

RESEARCH PAPER

Association of mitochondrial oxidative capacity with physical fitness in ageing: the Baltimore longitudinal study of ageing

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Abstract

Background: In younger individuals, fitness is mostly influenced by muscle mitochondrial oxidative phosphorylation (OxPhos) and cardiac output. However, compared with younger individuals, various impairments may also negatively affect fitness in older adults.

Objective: To investigate the relationship of OxPhos with cardiorespiratory fitness, the energetic cost of walking and aerobic resilience with respect to age.

Design: Cross-sectional.

Setting: Population.

Subjects: Six hundred and forty-nine Baltimore longitudinal study of ageing participants (mean age 64.5 years, 56.9% females).

Methods: Muscle mitochondrial OxPhos was measured as phosphocreatine recovery rate (k_{PCr}) through ^{31}P magnetic resonance spectroscopy. Based on age- and sex-specific k_{PCr} z-scores, we classified individuals with low (≤ -0.5 standard deviations [SD]), average (-0.5 to $0.5SD$) and high ($>0.5SD$) OxPhos. Cardiorespiratory fitness was measured as peak oxygen consumption (MVO_2 peak) during a treadmill testing. The energetic cost of usual pace walking was expressed as the average oxygen consumption per 100 metres. Aerobic resilience was the ratio between MVO_2 peak and average VO_2 during usual pace walking.

Results: Participants with higher k_{PCr} had 4.07 (95%CI: 2.88, 5.26) ml/kg/min higher MVO_2 peak and 0.19 (95%CI: 0.06, 0.32) higher aerobic resilience than those with lower k_{PCr} . The energetic cost of walking was greater by 0.84 (95% CI: 0.21, 1.47) ml/kg/100 m in those with high than low k_{PCr} . A multiplicative interaction between age and k_{PCr} was identified in the regressions predicting MVO_2 peak and aerobic resilience ($p_{interaction} = 0.01$), with differences between OxPhos groups attenuating after age 70.

Conclusion: Muscle mitochondrial OxPhos contributes to interindividual variability in cardiorespiratory fitness, especially in young and middle adulthood.

Keywords: ageing; mitochondria; energetic cost of walking; physical fitness; cohort study; older people

Key Points

- Higher muscle mitochondrial oxidative phosphorylation (OxPhos) is linked to greater cardiorespiratory fitness.
- Age modifies the association of OxPhos with cardiorespiratory fitness.
- OxPhos affects cardiorespiratory fitness more in younger than older adults.
- Adults with 1-SD lower OxPhos have cardiorespiratory fitness comparable to that of individuals 20 years older.

Introduction

The ageing process is widely heterogeneous, and older adults experience a vast range of changes in health and functional characteristics [1, 2]. Interestingly, respiratory and muscle function, two fundamental contributors to physical performance, have the broadest heterogeneity range in older individuals [1]. Detecting the factors that influence the heterogeneity of physical performance in old age is crucial for identifying new targets of intervention to maintain functional ability and achieve healthy ageing [3]. However, evidence on this topic has not yet been fully clarified.

Among other roles, mitochondria provide most of the energy used by cells to perform various biological processes, and their function is highly correlated with physical and cognitive performance [4, 5]. Mitochondrial volume and oxidative phosphorylation (OxPhos) gradually decline with ageing [6, 7]. However, changes in mitochondrial OxPhos with ageing depend on several factors, including the level of physical activity [8], acute and chronic diseases and even subtle systemic dysfunctions that disrupt the homeostatic balance of the organism [9, 10]. Ultimately, these changes may alter cardiorespiratory fitness and energy metabolism [11, 12], resulting in impaired physical performance, including reduced muscle strength [11, 13], slower walking speed [4, 13, 14] and greater fatigability [12, 15].

Cardiorespiratory fitness is typically assessed through a graded exercise test to exhaustion, representing the actual aerobic capacity of fit, healthy individuals. In the general population, cardiorespiratory fitness is often approximated by peak oxygen consumption (MVO₂ peak), defined as the highest rate of oxygen uptake achieved during a stress test when individuals may stop due to fatigue or other factors before reaching their maximal capacity [9]. Cardiorespiratory fitness depends on cardiovascular function and peripheral oxygen utilization, which are influenced mainly by parenchymal volume, perfusion and mitochondrial OxPhos. However, particularly in older persons, other factors, including musculoskeletal problems, pain, respiratory function and neurologic impairments [16], may also influence the level of effort an individual can afford and, consequently, MVO₂ peak.

