older people remains unclear. The aim of this analysis was to test the association between measures of skeletal muscle mitochondrial function and physical performance in older people.

Methods We analysed data from the Allopurinol in Functional Impairment trial. Participants aged 65 and over, who were unable to walk 400 m in 6 min, underwent 31P magnetic resonance spectroscopy of the calf after exercise at baseline and at 20 weeks follow up. The phosphocreatine recovery half-life time ($t_{1/2}$) was derived as a measure of mitochondrial function. Participants undertook the 6-min walk test and the Short Physical Performance Battery. Muscle mass measured using the Akern 101 bio-impedance analysis system. Bivariate correlations and multivariable regression analyses were conducted to determine associations between $t_{1/2}$ and baseline factors.

Results One hundred and seventeen participants underwent baseline 31P magnetic resonance spectroscopy, mean age 80.4 years (SD 6.0); 56 (48%) were female. Mean 6-min walk was 291 m (SD 80), mean SPPB score was 8.4 (SD 1.9); $t_{1/2}$ correlated significantly with Short Physical Performance Battery score ($r = 0.22$, $P = 0.02$) but not with 6-min walk distance ($r = 0.10$, $P = 0.29$). In multivariable linear regression, muscle mass and total body weight, but not $t_{1/2}$, were independently associated with Short Physical Performance Battery score and with 6-min walk distance. Change in $t_{1/2}$ was not significantly associated with change in Short Physical Performance Battery score ($r = 0.03$, $P = 0.79$) or with change in 6-min walk distance ($r = -0.11$, $P = 0.28$).

Conclusions Muscle mass, but not phosphocreatine recovery time, was consistently associated with Short Physical Performance Battery score and 6-min walk distance in older people with functional impairment.

Keywords Older people; Skeletal muscle; Phosphocreatine; Physical performance

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Background

Impaired physical performance is a common and important finding in older people. Performance impairments may reflect physiological derangements in a host of organ systems, including cardiac and respiratory systems, central and peripheral nervous system, bones and joints, but skeletal muscle dysfunction is an important overarching finding and major contributor to impaired physical performance.[1] Skeletal muscle dysfunction may manifest as reduced muscle strength and/or mass (sarcopenia), or impaired endurance. These impairments contribute to the frailty syndrome[2] and to a series of adverse consequences including limitation of activities of daily living, an increased need for care, increased risk of hospital admission and falls, increased length of stay in hospital, lower quality of life, and earlier death.[3–6]

Understanding the pathophysiology of impaired skeletal muscle function in older people is essential to find better ways to improve muscle function and hence improve physical performance. Many different pathophysiological pathways have been implicated, including mitochondrial dysfunction, oxidative stress, chronic inflammation, neuromuscular junction loss, derangements of neurohormonal systems, anabolic resistance, satellite cell loss, and cellular senescence. Work continues at pace to dissect out the relative contribution of these derangements, and the extent to which they contribute to deficits in muscle size, strength, and endurance.[7,8]

Mitochondrial dysfunction has been suggested to be an important component of skeletal muscle dysfunction with advancing age. Genetically inherited mitochondrial mutations are well known to cause mitochondrial myopathies.[9] Studies of mitochondrial function measured using 31P magnetic resonance spectroscopy (31P MRS) show that phosphocreatine recovery rate (a measure of how quickly mitochondria can generate ATP to replenish phosphocreatine after exercise) is much slower in older people with the frailty syndrome compared with healthy older people.[10] In contrast, phosphocreatine recovery rate does not appear to be significantly slower in healthy older people compared with younger people.[11,12]

It is not clear at present whether mitochondrial dysfunction is a major driver of impaired physical performance in older people. We therefore aimed to assess the relationship between PCr recovery rate and measures of physical performance in a population of older people with impaired physical function recruited to a randomized controlled trial.

