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Lower muscle mitochondrial energetics is associated with greater phenotypic frailty in older women and men: the Study of Muscle, Mobility and Aging

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Abstract

Background Phenotypic frailty syndrome identifies older adults at greater risk for adverse health outcomes. Despite the critical role of mitochondria in maintaining cellular function, including energy production, the associations between muscle mitochondrial energetics and frailty have not been widely explored in a large, well-phenotyped, older population.

Methods The Study of Muscle, Mobility and Aging (SOMMA) assessed muscle energetics in older adults

($N = 879$, mean age = 76.3 years, 59.2% women). ^{31}P magnetic resonance spectroscopy measured maximal production of adenosine triphosphate (ATP_{max}) *in vivo*, while *ex vivo* high-resolution respirometry of permeabilized muscle fibers from the vastus lateralis measured maximal oxygen consumption supported by fatty acids and complex I- and II-linked carbohydrates (e.g., Max OXPHOS_{CI+CII}). Five frailty criteria, shrinking, weakness, exhaustion, slowness, and low activity, were used to classify participants as robust (0, $N = 397$), intermediate (1–2, $N = 410$), or frail (≥ 3 , $N = 66$). We estimated the proportional odds ratio (POR) for greater frailty, adjusted for multiple potential confounders.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11357-023-01002-1>.

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Results One-SD decrements of most respirometry measures (e.g., Max OXPHOS_{CI+CII}, adjusted POR = 1.5, 95%CI [1.2,1.8], $p = 0.0001$) were significantly associated with greater frailty classification. The associations of ATP_{max} with frailty were weaker than those between Max OXPHOS_{CI+CII} and frailty. Muscle energetics was most strongly associated with slowness and low physical activity components.

Conclusions Our data suggest that deficits in muscle mitochondrial energetics may be a biological driver of frailty in older adults. On the other hand, we did observe differential relationships between measures of muscle mitochondrial energetics and the individual components of frailty.

Keywords Phenotypic frailty · Mitochondria · Muscle energetics · Aging

Introduction

Frailty is a state of decreased physiologic reserves and resilience which increases an individual's vulnerability to adverse health outcomes. While different definitions of frailty exist, we focus on the “physical frailty phenotype” or “phenotypic frailty” as defined by Fried [1, 2]. Phenotypic frailty operationalizes the measurement of frailty as a syndrome assessing five components: shrinking, exhaustion, weakness, slowness, and low physical activity. The phenotype discriminates healthier agers from those with high vulnerability to stressors—which precipitate adverse events, including hospitalizations,

disability, and death [3–7]. More recent work has focused on identifying underlying biological pathways of frailty to suggest pathophysiologies that might be amenable to intervention [1, 8]. The presence of lower physical activity and greater feelings of exhaustion in frail older adults suggest that frailty may be related to insufficient cellular energy and that age-related declines in skeletal muscle mitochondrial energetics may be an underlying biological driver of frailty [1, 8, 9].

Skeletal muscle mitochondrial energetics includes multiple biochemical pathways: respiration through the electron transport system (ETS) and oxidative phosphorylation (OXPHOS) act in concert to produce ATP to support metabolic activity of cells [10]. Muscle energetics have been measured in older adults using numerous methodologies; the current report utilizes both *in vivo* and *ex vivo* muscle energetic measures from the Study of Muscle, Mobility and Aging (SOMMA) [11]. *In vivo* ³¹P-magnetic resonance spectroscopy (³¹P MRS) of the quadriceps following a short bout of exercise captures a phosphorous spectrum that enables the calculation of maximal rate of adenosine triphosphate (ATP_{max}) generation in post-exercise recovery. *Ex vivo* assessment was also applied, where high-resolution respirometry of permeabilized muscle fibers (hereafter referred to as “respirometry”) was used to determine oxygen consumption (O₂ flux) or oxidative phosphorylation (OXPHOS) supported by specific energy substrate combinations (i.e., fatty acids and complex I- and II-linked carbohydrates).

In older adult quadricep muscle, typically the vastus lateralis, we and others have employed both high-resolution respirometry and ³¹P MRS to show that greater muscle mitochondrial energetics associated with better muscle and physical performance [10–16]. The few studies that have linked frailty to muscle mitochondrial energetics have been small [9, 17, 18]. Notwithstanding, they suggest that there is a connection between muscle energetics and human frailty in aging. We sought to test the hypothesis that lower muscle mitochondrial energetics is associated with greater frailty. We also sought to determine if these measures were associated with specific frailty components. The expansive dataset from the SOMMA cohort [19] lends itself to this cross-sectional investigation of *in vivo* and *ex vivo* measures of muscle

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mitochondrial energetics and their relationship with phenotypic frailty.

Methods

Study cohort and recruitment

Details related to the design of the Study of Muscle, Mobility and Aging (SOMMA) have been published [19]. We used SOMMA data (<https://sommaonline.ucsf.edu/>) from the baseline visit collected between April 2019 and December 2021; 879 older adults aged 70 or older were recruited at University of Pittsburgh and Wake Forest University School of Medicine. Exclusion criteria included the following: inability to walk 400 m or climb a flight of stairs; gait speed ≤ 0.6 m/s; had BMI ≥ 40 kg/m²; an active malignancy or advanced chronic disease that would prevent completing baseline assessments or limit their likelihood of providing follow-up data; contraindication to a muscle biopsy; the presence of magnetic resonance (MR) incompatible implants; or a diagnosis of dementia or claustrophobia. All participants provided written informed consent. The Western IRB-Copernicus Group (WCG) Institutional Review Board approved the SOMMA (WCGIRB #20180764).