In this analysis, we examined whether the relationship of muscle mitochondrial OxPhos with fitness measures, including MVO₂ peak, the energetic cost of walking, and aerobic resilience (i.e. the ratio between MVO₂ peak and the average VO₂ during walking at usual pace), would change with ageing.

Methods

Study population

This study uses data from participants of the Baltimore longitudinal study of ageing (BLSA), a prospective study started in 1958 and currently conducted by the National Institute on Aging (NIA) Intramural Research Program [17, 18]. The BLSA study protocol was approved by the Institutional Review Board of the Intramural Research Program of the National Institutes of Health (03AG0325: IRP/NIA/NIH); written informed consent was obtained from all participants at each visit. In brief, community-dwelling individuals aged 20 years and older and free from major chronic diseases and functional or cognitive deficits are enrolled on a voluntary basis. After the baseline evaluation, participants are reassessed every 4, 2, or 1 year if aged <60, 60–79, and 80+ years, respectively.

In the study, we examined a cross-sectional sample at the time of the first muscle mitochondrial OxPhos assessment (since April 2013). From the initial sample of 795 participants who underwent ³¹P magnetic resonance spectroscopy (MRS), we excluded 80 individuals with no available data on cardiorespiratory fitness and 66 who did not have a valid ³¹P MRS test for OxPhos measurement (details can be found below), resulting in a final analytical sample of 649.

Data collection

Participant assessments were conducted by trained personnel using standardized protocols, which included administration of validated scales and questionnaires, personal interviews, physical examinations and laboratory and imaging tests. For the present study, we collected *sociodemographic, lifestyle and medical information* (detailed in **Appendix S1 in Supplementary Data**) along with the data described below.

Mitochondrial oxidative capacity

Mitochondrial OxPhos was assessed by the phosphocreatine (PCr) recovery rate. Briefly, participants underwent ³¹P MRS scanning of the quadriceps muscles of the left leg using a 3 T Achieva MRI scanner (Philips, Best, The Netherlands) to quantify phosphorous-containing metabolites [19]. For details, please see **Appendix S2 in Supplementary Data**. In this study, participants were categorised according to mitochondrial OxPhos. Since the current literature shows that mitochondrial OxPhos varies by age and sex, we computed z-scores of k_{PCr} by sex and age (considering the following

categories: ≤ 55 , 56–60, 61–65, 66–70, 71–75, 76–80, 81–85, 86+). Consequently, as shown in **Appendix S3**, participants were classified into three groups: lower mitochondrial OxPhos (k_{PCr} z-scores ≤ -0.5 standard deviation [SD]), average OxPhos (k_{PCr} z-scores between -0.5 and $+0.5$ SD) and higher OxPhos (k_{PCr} z-scores >0.5 SD). The value of 0.5 SD is generally used as a cut-off to define a medium effect size of standardized mean differences [20].

Energetic cost of walking

Energy expenditure during walking was measured during a 2.5 minute walking test at the usual pace over a 20-metre course in an uncarpeted corridor. Participants were instructed to walk the course continuously for 2.5 minutes at their preferred pace. During the test, participants wore a portable indirect calorimeter (Cosmed K5, Cosmed, Rome, Italy) that monitored and recorded their average oxygen consumption and carbon dioxide production for each 30-s interval through breath-by-breath measures [21]. The total distance walked during the test was also recorded. In line with previous studies [21, 22], the average VO_2 per kilogramme of body weight per minute (ml/kg/min), expressing the mean oxygen consumption during walking, was derived after excluding the first 1.5 minutes of the test in order to estimate the VO_2 once the participant had reached a metabolic steady state. To obtain the energetic cost of walking at the usual speed, we computed the average oxygen consumption per metre walked (ml/kg/m) using the following formula: VO_2 (ml/kg/min) $\times 2.5$ min / total distance (m). From this value, in order to facilitate data interpretation, we derived the average oxygen consumption per 100 metres walked (ml/kg/100 m).

Cardiorespiratory fitness

Treadmill testing for measurements of MVO_2 peak was performed on all participants without high-risk cardiovascular diseases (e.g. moderate to severe aortic stenosis, ischemic heart diseases, or high-grade AV block). Participants underwent a modified Balke protocol in a motor-driven treadmill with constant speed (3.5 mph for men and 3.0 mph for women) and a slope that, starting from 0%, increased by 3% every 2 min. The test continued until participant exhaustion or symptom onset. During treadmill testing, VO_2 (expressed in ml/kg/min) was measured by indirect calorimetry in participants wearing two-way non-rebreathing masks through the Vyntus CPX system (Jaeger Medical America, Inc., Moreno Valley, California, USA). VO_2 assessment was performed every 30 seconds, and the highest value recorded during the test (MVO_2 peak) was used as an indicator of individual aerobic capacity and cardiorespiratory fitness [23].