Methods

Study population

The analyses described in this paper used data from the ALFIE (Allopurinol for Functional Impairment) trial.[13] The trial enrolled 124 people aged 65 and over with impaired physical performance (6-min walk distance $<$400 m) and randomized them to receive 600 mg allopurinol or matching placebo for 20 weeks. Exclusion criteria have been described in full elsewhere but included contraindications to allopurinol or to MRI scanning, and diseases likely to alter skeletal muscle function by alternative mechanisms to those seen in the sarcopenia of ageing (including chronic heart failure, severe COPD, peripheral arterial disease, steroid-induced myopathy, or active malignancy). Ethics approval was given by the East of Scotland Research Ethics committee (approval number 14/ES/1092), and written informed consent was obtained from all participants. The study was performed in line with the principles of the Declaration of Helsinki. The trial was approved by the UK Medicines and Healthcare products Regulatory Authority (CTA 2014-004122-18) and was registered at www.isrctn.com (ISRCTN03331094).

31P magnetic resonance spectroscopy

31P magnetic resonance spectroscopy (MRS) of the calf was performed before and after participants exercised by repeated plantarflexion of the foot of their non-dominant leg. Plantarflexion was performed against resistance using an MRI-safe, weight-adjusted system. The weight was set to provide resistance based on 15% of lean body mass[14] derived from bioimpedance analysis (BIA). Both sets of tests were conducted at baseline and at 20 weeks after the intervention. The full 31P MRS acquisition and analysis details have been published previously.[13] In brief, participants were scanned using a 3T Siemens MRI scanner (Trio/PrismaFit, Siemens Healthineers, Erlangen, Germany). A dual-tuned $^{1}H/^{31}P$ coil was used to acquire spectra every 4.2 s before exercise and during recovery starting immediately after cessation of plantarflexion exercise. Spectroscopy data were processed using JMRUI[15] to obtain a series of 31P MRS measures including phosphocreatine recovery rate, ATP, inorganic phosphate and total phosphocreatine levels pre- and post-exercise as previously described.[13,16] The maximal rate of PCr recovery was calculated from the maximum slope of the fitted graph for each recovery curve, with results presented both un-normalized and normalized for PCr depletion. The PCr recovery half time was calculated by multiplying the time constant by ln(2); the time constant was taken as the reciprocal of the rate constant derived from the PCr recovery curve. The percentage PCr depletion with exercise was calculated (previous studies have suggested that depletion should be between 30% and 70% to ensure sufficient depletion without generating an acidotic environment). Resting pH and the Pi:PCr were also calculated.

Measures of physical performance and muscle mass

The Short Physical Performance Battery (SPPB) was performed as previously described.[4] The gait speed component was assessed over a 2.44 m walking course. The SPPB assesses lower limb strength and balance and is a powerful predictor of adverse outcomes including future disability, death, and need for care.[4]

The 6-min walk test was conducted in a flat corridor on a 25 m course.[17] Standardized encouragement was given, and the distance walked was recorded to the nearest meter. A single test was conducted at each timepoint. The 6-min walk test is a submaximal test of exercise capacity and reflects endurance as well as muscle strength; in older people, it correlates significantly with measures of maximal exercise tolerance including $VO_{2max.}$[18–20]

Bioimpedance analysis was used to derive lean body mass using the BIA 101 analysis system (Akern Biosciences, Pontassive, Italy). Measurements were taken in the supine position. Appendicular lean body mass was derived using the Sergi equation[21] and adjusted for height squared to derive the appendicular skeletal muscle mass index (ASMMI); this equation was derived in a similar population of older Europeans, using the same BIA 101 analysis system as used in the current study.

Other covariates

In addition, a range of covariates measured during the trial were selected for inclusion in the analyses. Blood pressure was measured in the supine position after 5 min of rest using an OMRON HEP-705 oscillometric device; three readings were recorded and the mean of the second and third readings were taken as the measurement for analysis. Endothelial function was assessed by measuring flow-mediated dilation (FMD) of the Brachial artery in response to 5 min of forearm blood flow occlusion.[22] Brachial artery diameter was measured as previously described by using a Sequoia 512 ultrasound machine (Siemens, Camberley, UK) and an 8-MHz linear array ultrasound probe. 8-Hydroxydeoxyguanosine (8-OHDG), a circulating marker of muscle oxidative stress, was measured by an ELISA (Cusabio, Houston, TX, USA). Serum urate (Sigma-Aldrich/Merck, Darmstadt, Germany) and plasma marker of oxidative stress denoted by Thiobarbiturate Reactive Substances (TBARS) (Cayman Chemicals, Michigan USA) were measured by colorimetric assays.