General baseline questionnaires and measures

SOMMA baseline assessments [19] were obtained over three visits and included questionnaires that surveyed a range of demographic information from participants, including age based on self-reported date of birth, self-reported sex, race, ethnicity, education (\leq high school or other, some college, college graduate, post college), marital status, drinking (drinks per week), and smoking history (ever vs never), medical history, and height at age 25. The total number of prescription medications taken within the last 30 days was recorded by inventory on prescription bottles. Self-reported mobility was assessed by the mobility assessment tool-short form (MAT-sf), in which a participant rates their ability to complete a number of mobility tasks demonstrated in an animated video [20]. Height was measured on stadiometers and mass on digital scales; body mass index (BMI) was calculated as weight divided by square of height (kg/m²). Participants underwent whole-body magnetic

resonance (MR) scans that were analyzed using AMRA Researcher (AMRA Medical AB, Linköping, Sweden) to calculate the volumes of abdominal subcutaneous and visceral adipose tissue (ASAT; VAT) [21, 22]. Maximal grip strength in both hands was determined in duplicate trials with an adjustable, hydraulic, isometric dynamometer (Jamar) [23]. Walking speed (m/s) was calculated from 4-m usual pace that is part of the Short Physical Performance Battery (SPPB) [24, 25]. Physical activity was measured by the Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire; we calculated total time reported on energy expenditure across all intensities of activities (min/week) and the corresponding calorie expenditure (kcal/week) [26]. Depressive symptomology was assessed by the Center of Epidemiologic Studies Depression Scale, 10-item version (CESD-10) [27]. Whole body muscle mass was measured using deuterated creatine (D₃Cr) method as previously described [28, 29]. From the self-reported medical history, a modified Rochester Epidemiology Project [30] multimorbidity score (0-11) was calculated. This score included these age-related conditions: cancer (excluding non-melanoma skin), chronic kidney disease or renal failure, atrial fibrillation, lung disease (i.e., chronic obstructive pulmonary disease, bronchitis, asthma, or emphysema), coronary heart disease (i.e., blocked artery or myocardial infarction), heart failure, dementia, ≥ 10 score on CESD-10 (consistent with depressive symptoms), diabetes, stroke, and aortic stenosis.

Phenotypic frailty index

The frailty phenotype was defined closely following the criteria by Fried et al. from the Cardiovascular Health Study (CHS) [2] (Supp. Table 1), with frailty defined as three or more of the following components present: shrinking, weakness, slowness, low physical activity, and exhaustion. The presence of each frailty component was defined as: shrinking—the lowest sex-specific quintile of D₃Cr muscle mass/weight; weakness—maximal grip strength in the lowest quintile, stratified by sex and BMI quartile; slowness—the slowest quintile of walking speed, stratified by sex and median height; low physical activity—the lowest sex-specific quintile of energy expenditure from the CHAMPS; and exhaustion—answering “at least 3–4 days per week” to either of two questions from the

CESD-10 (“I felt that everything I did was an effort” and “I could not get going”). Participants who were unable ($N = 2$) or refused ($N = 1$) the grip strength measurement due to health and safety reasons were considered to have weakness. SOMMA did not assess unintentional weight loss over the previous year—which is a component in the CHS frailty definition. D_3Cr muscle mass/weight was used in lieu. Two secondary shrinking definitions were explored in sensitivity analyses: a cut point of BMI < 20 and the highest quintile of height loss since age 25. Participants with none of the components were considered robust, 1–2 components were intermediate, and 3 or more components were frail. For those missing data on 1–2 of the components, the frailty index was extrapolated based on the mean of the non-missing components.

Permeabilized muscle fibers for high-resolution respirometry measures

Participants were instructed to take prescription medications as usual with water except for oral medications or insulin for diabetes which they took after tissue sampling. If participants were asked to withhold aspirin, they were asked to stop taking it 3 days prior to tissue collection. Percutaneous muscle biopsy of vastus lateralis was performed as previously described [13, 31]. Myofiber bundles of 2–3mg were teased apart, chemically permeabilized with saponin, and washed in the presence of the myosin II ATPase inhibitor blebbistatin (25 μ M; to inhibit muscle contraction). Here, we used two standardized substrate uncoupler inhibitor titration (SUIT) protocols [11], run in duplicate, with steady-state respiration normalized to wet weight, to assess the activity of mitochondrial electron transport system in permeabilized muscle fiber (PMF) bundles with Oxygraph 2K instruments (Oroboros Inc., Innsbruck, Austria). All assays were run at 37 °C in MiRO5 supplemented with blebbistatin (25 μ M), and O_2 concentrations were between 400 and 200 μ M. Data were collected and analyzed with Datlab 7.4.

Two protocols were used. In protocol 1, respirometry measures include complex I–supported leak respiration (leak or state 4) which was determined through the addition of pyruvate (5 mM) and malate (2 mM). Sequential additions of adenosine diphosphate (ADP; 25 μ M, 200 μ M, 4.2 mM) were added to induce complex I–supported oxidative phosphorylation (Submax

OXP_{OS}_{CI} and OXP_{OS}_{CI}), and glutamate (10 mM) was added to elicit maximal complex I–supported OXP_{OS} (Max OXP_{OS}_{CI}). Succinate (10 mM) was added, eliciting maximal complex I- and II–supported OXP_{OS} (Max OXP_{OS}_{CI+II}). Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) was titrated (0.5–3.0 μ M) until maximal uncoupled complex I- and II–supported electron transfer system (Max ETS) capacity was achieved. In protocol 2, respirometry measures differed from protocol 1 by the addition of palmitoylcarnitine (25 μ M) in place of pyruvate, an ADP final concentration of 4.0 mM, and no uncoupling with FCCP was assessed. The nomenclature of these respirometry measures from protocol 1 and 2 is summarized (Supp. Table 2). Respiratory control ratio (RCR) is defined as the ratio of ADP-stimulated respiration to non-ADP (leak) respiration. The average RCR was 7.1 ± 2.9 for protocol 1 ($N = 747$) and 3.5 ± 1.7 for protocol 2 ($N = 547$).