Aerobic resilience

The ratio between the MVO_2 peak and the average VO_2 during walking at usual pace (both measures expressed as ml/kg/min) was computed as an indicator of aerobic resilience. This parameter assesses how much the individual

can increase their aerobic metabolism from usual activities to maximal effort. Higher values indicate that the individuals can perform a level of effort that requires an energy cost substantially higher than that of standard walking, while a lower value means that the energy cost of maximal effort is close or equal to the effort required during standard walking.

Statistical analysis

Comparison of participants' characteristics by mitochondrial OxPhos was performed using ANOVA or Chi-squared tests, as appropriate.

Relationships between mitochondrial OxPhos and fitness-related parameters were tested using simple correlation and expressed as Pearson r coefficients. Associations of energetic parameters with age were tested by linear regression models. The possible non-linearity of the relationships was explored by plotting residuals vs fitted values, assessing the Mean Absolute Percentage Errors, and goodness of fit (Bayesian Information Criterion, Akaike Information Criterion). These analyses indicated that the relationship between age and VO_2 (peak or at usual pace) was approximately linear; whereas the energetic cost of walking exhibited a non-linear trend with age, with a steeper increase after the age of 65.

Linear regressions were performed to estimate the mean difference in MVO_2 peak and aerobic resilience between mitochondrial function groups. Analyses were adjusted for age, sex, ethnicity, physical activity level and PCr percent depletion with exercise, considering their potential confounding role in the studied associations. Further regression models were evaluated after including multiplicative interactions between mitochondrial OxPhos level (categorised) and age. From the parameters of the regression equation, we estimated the intercept and slope values of the MVO_2 peak and aerobic resilience versus OxPhos curves from younger to older age. Predicted values were shown in regression plots. Estimates were expressed as β -coefficient and 95% Confidence Intervals (95%CI) and were provided for the total sample and after sex-stratification. Concerning the energetic cost of walking, we used a regression spline function and tested the interaction between age (modelled with a B-spline with three degrees of freedom) and mitochondrial OxPhos groups after adjusting the model for the same covariates listed above.

To further explore the modifying effect of mitochondrial OxPhos on the relationship between age and MVO_2 peak and aerobic resilience, we derived point estimates from sex-stratified linear regression models, including age, ethnicity, physical activity level, PCr percent depletion with exercise, k_{PCr} and the interaction age $\times k_{PCr}$. The mean or mode of quantitative or categorical covariates, respectively, was used to generate the point estimates.

As a sensitivity analysis, we performed quantile regression analysis to evaluate whether the differences in energetic metabolism variables by mitochondrial OxPhos depended on the outcome distribution. Moreover, linear regressions

were repeated after categorising participants by sex-specific z-scores of k_{PCr} .

All statistical tests were performed using R statistical software, and a P -value $< .05$ was considered statistically significant.

Results

Characteristics of the 649 study participants are reported in Table 1. The mean age of the sample was 64.5 years (min-max 22–92, with 56.5% aged ≥ 65 years), 43.1% were males, and 24.5% were Black. The sample had high educational attainment of 17.7 mean years of schooling and cognitive performance with a mean MMSE score of 28.6, and 49.6% reported a moderate or very active lifestyle. When comparing sample characteristics based on mitochondrial OxPhos levels (Table 1), those with low values (k_{PCr} z-scores ≤ -0.5) were more likely to be Black and physically inactive and to report fewer years of schooling and a positive history of diabetes, chronic heart failure, PAD and COPD than those with average or high OxPhos. MVO_2 peak and the energetic cost of walking increased significantly from the low to the high mitochondrial OxPhos groups.

In simple correlation analyses, mitochondrial OxPhos measured as k_{PCr} showed a positive correlation with MVO_2 peak ($r = 0.44$, $P < .001$) and aerobic resilience ($r = 0.29$, $P < .001$), while there was no significant association with the energetic cost of walking (Figure 1).