Statistical analyses

Statistical analyses were performed using SPSS v25 (IBM, New York, USA); a two-sided P value of <0.05 was taken as significant for all analyses. The analysis dataset comprised all participants who underwent 31P MRS at baseline; descriptive baseline statistics were produced for this cohort. For analysis of baseline cross-sectional data, bivariate correlations between baseline physical performance measures and baseline 31P MRS measures and other covariates were generated. Continuous variables were compared using Spearman's rank correlation coefficient as MRS measures were not normally distributed. Categorical variables were compared using Student's t-test. Multivariable analysis was then performed using linear regression models with SPPB and 6-min walk distance as dependent variables to explore whether 31P MRS measures were independently associated physical performance. Several of the MRS variables were not normally distributed, so the log or square root of the variable was taken to achieve a normal distribution.

Associations between the change in physical performance between baseline and follow up and change in 31P MRS and other covariates were similarly analysed; change scores for each variable were calculated, then compared using Spearman's rank test due to the data not being normally distributed.

Results

One hundred and seventeen participants had MRS data collected at baseline and formed the study population, baseline details are given in *Table 1*. The mean age of participants was 80.4 years (SD 6.0); 56 (48%) were female. All participants had significantly impaired physical performance; the mean 6-min walk was 291 m with a range of 84 to 398 m, and the mean SPPB score was 8.4, with a range of 3 to 12. The mean resting skeletal muscle pH was 7.23 (SD 0.09), and the mean percentage PCr depletion with exercise was 61% (SD 18).

Baseline cross-sectional correlations

The relationship between the measures of physical performance and phosphocreatine recovery half time ($t_{1/2}$) are show in *Figure 1*, along with the relationship between age and $t_{1/2}$. A significant association was seen between $t_{1/2}$ and the SPPB score (rho = 0.22, P = 0.02) but not between $t_{1/2}$ and the 6-min walk distance (rho = 0.10, P = 0.29). The mean $t_{1/2}$ for the study population was 35 (SD 22) seconds (median 31 s; interquartile range 22 to 41 s).

Table 2 shows the association between all baseline variables and the two measures of physical performance. Several measures of phosphorus metabolism were associated with SPPB, but only un-normalized PCr recovery rate was associated with 6-min walk distance; no significant association was seen after normalization for PCr depletion. None of the studied co-morbidities showed a significant association with

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Table 1 Baseline descriptors (n = 117)

Mean age (years) (SD)	80.4 (6.0)
Female sex (%)	56 (48)
Ischaemic heart disease (%)	19 (16)
Hypertension (%)	69 (59)
Dyslipidaemia (%)	62 (53)
Stroke or transient ischaemic attack (%)	12 (10)
Diabetes mellitus (%)	18 (15)
Median weekly alcohol intake (units) (IQR)	2 (0–5)
Current smoker (%)	8 (7)
Systolic blood pressure (mmHg) (SD)	143 (18)
Diastolic blood pressure (mmHg) (SD)	77 (9)
Body mass index (kg/m^2) (SD)	28.3 (4.8)
Six-min walk distance (m) (SD)	291 (80)
Lean body mass (kg) (SD)	
Men	33.6 (7.6)
Women	24.5 (4.0)
Appendicular skeletal muscle mass index (kg/m^2) (SD)	
Men	11.3 (2.4)
Women	10.0 (1.7)
Mean short physical performance battery (SD)	8.4 (1.9)
Median total number of medications (IQR)	5 (3–8)
ACE inhibitor (%)	29 (25)
Beta-blocker (%)	20 (17)
Calcium channel blocker (%)	36 (31)
Alpha blocker (%)	12 (10)
Thiazide (%)	28 (24)
Loop diuretic (%)	10 (9)
Aldosterone antagonist (%)	4 (3)
Angiotensin receptor blocker (%)	11 (9)
Statin (%)	49 (42)
Antiplatelet (%)	28 (24)
Insulin (%)	1 (1)
Antidiabetic (%)	11 (9)

either SPPB or the 6-min walk distance. Body mass index showed a significant negative association with both SPPB and walk distance as expected; in contrast, lean body mass showed a positive association with both SPPB and walk distance.