³¹P MRS

³¹Phosphorous magnetic resonance spectroscopy (³¹P MRS) of the quadriceps was used to determine the *in vivo* rate of phosphocreatine (PCr) recovery following an acute bout of knee extensor exercise as previously described [11]. In brief, a 12" ³¹P/¹H dual-tuned, surface RF coil (PulseTeq, Limited) was used with standard one pulse experiments to capture the phosphorous spectra (levels of PCr, Pi, PDE, and ATP peak areas) of the quadriceps with a 3 Tesla MR magnet (Siemen’s Medical System—Prisma [Pittsburgh] or Skyra [Wake Forest]). The software jMRUI v.6.0 was used to determine relative changes in PCr as described [11]. ATP_{max} was calculated by fitting the post-exercise recovery of PCr, assuming a resting PCr concentration of 24.5 mM [32].

Statistical analyses

Characteristics were summarized across the three-category frailty variable (robust, intermediate, frail). Tests for linear trend of characteristics across frailty categories included linear regression for normally distributed continuous variables and two-sided Jonckheere-Terpstra for skewed and categorical variables. We tested for interaction with sex for all muscle mitochondrial measures by including the main effects (sex and the muscle mitochondrial measures) and

their product (sex*muscle mitochondrial measure); all p -interaction > 0.10 . Spearman's correlation coefficients were used to quantify the bivariate relationships between MAT-sf and phenotypic frailty scores.

Associations of muscle mitochondrial energetics with frailty scores were examined with linear regression models. Muscle mitochondrial energetics was presented as adjusted means along with their 95% confidence intervals (CI) across the three categories of frailty. Where we presented results on linear trend across frailty categories, frailty was used as an independent, ordinal variable in models; some tests were performed comparing categories to the reference (robust). Results from models with 1 unit increase in frailty (and by each component) were shown as beta coefficients with 95% CI. We also estimated the proportional odds ratio (POR) for greater frailty status, expressed per 1 standard deviation (SD) decrement of muscle mitochondrial energetics. Each measure was examined in a separate model; the proportionality assumption was met for all models, allowing us to report a single odds ratio that reflects the likelihood of being a greater frailty category. We report two sets of adjusted models: model 1 adjusted for technician/site; model 2 adjusted for technician/site, age, self-reported sex, race, education, marital status, adiposity (ASAT and VAT), height, smoking, alcohol use, and number of multimorbidity conditions. At one site, one technician performed all respirometry measures while several technicians ($N = 6$) contributed at the other site. To control for both site and technician, we created a categorical variable that grouped site and technicians, enabling a singular but combined adjustment. Analyses were completed in SAS version 9.4.

Analysis sample

Of the 879 participants who enrolled in SOMMA and completed baseline measurements (Fig. 1), only six participants did not have usable data for both high-resolution respirometry of permeabilized muscle fibers and ^{31}P MRS of quadriceps. Some ($N = 61$) did not have respirometry performed, and others ($N = 34$) were missing this measure due to safety reasons, leaving a total of 778 participants with frailty and respirometry data. A few ($N = 20$) were missing ^{31}P MRS data which resulted in a total of 853 participants with the ^{31}P MRS measures and frailty assessment.

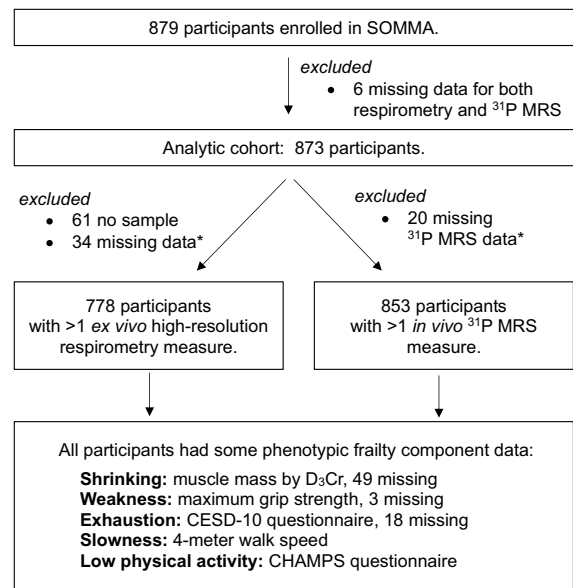


Fig. 1 SOMMA participants with data for frailty and muscle mitochondrial energetics. Participants with missing data for these measures were due to *safety reasons preventing sampling and testing, or the data could not be calculated. CESD-10, 10-Item Center for Epidemiological Studies Depression Scale; CHAMPS, Community Health Activities Model Program for Seniors; D_3Cr , D_3 -creatine dilution method; ^{31}P MRS, phosphorous magnetic resonance spectroscopy; respirometry, high-resolution respirometry; SOMMA, Study of Muscle, Mobility and Aging

Results

At baseline, 397 (45.5%) SOMMA older adults were categorized as robust, 410 (47.0%) were intermediate, and 66 (7.6%) were frail (Table 1). As expected, the individual measurements used to define the frailty index were related to frailty level. Those with greater levels of frailty had lower muscle mass, weaker grip strength, slower 4-m walk speed, lower physical activity, and were more likely to report exhaustion. Those with greater levels of frailty had less education, lower alcohol use, greater adiposity (ASAT, VAT, BMI), older age, greater multimorbidity, and higher number of medications. There was no significant interaction (p -interaction > 0.10 for all) detected when we assessed the interaction of sex and the muscle mitochondrial energetics' measures. We also show the muscle mitochondrial bioenergetic capacities across MAT-sf scores and by frailty status (Supplementary Figure 2).