In multivariable linear regression analyses (Table 2), participants with average (k_{PCr} z-scores from -0.5 to 0.5 SD) or higher (k_{PCr} z-scores > 0.5 SD) mitochondrial OxPhos had 1.59 (95%CI: 0.56, 2.63) and 4.07 mL/kg/min (95%CI: 2.88, 5.26) higher MVO_2 peak, respectively, than the lower k_{PCr} group. Similarly, participants with high k_{PCr} had, on average, 0.19 higher aerobic resilience (95% CI: 0.06, 0.32) compared to those with low k_{PCr} . Participants with average and high k_{PCr} showed energetic cost of walking values greater by 0.71 (95% CI 0.16, 1.26; $P = .01$) and 0.84 (95% CI: 0.21, 1.47; $P = .009$) mL/kg/100 m than those with low k_{PCr} , respectively (data not shown). When stratifying the analyses by sex, we observed greater effect sizes for the associations related to MVO_2 peak and aerobic resilience in males than females, with no significant sex differences identified in the findings regarding the energetic cost of walking (Table 2, Figure 2).

There was a significant interaction between age and k_{PCr} in regression models with MVO_2 peak and aerobic resilience as dependent variables (for both models: $p_{interaction} = 0.01$), but not in the regression analysis for the energetic cost of walking. Individuals with higher k_{PCr} had higher MVO_2 peak and aerobic resilience from younger to older age, although the strength of this association substantially attenuated with increasing age, especially after 70 years (Figure 2 and Appendix S4 in Supplementary Data). For example, examining point estimates of MVO_2 peak and aerobic resilience

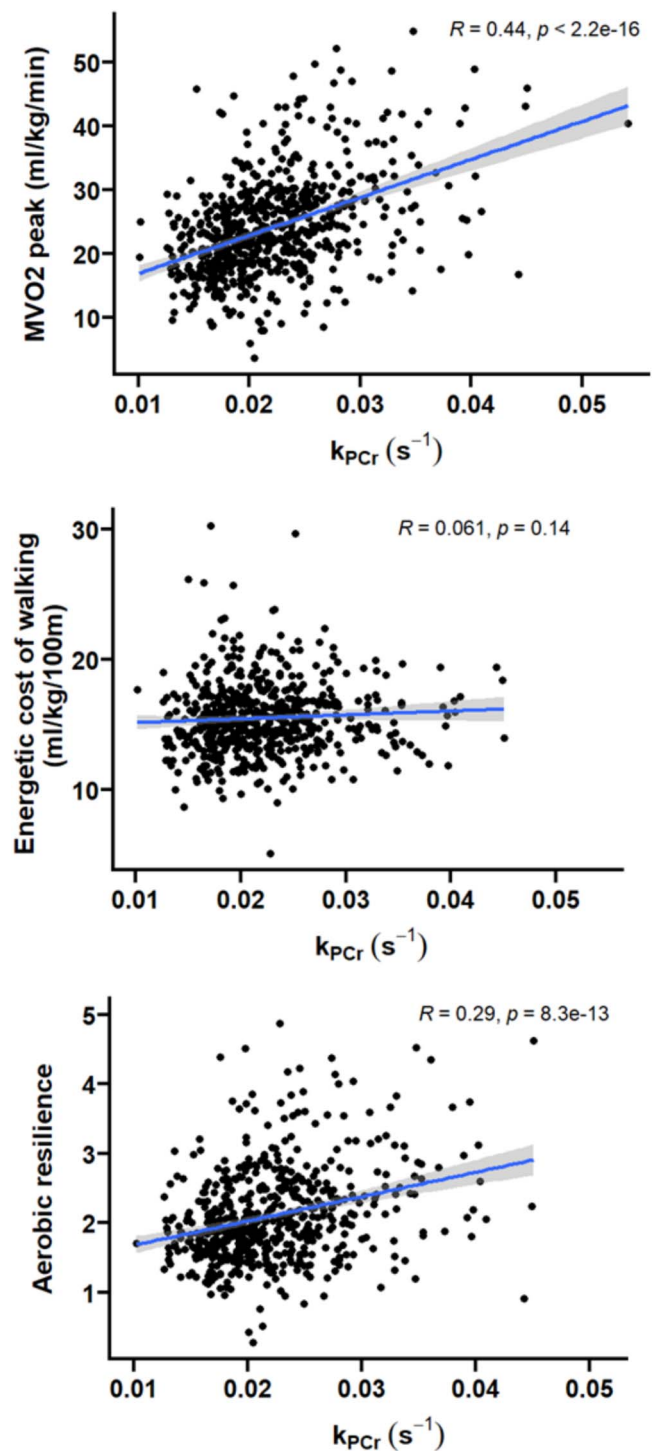


Figure 1. Scatterplot and correlation coefficient of k_{PCr} with peak oxygen consumption, the energetic cost of walking and aerobic resilience. Abbreviations: k_{PCr} , phosphocreatine recovery rate; MVO_2 peak, peak oxygen consumption.

by sex, age and mitochondrial OxPhos (see Appendix S5 in Supplementary Data), an individual at age 40 with low k_{PCr} had approximately the same MVO_2 peak as a 60-year-old of the same sex but with high k_{PCr} . This gap progressively narrowed with age and was less marked for aerobic resilience.