Baseline multivariable analyses

Table 3 shows the results of multivariable analyses, relating each measure of physical performance to measures of phosphorus metabolism together with body weight, muscle mass and (for the 6-min walk) height. Muscle mass and body weight were significantly associated with both 6-min walk distance and SPPB, but none of the MRS measures was independently associated with 6-min walk distance or the SPPB score.

Associations with change in physical performance over time

Changes in normalized PCr recovery between baseline and 20 weeks showed a weak but significant correlation with changes 6-min walk distance, but changes in other MRS measures, urate, or measures of oxidative stress did not correlate with changes in SPPB or 6-min walk distance. Results are shown in *Table 4*.

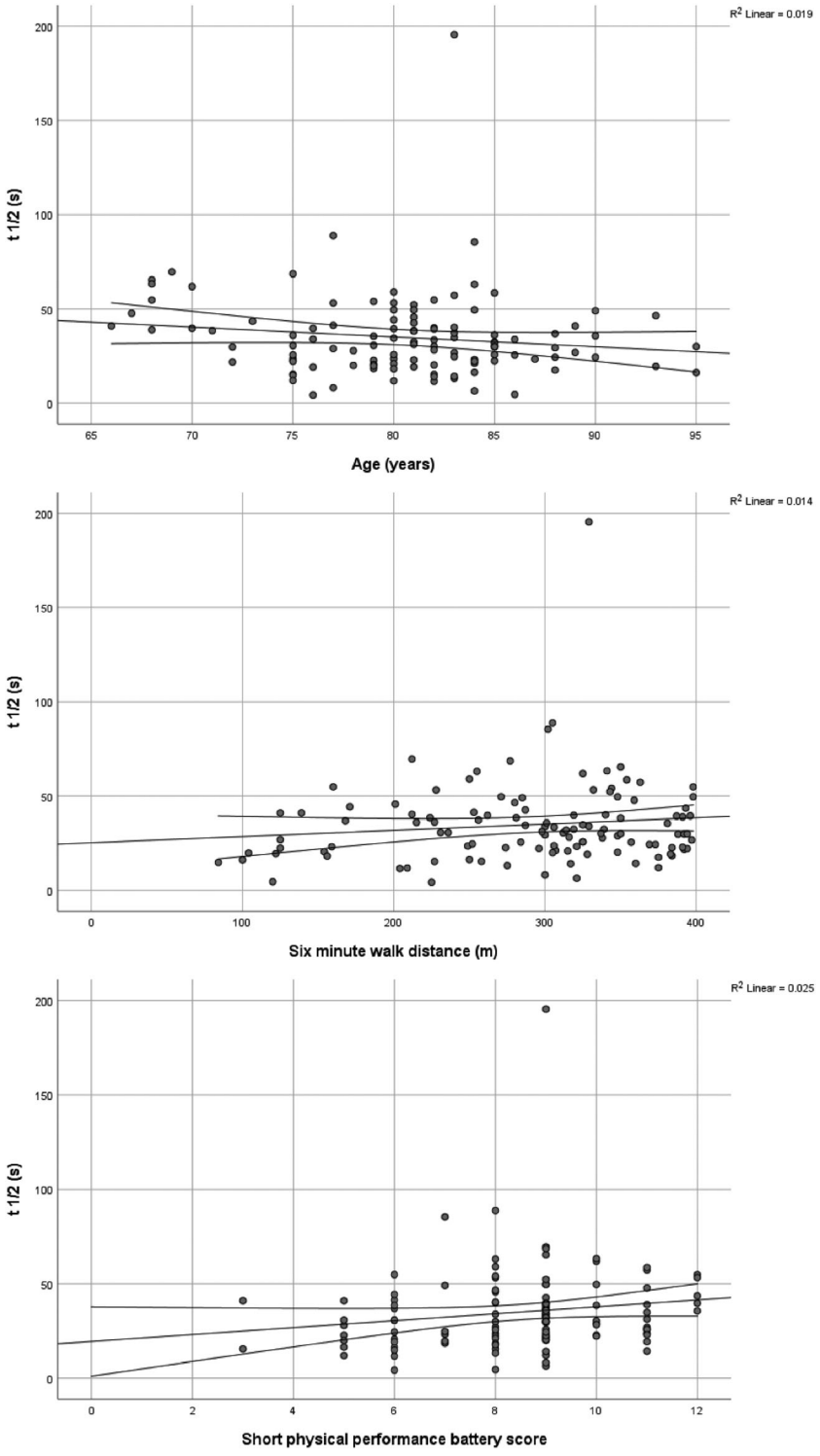
Discussion

In this study, we found that mitochondrial function measured by phosphocreatine recovery half time was not independently associated with physical performance in this group older people with functional impairment. Multivariable analysis found that body weight and muscle mass were more closely associated with physical performance than MRS measures of muscle function. We also found that PCr recovery time in this population was similar to that found in healthy older populations and younger people. The lack of correlation between most measures of change in PCr recovery rate and change in physical performance over time suggests that interventions to improve mitochondrial function as measured by PCr recovery rate are unlikely to deliver improvements in physical function in this group of older people.

Previous studies on healthy older people have found PCr recovery half times similar to that found in the current study. Mean $t_{1/2}$ was 36 s in a group of 16 active older people with a mean age of 85 years[11] and was 32 s in a group of six healthy older people with ages ranging from 70 to 83 years.[12] A study of healthy older people aged 65 and over measured a mean $t_{1/2}$ of 30 s when examining quadriceps function.[23] We found an association between older age and longer PCr recovery rate, similar to that found in previous studies.