Table 1 Baseline characteristics of SOMMA participants (aged ≥ 70 years) by frailty status

Characteristics	Frailty status			Overall ($N = 873$)	p trend
	Robust ($N = 397$)	Intermediate ($N = 410$)	Frail ($N = 66$)		
Phenotypic frailty components:					
Shrinking: D ₃ Cr muscle mass, kg/wt	23.36 \pm 6.57	21.37 \pm 6.29	18.13 \pm 4.81	22.05 \pm 6.48	< .0001
Weakness: Grip strength, kg	29.23 \pm 8.67	24.72 \pm 8.83	19.89 \pm 7.42	26.40 \pm 9.11	< .0001
Slowness: 4m walk speed, m/s	1.13 \pm 0.19	0.99 \pm 0.18	0.80 \pm 0.10	1.04 \pm 0.20	< .0001
Low activity: Physical activity ^a , cal/wk	4727.01 \pm 2836.26	3201.42 \pm 3086.28	1441.47 \pm 1521.84	3762.13 \pm 3045.12	< .0001
Exhaustion: “yes”	0	9 (2.2)	5 (8.3)	14 (1.6)	< .0001
Site:					0.12
Pittsburgh	207 (52.1)	203 (49.5)	26 (39.4)	436 (49.9)	
Wake Forest	190 (47.9)	207 (50.5)	40 (60.6)	437 (50.1)	
Age, years	75.34 \pm 4.44	76.81 \pm 5.05	79.52 \pm 6.17	76.34 \pm 5.01	< .0001
Women	232 (58.4)	244 (59.5)	40 (60.6)	516 (59.1)	0.69
Non-Hispanic White	339 (85.4)	343 (83.7)	54 (81.8)	736 (84.3)	0.38
Not Married	170 (43.0)	216 (52.8)	41 (63.1)	427 (49.1)	< .001
Education:					< .0001
\leq High school/ other	30 (7.6)	73 (18.1)	17 (26.6)	120 (13.9)	
Some college	88 (22.2)	104 (25.7)	16 (25.0)	208 (24.1)	
College graduate	108 (27.3)	102 (25.3)	13 (20.3)	223 (25.8)	
Post college work	170 (42.9)	125 (30.9)	18 (28.1)	313 (36.2)	
Never smoked	223 (56.3)	229 (56.1)	38 (59.4)	490 (56.5)	0.84
Alcohol, drinks/wk	3.39 \pm 4.61	2.28 \pm 4.08	1.76 \pm 5.48	2.76 \pm 4.47	< .0001
ASAT volume, L	7.08 \pm 2.99	8.04 \pm 3.24	8.58 \pm 3.48	7.64 \pm 3.19	< .0001
VAT volume, L	3.88 \pm 2.16	4.46 \pm 2.34	4.86 \pm 2.44	4.22 \pm 2.29	< .0001
Body mass index, kg/m ²	26.64 \pm 4.19	28.29 \pm 4.68	29.06 \pm 4.96	27.60 \pm 4.57	< .0001
Height loss since age 25, m	0.04 \pm 0.03	0.04 \pm 0.04	0.05 \pm 0.05	0.04 \pm 0.04	< .0001
Multimorbidity, (0-11)	0.73 \pm 0.78	0.82 \pm 0.89	1.14 \pm 1.11	0.80 \pm 0.86	0.04
Rx medications, past 30 days, (0–20)	3.69 \pm 2.98	4.85 \pm 3.59	5.80 \pm 3.95	4.39 \pm 3.42	< .0001

All characteristics reported as mean \pm SD or N (%). P value for trends across frailty status (robust, intermediate, frail) for each characteristic

D₃Cr D₃ creatine, ASAT abdominal subcutaneous adipose tissue, VAT visceral adipose tissue.

^aPhysical activity derived from Community Health Model Activities Program for Seniors (CHAMPS) questionnaire where total energy expenditure of all exercises was summed

Lower skeletal muscle mitochondrial energetics, whether determined by *in vivo* or *ex vivo* measures, associated with greater frailty in minimally (site or technician) adjusted models (Model 1, Table 2). The fully adjusted results (model 2) were similar to the minimally adjusted with a few exceptions. Mean values of Leak_{CI}, Max ETS_{CI+CII}, OXPHOS_{CI+FAO}, Max OXPHOS_{CI+CII+FAO}, and ATP_{max} became non-significant after further adjustment (Table 2; Fig. 2).