Table 1. Baseline characteristics of the total sample and by mitochondrial oxidative capacity levels

	All (n = 649)	Mitochondrial OxPhos			P-value
		Low (n = 229)	Average (n = 250)	High (n = 170)	
Sex (male)	280 (43.1)	97 (42.4)	108 (43.2)	75 (44.1)	.94
Age (y)	64.48 (15.50)	64.37 (15.28)	64.74 (15.82)	64.25 (15.39)	.944
Ethnicity (Black)	159 (24.5)	88 (38.4)	43 (17.2)	28 (16.5)	<.001
Educational level (y)	17.65 (2.60)	17.24 (2.60)	17.73 (2.71)	18.09 (2.38)	.004
Physical activity					<.001
Inactive	53 (8.2)	29 (12.9)	18 (7.2)	6 (3.6)	
Light	271 (42.1)	110 (49.1)	109 (43.6)	52 (30.8)	
Moderate	198 (30.8)	58 (25.9)	80 (32.0)	60 (35.5)	
Very active	121 (18.8)	27 (12.1)	43 (17.2)	51 (30.2)	
Body Mass Index (kg/m ²)	26.91 (4.65)	27.42 (4.81)	26.77 (4.45)	26.42 (4.67)	.131
MMSE total score	28.59 (1.43)	28.40 (1.56)	28.73 (1.38)	28.66 (1.30)	.053
Chronic diseases					
Anaemia	82 (14.9)	36 (18.6)	33 (15.3)	13 (9.2)	.056
Depression	49 (8.9)	19 (9.8)	24 (11.1)	6 (4.2)	.069
Cancer	61 (11.1)	29 (14.9)	18 (8.3)	14 (9.9)	.09
Hypertension	244 (44.2)	94 (48.5)	81 (37.5)	69 (48.6)	.039
Diabetes	51 (9.2)	28 (14.4)	16 (7.4)	7 (4.9)	.006
Chronic heart failure	35 (6.3)	19 (9.8)	11 (5.1)	5 (3.5)	.042
Stroke	49 (8.9)	21 (10.8)	21 (9.7)	7 (4.9)	.147
Peripheral artery disease	20 (3.6)	12 (6.2)	3 (1.4)	5 (3.5)	.034
Chronic Obstructive Pulmonary Disease	81 (14.7)	38 (19.6)	31 (14.4)	12 (8.5)	.017
Joint diseases	292 (52.9)	100 (51.5)	119 (55.1)	73 (51.4)	.71
Parkinson's disease	3 (0.5)	0 (0.0)	3 (1.4)	0 (0.0)	.096
k _{PCr} (s ⁻¹ *1000)	22.00 (5.81)	17.05 (2.30)	21.72 (2.68)	29.07 (5.28)	<.001
PCr depletion (%)	55.58 (11.69)	56.93 (11.58)	56.57 (11.73)	52.23 (11.17)	<.001
MVO ₂ peak (ml/kg/min)					
Males	26.74 (8.65)	24.4 (7.36)	26.2 (8.62)	30.5 (9.07)	<.001
Females	21.99 (6.39)	20.1 (5.73)	22.4 (5.87)	24.0 (7.28)	<.001
Energetic cost of walking (ml/kg/100 m)	15.50 (2.80)	14.81 (2.74)	15.88 (3.00)	15.82 (2.41)	<.001
Aerobic resilience					
Males	2.32 (0.77)	2.24 (0.70)	2.27 (0.74)	2.49 (0.85)	.093
Females	1.94 (0.57)	1.87 (0.53)	1.98 (0.58)	1.97 (0.60)	.305

Notes: Values are expressed as mean (standard deviations) for quantitative variables, and as count (%) for categorical variables. Mitochondrial OxPhos groups are categorised based on age- and sex-specific z-scores of k_{PCr} ≤ -0.5 (low), between -0.5 and 0.5 (average) and > 0.5 (high). P-values refer to the difference between mitochondrial OxPhos groups. Some data were missing in educational level (n = 1), physical activity (n = 7), body mass index (n = 106), MMSE (n = 108), chronic diseases (n = 102 for each of the listed diseases), energetic cost of walking (n = 85) and aerobic resilience (n = 80). Aerobic resilience is the ratio between MVO₂ peak (ml/kg/min) and VO₂ during usual walking (ml/kg/min). Abbreviations: MMSE, Mini-Mental State Examination; MVO₂ peak, peak oxygen consumption; OxPhos, oxidative phosphorylation; VO₂, oxygen consumption.