[12,23] Our results are consistent with those from Santanasto *et al*.[24] who found a relationship between walk speed and PCr recovery rate in high-functioning older people but found no such relationship in people with functional impairment despite similar mean PCr recovery rates between the two groups. Of note, the mean SPPB in our study (8.4) was very similar to that seen in the functionally impaired group studied by Santanasto *et al*. (7.8). Other studies[23,25] have also found significant relationships between PCr recovery rate and walk speed over longer distances or at faster pace, although again these studies enrolled healthy older people.

Some of the differences seen in PCr recovery rate with age may be due to the level of physical activity; older people who were sedentary had much longer PCr recovery half-times (50 s) than those who were physically active (25 s)[26]; older people who were physically active showed similar PCr recovery half-times to young active individuals. Interestingly, in this study, measures of mitochondrial function were not significantly associated with gait speed, chair stand time, or stair climb time on univariate analysis but were significantly associated with 1-repetition maximum strength and stair climb tests when adjusted for muscle mass, age, and sex. A similar relationship was seen between PCr recovery time

Figure 1 Relationship between baseline phosphocreatine recovery half-time, age, and physical performance measures.



and physical activity in healthy people enrolled in the Baltimore Longitudinal Study of Ageing.[27] These differences in physical activity may also underlie the differences in PCr recovery rate seen between older women who were non-frail, pre-frail, and frail[28]; similar results were seen in another small study comparing older people living with prefrailty with those who were not frail.[29] Frailty was classified by the Fried frailty criteria in these studies, and low levels of physical

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Table 2 Associations between baseline covariates and baseline measures of physical performance continuous