These data show that lower muscle mitochondrial energetics is associated with greater frailty in older adults independent of site/technician, age, sex, race, education, marital status, adiposity, height, smoking, alcohol use, and multimorbidity burden. To assess the likelihood of increasing frailty with decreasing levels of muscle energetics, we used proportional odds models. We found that every 1 SD decrement of most measures of muscle mitochondrial energetics was

Table 2 Adjusted means of muscle mitochondrial energetics by frailty in participants (age ≥ 70 yrs)

	<i>M</i>	<i>N</i>	Beta (95% CI) Per 1 unit	Adjusted means (95% CI)			<i>p</i> trend
				Robust (0)	Intermediate (1–2)	Frail (3+)	
Leak _{CI}	1	747	− 0.28 (− 0.44, − 0.13)***	5.24 (5.03, 5.46)	4.77 (4.56, 4.99)**	4.47 (3.93, 5.02)*	< 0.001
	2	687	− 0.15 (− 0.32, 0.02)	5.13 (4.91, 5.36)	4.84 (4.62, 5.06)	4.78 (4.19, 5.38)	0.08
Submax OXPHOS _{CI}	1	612	− 0.71 (− 1.08, − 0.35)***	12.85 (12.38, 13.33)	12.05 (11.53, 12.57)*	10.36 (8.98, 11.74)***	< 0.001
	2	569	− 0.50 (− 0.92, − 0.09)*	12.74 (12.24, 13.24)	12.23 (11.68, 12.78)	10.91 (9.41, 12.40)*	0.03
OXPHOS _{CI}	1	747	− 2.21 (− 3.03, − 1.38)***	32.50 (31.33, 33.66)	29.48 (28.32, 30.64)***	25.27 (22.30, 28.23)***	< 0.0001
	2	687	− 1.37 (− 2.29, − 0.46)**	31.86 (30.67, 33.05)	30.01 (28.82, 31.20)*	27.24 (24.04, 30.44)**	< 0.01
Max OXPHOS _{CI}	1	743	− 2.24 (− 3.11, − 1.37)***	35.90 (34.67, 37.13)	32.80 (31.58, 34.01)***	28.52 (25.41, 31.64)***	< 0.0001
	2	684	− 1.40 (− 2.37, − 0.43)**	35.32 (34.05, 36.58)	33.33 (32.07, 34.59)*	30.53 (27.15, 33.91)*	< 0.01
Max OXPHOS _{CI+CIII}	1	745	− 4.05 (− 5.37, − 2.73)***	63.52 (61.65, 65.39)	57.22 (55.36, 59.08)***	51.22 (46.47, 55.97)***	< 0.0001
	2	686	− 2.82 (− 4.25, − 1.38)***	62.72 (60.85, 64.59)	58.14 (56.27, 60.00)**	54.40 (49.39, 59.42)**	0.0001
Max ETS _{CI+CIII}	1	612	− 3.24 (− 5.12, − 1.37)***	82.39 (79.95, 84.83)	79.02 (76.37, 81.68)	69.47 (62.42, 76.53)***	< 0.01
	2	570	− 1.88 (− 3.90, 0.15)	81.51 (79.06, 83.95)	79.99 (77.30, 82.67)	72.18 (64.84, 79.51)*	0.06
Leak _{CI+FAO}	1	547	− 0.12 (− 0.29, 0.05)	4.58 (4.35, 4.82)	4.48 (4.26, 4.71)	3.71 (3.07, 4.35)*	0.06
	2	505	− 0.01 (− 0.20, 0.17)	4.44 (4.19, 4.69)	4.49 (4.26, 4.72)	3.89 (3.23, 4.54)	0.47
OXPHOS _{CI+FAO}	1	547	− 0.59 (− 1.00, − 0.17)**	13.41 (12.83, 13.99)	12.34 (11.80, 12.88)**	11.46 (9.89, 13.02)*	< 0.01
	2	505	− 0.34 (− 0.80, 0.11)	13.23 (12.61, 13.85)	12.49 (11.92, 13.06)	12.02 (10.40, 13.64)	0.06
Max OXPHOS _{CI+FAO}	1	546	− 1.25 (− 2.06, − 0.43)**	29.31 (28.16, 30.46)	27.06 (25.99, 28.14)**	25.07 (21.97, 28.16)*	0.001
	2	504	− 0.76 (− 1.64, 0.13)	28.94 (27.74, 30.14)	27.32 (26.21, 28.43)	26.44 (23.28, 29.60)	0.04
Max OXPHOS _{CI+CIII+FAO}	1	545	− 2.68 (− 4.20, − 1.17)***	60.19 (58.04, 62.34)	55.60 (53.59, 57.61)**	51.54 (45.76, 57.31)**	< 0.001
	2	504	− 1.16 (− 2.75, 0.43)	58.84 (56.69, 61.00)	56.25 (54.27, 58.24)	55.23 (49.58, 60.88)	0.08
ATP _{max}	1	812	− 0.02 (− 0.03, − 0.01)***	0.56 (0.54, 0.57)	0.53 (0.52, 0.54)**	0.50 (0.46, 0.54)**	< 0.001
	2	752	− 0.01 (− 0.02, 0.00)	0.55 (0.54, 0.57)	0.53 (0.52, 0.55)	0.53 (0.49, 0.57)	0.13

Model 1 was adjusted for technician/site; model 2 was adjusted for model 1 + age, sex, race, education, marital status, adiposity, height, smoking, alcohol, and multimorbidity. T-distribution was used to compare a specific frailty category to the referent (0 = robust) or per 1 unit increase in frailty

CI/III complex *III*, *OXPHOS* oxidative phosphorylation, *ETS* electron transport system, *FAO* fatty-acid oxidation, *PCr* phosphocreatine, *ATP_{max}* maximal adenosine triphosphate