Similar results were observed when performing quantile regression analyses (**Appendices S6 and S7, Supplementary Data**), and when considering sex-specific z-scores of k_{PCr} (**Appendices S8 and S9, Supplementary Data**).

Discussion

Our study reinforces the hypothesis that muscle mitochondrial OxPhos plays a significant role in cardiorespiratory fitness and aerobic resilience from younger to older age. Individuals with greater mitochondrial OxPhos exhibited higher MVO₂ peak and aerobic resilience across all age groups; however, this advantage appears to diminish with increasing age. In contrast, muscle mitochondrial OxPhos capacity does not appear to be substantially related to the energetic cost of walking across different ages.

Previous findings support a direct association between mitochondrial OxPhos and VO₂ max in both young and older adults, with skeletal muscle mitochondrial energetics accounting for approximately one-third of the variability in VO₂ max [11, 24]. Such a relationship is supported by several mechanisms. For instance, greater mitochondrial efficiency and capacity to restore PCr from inorganic phosphate (Pi) affect fatigability [12], which conditions an individual's ability to undergo greater physical efforts. In addition, mitochondria are involved in diverse biological activities beyond OxPhos, such as regulating intracellular calcium homeostasis [25], apoptosis, and atrophic processes in skeletal muscle [26, 27], which could influence muscle quality and, indirectly, MVO₂ peak. Alterations in mitochondrial efficiency reflect a higher production of reactive oxygen species, which promote the establishment of a chronic

Table 2. Linear regression for the association of k_{PCr} with peak oxygen consumption and aerobic resilience stratified by sex

	All		Males		Females	
	β (95%CI)	P-value	β (95%CI)	P-value	β (95%CI)	P-value
Outcome: MVO₂ peak (ml/kg/min)						
Low k_{PCr}	[ref]		[ref]		[ref]	
Average k_{PCr}	1.59 (0.56, 2.63)	.003	1.88 (0.07, 3.69)	.042	1.33 (0.16, 2.50)	.026
High k_{PCr}	4.07 (2.88, 5.26)	<.001	5.42 (3.32, 7.51)	<.001	2.80 (1.45, 4.14)	<.001
Outcome: Aerobic resilience						
Low k_{PCr}	[ref]		[ref]		[ref]	
Average k_{PCr}	0.11 (−0.00, 0.23)	.051	0.10 (−0.09, 0.29)	.291	0.12 (−0.02, 0.26)	.098
High k_{PCr}	0.19 (0.06, 0.32)	.004	0.28 (0.07, 0.50)	.011	0.10 (−0.06, 0.26)	.215

Notes: Mitochondrial oxidative capacity groups are categorised based on age- and sex-specific z-scores of $k_{PCr} \leq -0.5$ (low), between -0.5 and 0.5 (average) and > 0.5 (high). The linear regression model is adjusted for age, sex, ethnicity, physical activity level and PCr percent depletion with exercise. Analyses include 613 (for MVO₂ peak) and 548 (for aerobic resilience) individuals with no missing data in the covariates. Bold values indicate statistically significant estimates. Abbreviations: MVO₂ peak, peak oxygen consumption.

inflammatory status through multiple pathways [28, 29]. Moreover, mitochondrial dysfunction is linked to a massive release into the cytosol of damage-associated molecular species, including mtDNA (mainly in its oxidised forms) and cardiolipin, a structural inner-membrane phospholipid [30, 31]. Overall, these effects may have a central role in the development of sarcopenia and physical impairments [32, 33]. However, the contribution of these mechanisms to age-related changes in physical fitness observed in our study remains undetermined. In fact, whether OxPhos capacity correlates with other mitochondrial functions is unknown because no standard method exists to assess non-OxPhos mitochondrial function in humans.