	6-min walk distance		SPPB	
Continuous covariates	*r*	*P*	*r*	*P*
Age	−0.13	0.18	−0.12	0.20
PCr measures				
$t_{1/2}$	0.10	0.29	0.22	0.02
Normalized PCr recovery rate	0.01	0.94	0.00	0.97
Non-normalized PCr recovery rate	0.22	0.02	0.26	0.005
Resting pH	−0.10	0.29	−0.09	0.32
Pi:PCr ratio	0.07	0.46	0.03	0.78
%PCr depletion	−0.15	0.10	−0.18	0.054
Uric acid	−0.10	0.28	0.05	0.61
8OHDG	−0.27	0.004	−0.24	0.01
TBARS	0.06	0.54	0.05	0.60
Flow-mediated brachial artery dilatation	−0.22	0.06	−0.23	0.05
Weight	−0.13	0.17	−0.12	0.22
Height	0.19	0.04	0.22	0.02
Body mass index	−0.29	0.002	−0.31	0.001
Lean body mass	0.23	0.01	0.26	0.005
Categorical covariates	**Mean (SD)**	***P***	**Mean (SD)**	***P***
Sex				
Male	298 (83)	0.32	8.8 (1.6)	0.03
Female	283 (77)		8.0 (2.1)	
Ischaemic heart disease				
Yes	288 (79)	0.85	8.5 (1.6)	0.88
No	291 (81)		8.4 (2.0)	
Hypertension				
Yes	270 (82)	<0.001	8.1 (1.9)	0.04
No	321 (68)		8.9 (1.8)	
Dyslipidaemia				
Yes	278 (76)	0.06	8.3 (1.9)	0.48
No	306 (83)		8.5 (2.0)	
Stroke/transient ischaemic attack				
Yes	284 (52)	0.75	8.6 (1.7)	0.74
No	292 (83)		8.4 (2.0)	
Diabetes mellitus				
Yes	244 (83)	0.006	7.7 (2.0)	0.07
No	299 (77)		8.5 (1.9)	
Current smoker				
Yes	309 (98)	0.51	8.3 (2.3)	0.81
No	289 (79)		8.4 (1.9)	

PCr, phosphocreatine. Pi, inorganic phosphate. TBARS, thiobarbiturate reactive substances. 8OHDG, 8-hydroxydeoxyguanosine.

Table 3 Multivariable analyses

	Log $t_{1/2}$		Square root of non-normalized initial PCr recovery rate	
	B (95% CI)	*P*	*B* (95% CI)	*P*
SPPB				
Muscle mass	0.117 (0.060 to 0.175)	<0.001	0.107 (0.050 to 0.164)	<0.001
Weight	−0.051 (−0.081 to −0.021)	0.001	−0.045 (−0.075 to −0.016)	0.003
MRS measure	1.21 (−0.143 to 2.55)	0.08	0.004 (−0.001 to 0.009)	0.09
6 min walk distance				
Muscle mass	4.29 (1.69 to 6.89)	0.001	3.58 (1.01 to 6.16)	0.007
Weight	−2.87 (−4.12 to −1.60)	<0.001	−2.35 (−3.63 to −1.08)	<0.001
Height	139.35 (−26.8 to 305.5)	0.10	122.72 (−50.26 to 295.69)	0.16
MRS measure	37.40 (−146.5 to 347.66)	0.18	0.100 (−0.094 to 0.298)	0.30

MRS, magnetic resonance spectroscopy; SPPB, short physical performance battery.

activity are an important component of the 'spiral of decline' posited by the Fried frailty model.[30]

Another explanation may be that in older people with functional impairment, factors other than mitochondrial function are more important in determining physical performance. Co-morbid disease, particularly arthritis, heart and lung disease, but also neuromuscular disease, such as previous stroke, chronic pain syndrome, and parkinsonian

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Table 4 Associations between changes in covariates and changes in measures of physical performance between baseline and 20 weeks

	6-min walk distance change		SPPB change	
	r	*P*	*r*	*P*
MRS measures				
$t_{1/2}$	−0.11	0.28	0.03	0.80
Normalized PCr recovery rate	0.20	0.04	0.13	0.17
Non-normalized PCr recovery rate	0.18	0.06	0.16	0.10
Resting pH	0.02	0.82	−0.04	0.67
Pi:PCr ratio	0.18	0.06	0.12	0.23
%PCr depletion	0.14	0.14	0.15	0.13
Uric acid	−0.10	0.30	0.08	0.43
8OHDG	0.13	0.20	0.10	0.34
TBARS	−0.003	0.98	0.02	0.88
Flow-mediated dilatation of brachial artery	0.01	0.91	−0.18	0.13
Muscle mass	0.03	0.80	−0.00	0.98

PCr, phosphocreatine; Pi, inorganic phosphate; TBARS, thiobarbituric reactive substances; 8OHDG, 8-hydroxydeoxyguanosine.

syndromes, may all impair physical performance in complex ways without mitochondrial function being the limiting factor. Skeletal muscle perfusion has been suggested as a limiting factor for skeletal muscle function in some older people[31] although our results did not show an association between better endothelial function at the brachial artery and better physical performance.