* < 0.05; ** < 0.01; *** < 0.001

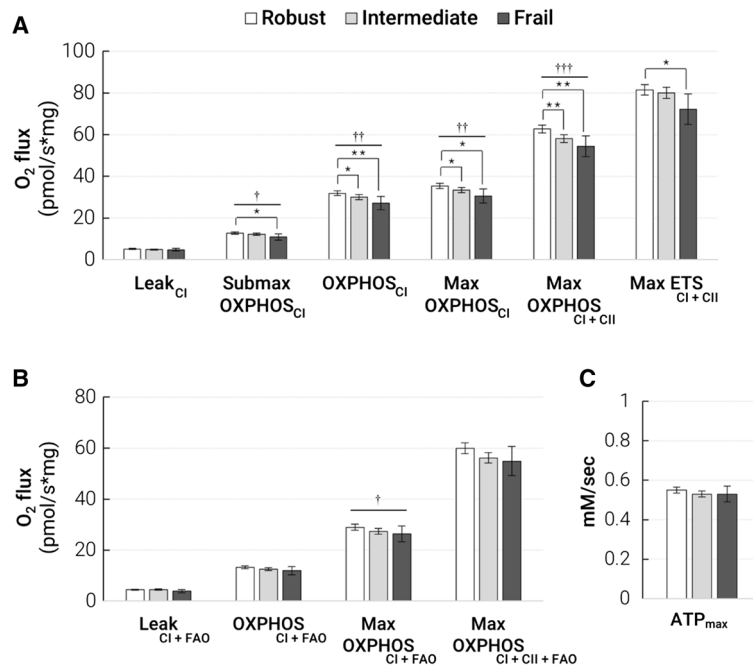


Fig. 2 Lower muscle mitochondrial energetics is associated with greater frailty in SOMMA older adults. Multivariate-adjusted means (age, sex, race, education, marital status, adiposity, height, smoking, alcohol, multimorbidity) of muscle mitochondrial measures in SOMMA older adults (age ≥ 70 years) by frailty status are shown with 95% confidence intervals (CI). **A** Oxidative phosphorylation (OXPHOS) measured with protocol 1 (carbohydrates) in permeabilized myofibers by high-resolution respirometry, $N \geq 569$ for all measures. **B**

OXPHOS measured with protocol 2 (carbohydrates and fatty acid (FAO)), $N \geq 504$ for all measures. **C** P^{31} MRS measure of maximal adenosine triphosphate production (ATP_{max}), $N \geq 534$. Significance levels for the comparison of the referent group (robust) to the intermediate and frail groups are denoted as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. The p -trend across the three groups were denoted with † $p < 0.05$; †† $p < 0.01$; ††† $p < 0.001$. Details of adjusted means and variable names are available in Supplementary Tables 2 and 3

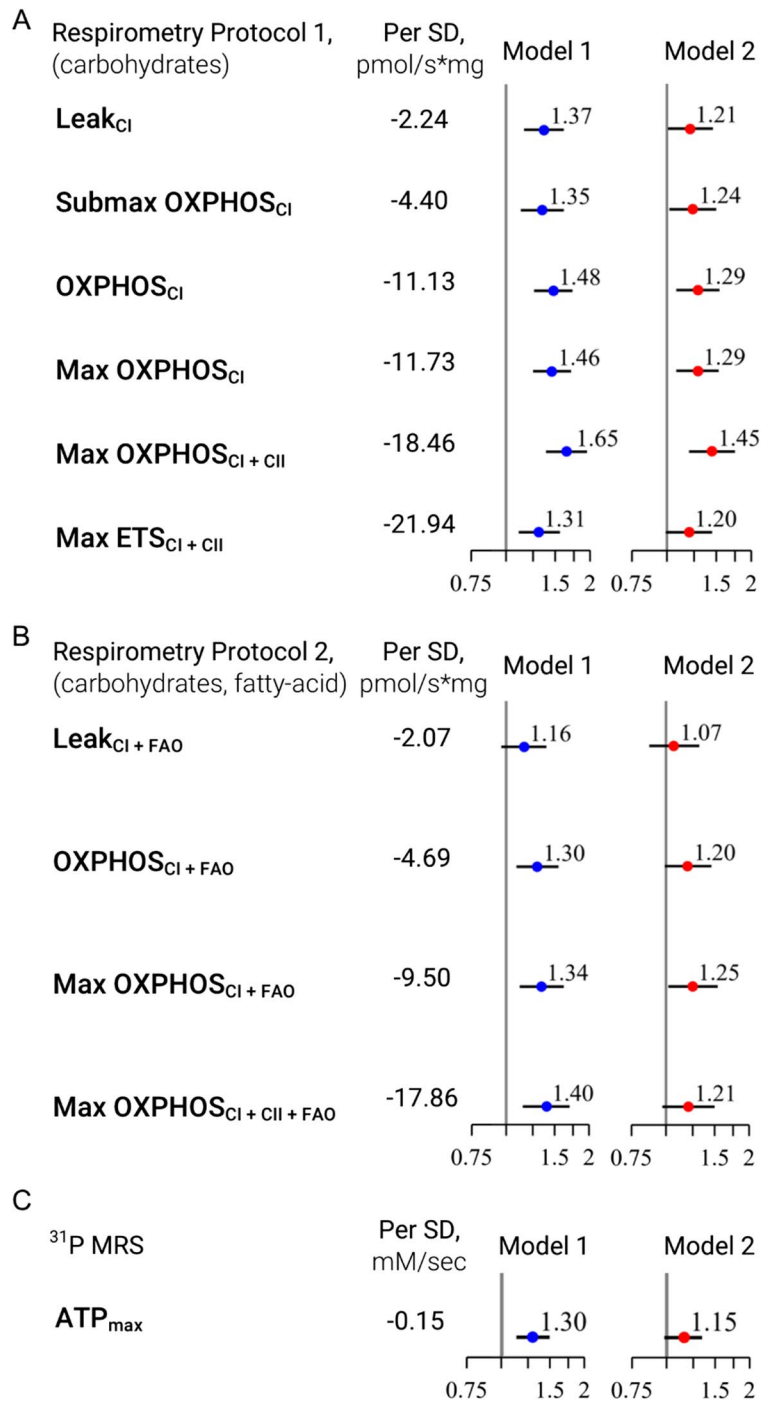
associated with a higher likelihood of greater frailty (Fig. 3). Most of these associations retained statistical significance in fully adjusted models.