Interestingly, the difference in MVO₂ peak between individuals with varying levels of mitochondrial OxPhos gradually diminished with ageing. This gap was particularly pronounced in younger individuals, in whom a difference of one SD in mitochondrial OxPhos corresponded to a cardiorespiratory fitness of nearly 20 years older. Conversely, by the age of 70, the difference in fitness accounted for by differences in OxPhos was minimal, suggesting that additional factors other than mitochondrial oxidative capacity may have a strong influence on cardiorespiratory fitness with advancing age. Notably, these findings were derived from regression analyses adjusted for physical activity levels. This likely buffered the differences observed between groups since physical activity may be either a confounder (by influencing mitochondrial OxPhos and cardiorespiratory fitness [7]) or a mediator of the studied association since mitochondrial OxPhos may affect the level to which individuals can be engaged in exercise, especially in older age. A second hypothesis concerns the possible greater relevance of the decrease in muscle mass compared to mitochondrial energetics to older people's MVO₂ peak. This interpretation would contrast with previous studies that reported mitochondrial OxPhos reduction to be important and independent of the loss of muscle mass in determining age-related changes in VO₂

max [34]. A third hypothesis is that the accumulation of deficits and chronic diseases associated with ageing may significantly impact peak MVO₂ in the oldest individuals, thereby accounting for a large portion of the variability in cardiorespiratory fitness above and beyond the role played by mitochondrial OxPhos [35]. Moreover, besides the number of chronic diseases, individuals in the oldest age classes exhibit a higher frequency of cardiovascular, metabolic and respiratory conditions, which can substantially affect aerobic capacity [35]. Finally, participants in the oldest age groups who could perform the physical performance tests were likely the healthiest. Therefore, although age-specific z-scores of k_{PCr} should take into account the physiological decline of OxPhos with advancing age, selective survival could have mitigated the differential impact of mitochondrial OxPhos on the outcomes. Accordingly, the variability in k_{PCr} values with advancing age gradually reduced.

Similar results were observed for aerobic resilience, a measure obtained from the ratio of MVO₂ peak to average VO₂ during walking, which indicates the extent to which individuals can increase their aerobic metabolism from usual activities to maximal effort. In our sample, adults with higher mitochondrial OxPhos showed greater aerobic resilience; however, the difference in aerobic resilience by OxPhos level attenuated in older participants. This trend was likely driven by differences in MVO₂ peak, as mitochondrial OxPhos did not appear to be substantially associated with the energetic cost of walking. In particular, the energetic cost of walking did not correlate with mitochondrial OxPhos, and only a minimal difference in this parameter (less than 0.01 mL/kg/m) emerged comparing individuals with average or high vs. low OxPhos. This result leaves room for various interpretations. On the one hand, the sample included individuals who were physically fit enough to undergo the treadmill test and could be relatively homogeneous in terms of energetic walking cost. In this context, differences in physical fitness may more markedly emerge when considering