Strengths of our study were that we were able to study MRS measures in a comparatively large group of older people, with a mean age of over 80 years old and that we were able to compare MRS measures with a measure of lower limb function reflecting strength (the SPPB) as well as a measure of endurance (the 6-min walk) reflecting complementary aspects of skeletal muscle function. Unlike most other large studies using MRS in older people, we focused on a group of older people with impaired physical function —a group at risk of activity impairment and further deterioration, and thus highly relevant to clinical practice.

There are a number of limitations to our study; despite enrolling older people with functional impairment, only two participants fulfilled the criteria for low muscle mass according to the revised EWGSOP criteria for sarcopenia. In addition, we did not measure handgrip strength in this study, which would have given additional useful information on muscle function. Participants in any randomized controlled trial tend to be highly selected and somewhat healthier than the population from which they are drawn, although our study population had significant multimorbidity. Phosphocreatine recovery rate is only one way of interrogating mitochondrial function. It has the advantage of not requiring muscle biopsy and provides a window into real-time physiological response of muscle to an exercise challenge. It is possible that other aspects of mitochondrial function are more important in determining muscle function in older people, but we did not undertake muscle biopsy to enable exploration of other measures of mitochondrial structure, density, or function.[11,29] Alternatively, other factors, such as mitochondrial number or delivery of substrates to mitochondria, may provide the underlying cause for impaired skeletal muscle function.[31,32]

It is unclear how well correlated MRS measures are between different muscle groups in older people with functional impairment. We studied calf muscle MRS measures, but quadriceps measures would have been arguably more relevant to performance in both the SPPB and 6-min walk. Such measures are more difficult to acquire and exercising the quadriceps to exhaustion within the MRI scanner is technically more challenging, requiring heavier weights and larger equipment although previous studies in healthy older people have accomplished this.[27] Similarly, we derived an estimate of appendicular muscle mass from whole-body bioimpedance rather than measuring leg muscle mass directly using DXA or MRI, which may have weakened correlations between mass and MRS measures. We were unable to include physical activity as a covariate in our analyses as this was not measured in the trial.

We suggest that future observational studies in this area should focus on patients with a confirmed diagnosis of sarcopenia or physical frailty; such patients are those most likely to manifest derangements of normal skeletal muscle physiology. Future studies should incorporate measures of physical activity given the important modifying influence that inactivity appears to have on mitochondrial function. The addition of muscle biopsy alongside MRS measures would further augment the ability of future studies to both delineate the mitochondrial pathology underlying sarcopenia and frailty but would also help to identify which MRS measure most closely correlated with biopsy-based measures of mitochondrial function. Including MRS assessment of quadriceps function would provide data on a muscle group crucial to daily activity in older people (such as climbing stairs and rising from a chair).

Our results do not support strategies to improve mitochondrial function as the main target for improving skeletal

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muscle function in this group of older people. However, such a strategy may still be worth exploring in older people with worse muscle function—that is, those who are physically inactive, those with sarcopenia or those with more advanced physical frailty. Future studies should therefore target these groups for proof-of-concept studies aimed at improving mitochondrial function. For older people with impaired function but without sarcopenia or frailty, alternative strategies (e.g. nutrition, senolytics, myostatin system inhibitors, or improving skeletal muscle perfusion)[1,31,32] should be explored further to both improve muscle mass and improve skeletal muscle function.

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Conflict of interest

Allan D. Struthers has applied for a patent on the use of xanthine oxidase inhibitors to treat angina pectoris.

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