To understand which frailty components may have the strongest relationships with muscle mitochondrial energetics, we also determined the means for each of the muscle mitochondrial variables according to the presence or absence of each individual frailty component (i.e., shrinking, weakness, exhaustion, slowness, and low physical activity). The fully adjusted means are shown (Fig. 4) with statistics reported for both the minimally (model 1; denoted with *) versus fully adjusted (model 2; denoted with †) models. Muscle mitochondrial energetics differed most consistently based on the presence versus absence of slowness (walking speed) and low physical activity (CHAMPS questionnaire). Notably, the measure of OXPPOS_{C1} differed by the presence of nearly all the frailty components except

exhaustion—though this may have been due to very small number of participants ($N = 9$) endorsing this component. This data suggests that the associations of muscle mitochondrial energetics and frailty could be primarily driven by lower muscle energetics associated with low physical activity and/or low gait speed.

In sensitivity analyses, we explored two alternative definitions of the shrinking component based on low BMI (< 20) and greater height loss and found little overlap in participants identified (Supp. Figure 1). In fact, none overlapped between all three definitions of shrinking. The frailty index using low muscle mass resulted in higher correlations with the mobility assessment tool-short form (MAT-sf) scores ($r = -0.38$) than when definitions with low BMI ($r = -0.32$) or greater height loss ($r = -0.32$, all $p < 0.001$) were used (Supp. Table 3).

Fig. 3 Older adults with lower muscle mitochondrial energetics have higher odds of greater frailty. Measures of association are proportional odds ratios (POR). Model 1 associations were adjusted for technician or site. Model 2 associations were adjusted for model 1 plus age, sex, race, education, marital status, adiposity, height, smoking, alcohol, and number of conditions. Panel shows POR of greater frailty by 1 SD decrement of **A** respirometry protocol 1 measures, $N \geq 569$, **B** respirometry protocol 2 measures, $N \geq 504$, and **C** ATP_{max} by ³¹P MRS, $N \geq 534$. Use Supplementary Table 2 for variable name reference



Discussion

Frailty may stem from individuals having diminished or scarcer energy reserves in musculoskeletal systems [8, 33, 34], where some older adults could become

frail sooner due to an accelerated onset of muscle and physical decline. This study is one of the first to employ a comprehensive assessment of *in vivo* and *ex vivo* skeletal muscle mitochondria energetics in a large group ($N = 879$) of older women and men with