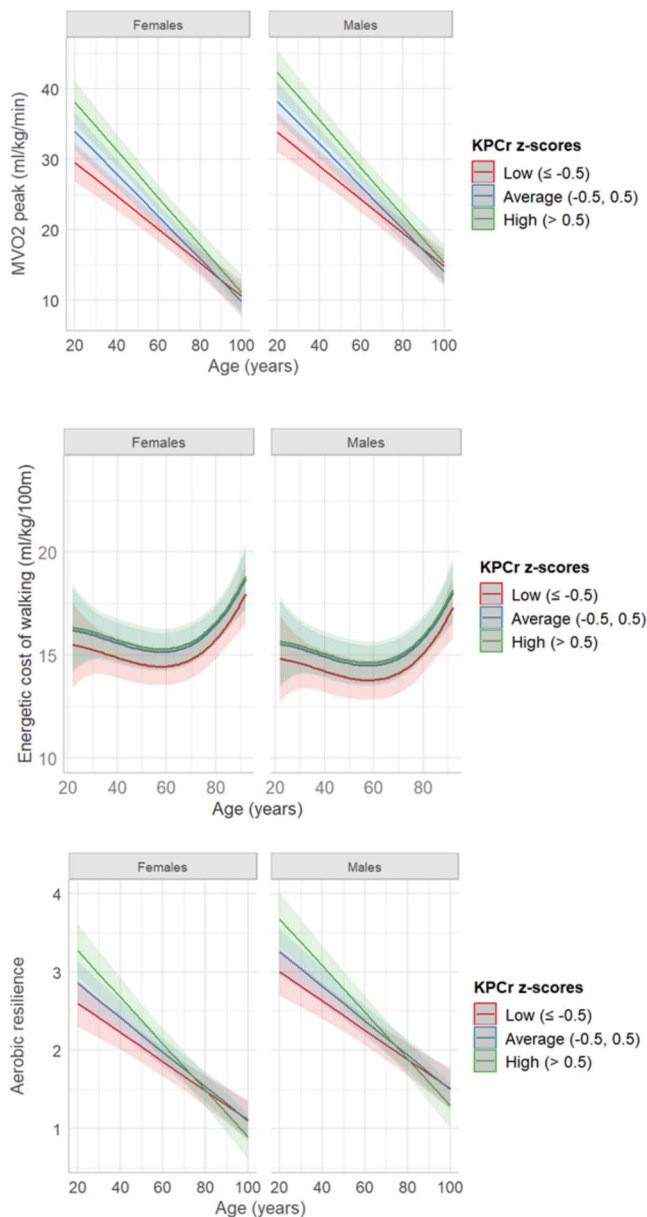


Figure 2. Predicted peak oxygen consumption, energetic cost of walking and aerobic resilience as a function of age, by sex and mitochondrial oxidative capacity levels. *Notes:* Plots illustrate predicted values from linear regression models adjusted for age, sex, ethnicity, physical activity level and PCr percent depletion with exercise. Analyses include 613 (for MVO₂ peak) and 548 (for aerobic resilience) individuals with no missing data in the covariates. Mitochondrial oxidative capacity groups are categorised based on age- and sex-specific z-scores of $k_{PCr} \leq -0.5$ (low), between -0.5 and 0.5 (average) and > 0.5 (high).

aerobic capacity during maximal effort than during usual activities. On the other hand, our findings might suggest that walking energetics is influenced more by the integrity and functionality of the biomechanical and kinesiological systems that optimise energy consumption during gait [22, 36, 37] than mitochondrial efficiency [38]. Concerning

the former, changes in coordination, balance, and other walking mechanics' features (e.g. stride length, joint ranges of motion, antagonist muscle co-activation timing) in older age may be attributed to common symptoms (e.g. osteoarticular pain) and multisystemic dysfunctions, including neurodegenerative processes. Accordingly, previous studies have demonstrated that certain biomarkers of neurodegeneration, such as brain and hippocampal atrophy, as well as amyloid-beta deposition, are inversely associated with the energetic cost of walking [39, 40].

Our analysis of sex-related differences revealed that the disparity in aerobic capacity and resilience between the higher and lower mitochondrial OxPhos groups was nearly twice as pronounced in males as in females. These results extend current knowledge by emphasising sex-specific patterns in the association between mitochondrial OxPhos and mobility performance [41]. Although some evidence suggests higher intrinsic mitochondrial function in females [42], other studies have found similar respiration rates in both sexes, but differences in mitochondrial membrane microviscosity, fatty acid oxidation and ADP sensitivity [41, 43, 44]. Moreover, males and females likely have differences in the biomechanics of walking, which may also help explain our results [45].

The strengths of our study include the relatively large sample of individuals across different age groups with assessments of mitochondrial OxPhos and maximal aerobic capacity using high-quality and validated approaches. Moreover, in addition to the MVO₂ peak, measuring energy expenditure during walking at the preferred pace through indirect calorimetry allowed us to derive a measure of aerobic resilience, which represents a novelty of this work. On the other hand, possible limitations include the cross-sectional study design and the lower representation of individuals in the younger age group, which may have affected the statistical power of the analysis on age-related changes. Additionally, we acknowledge that the participants who underwent the treadmill test may not be representative of the general older population, particularly the frailest individuals and those with high-risk cardiovascular conditions, who did not take the test. Accordingly, the key characteristics of the study participants include high educational attainment, healthy lifestyle and preserved cognitive function.

In conclusion, our study underscores the critical role of mitochondrial OxPhos in elucidating the differential age-related decline in aerobic capacity. However, our findings also suggest that, in older age, other factors beyond mitochondrial OxPhos become increasingly influential in determining fitness and play a vital role in enhancing the capacity for aerobic metabolism under greater exertion. Reduced physical fitness is a driving determinant of physical performance impairment and loss of functional ability, which may lead to the development of disability and the need for higher assistance and healthcare services. Larger studies with a longitudinal design are required to further verify the role of muscle mitochondrial OxPhos and deepen our understanding of the additional factors that modulate individual functional trajectories from adult to older age. Moreover, these insights emphasise the

importance of investigating the effectiveness of multifactorial interventions targeted but not be focused exclusively to mitochondrial efficiency, to mitigate the detrimental effects of ageing on physical function and self-sufficiency.

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Supplementary Data: Supplementary data is available at *Age and Ageing* online.

Declaration of Conflicts of Interest: None declared.

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