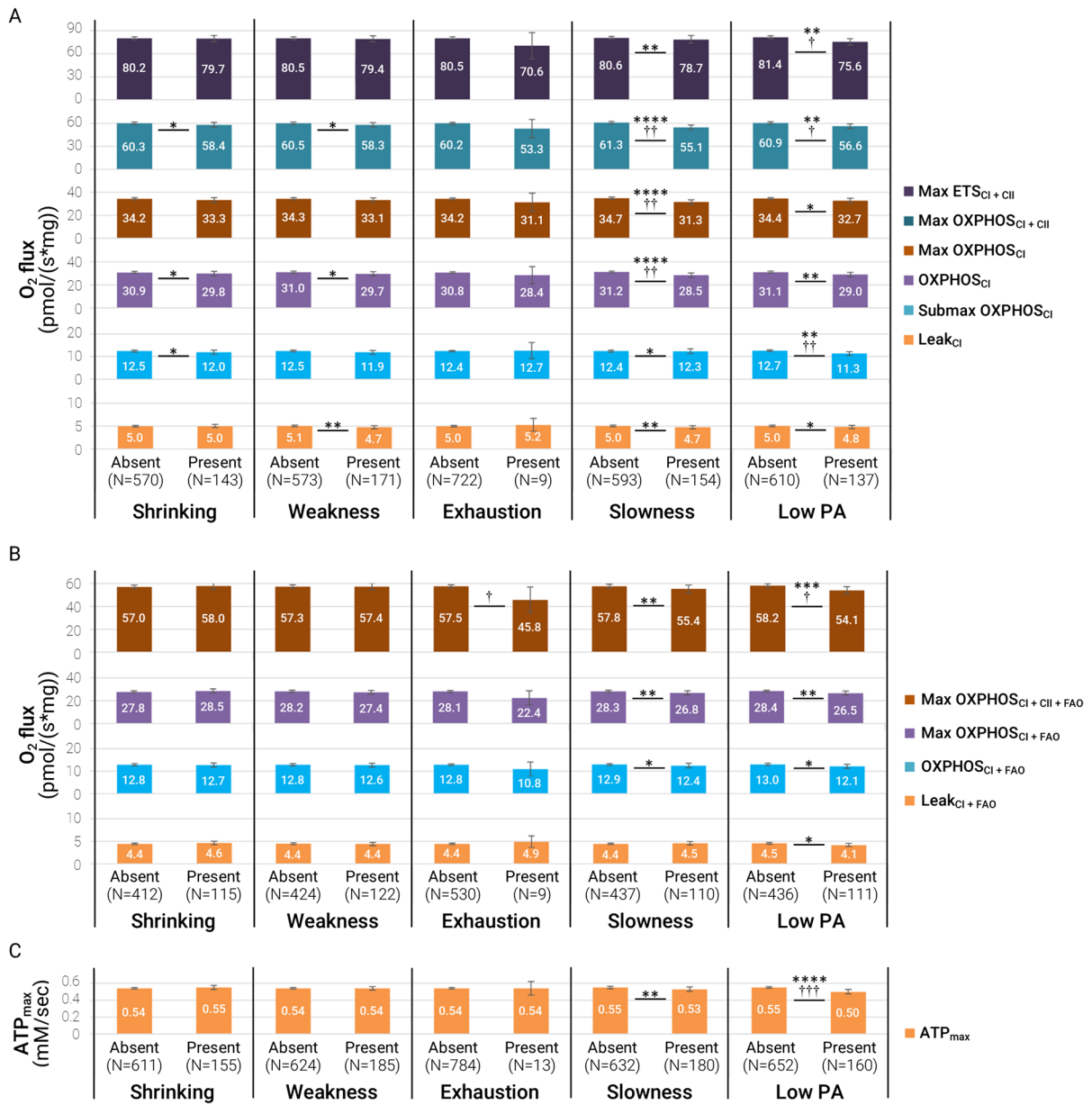


Fig. 4 Muscle mitochondrial energetics is differentially associated with phenotypic frailty components. Comparisons were between older adults with an absence versus presence of each frailty component. The bar chart shows fully adjusted means of each group with 95% confidence intervals (CI) from model 2 (site or technician, age, sex, race, education, marital status, adiposity, height, smoking, alcohol, and number of multimorbidities with corresponding p -values: † < 0.05, †† < 0.01, and †††

† < 0.001. p values from model 1 (site or technician) were also included: * < 0.05, ** < 0.01, *** < 0.001, and **** < 0.0001. **A** Protocol 1 respirometry supported by complex I- and II-linked carbohydrates, **B** protocol 2 respirometry supported by complex I- and II-linked carbohydrates and fatty-acids, and **C** ^{31}P MRS measure of ATP_{max} . The N reported is an approximation, as different participants could be missing various respirometry or ^{31}P MRS measures

wide-ranging function. Whether muscle mitochondrial energetics were assessed by *in vivo* ATP_{max} or *ex vivo* OXPPOS (supported by fatty acids and/or

complex I- and II-linked carbohydrates), we found that our data largely supported this conclusion: lower skeletal muscle mitochondrial energetics associated

with greater phenotypic frailty in older adults. To our knowledge, we are also the first to observe that these associations were driven by older adults with the presence of two specific frailty components: slowness and low physical activity. Many of these associations were independent of suspected confounders, including key demographics (i.e., age, race, sex, marital status, education), smoking, alcohol, body composition (i.e., height, abdominal subcutaneous, and visceral adipose tissue), and the burden of age-related conditions. Our data here substantiate Fried et al. [1] who discussed frailty as a state of reduced energy production and utilization; frailty is more than a phenotype and likely represents a state of compromised physiological dynamics.

Phenotypic frailty and ex vivo muscle mitochondrial energetics (respirometry)

The hypothesis that muscle mitochondrial activity and functional declines in aging contribute to the etiology of sarcopenia has been supported by a range of studies where we and others have described associations between muscle mitochondrial energetics and physical performance [10–16, 35–37]. Albeit not in the context of frailty in older adults, we and others have also demonstrated that muscle mitochondrial energetics affect walk speed [13, 14, 37], but there is less evidence supporting its relationship with grip strength [15] and muscle mass.[35] A strength of this current study is that SOMMA older adults have a wide range of function; on average, these older adults had slower gait speed and lower grip strength than the studies aforementioned [13–15]. We have shown, in a smaller study, that compared to young inactive women, frail older women have reduced muscle mitochondrial respiration due to decreased mitochondrial content [18]. Our data in this study are consistent with these antecedents. After adjusting for potential confounders and mediators, muscle mitochondrial energetics remained strongly associated with only slowness and low physical activity but not weakness and shrinking. However, the analysis does not account for older adults with only one component present versus those with multiple components. Our fully adjusted means showed a difference of approximately 5–6 pmol/s*mg between older adults with the presence of slowness or low activity versus those without. Exercise training in women with obesity

[38] and older adults [15] can improve, on average, approximately 10–15 pmol/s*mg of maximal muscle mitochondrial respiration (e.g., Max OXPHOS_{CI+CII}, Max OXPHOS_{CI+CII+FAO}, Max ETS) [39]. In the unadjusted means (not reported), the differences were approximately 10 pmol/s*mg, and this observation supported the importance of adjusting for potential confounders (i.e., sex, adiposity, multimorbidity burden). Taken together, adjusting for confounders and the overlap in older adults with a different number of components could contribute to the lower difference (5–6 pmol/s*mg) observed in these individual component analyses. Our data show that the differences in muscle mitochondrial energetics noted between those with or without low activity and slowness have comparable physiological relevance in the context of how much exercise can improve these metrics in older adults [15].

We measured muscle mitochondrial energetics supported by (1) carbohydrate and (2) fatty acid (FA) with carbohydrate energy substrates. There were some differences between both respirometry protocols in the association with frailty that warrant consideration. Aside from palmitoylcarnitine, the FA with carbohydrate respirometry protocol differed from the carbohydrate protocol in that it lacked pyruvate, which may explain the more robust difference in carbohydrate-associated respiration across frailty categories. Alternatively, as an explanation for the more subtle group differences with the FA with carbohydrate respirometry protocol, the addition of palmitoylcarnitine may have corrected for deficits in carnitine metabolism, which is essential to fatty acid oxidation and associated with frailty [8, 33]. Reports of elevated fatty acids in circulation and deposited within muscle, a feature highly predictive of mobility loss, support this [40, 41].

We measured submaximal OXPHOS_{CI} by titrating adenosine diphosphate (ADP) within a physiological range (20–165 μ m) which provides an estimate of ADP sensitivity [42]. The study that established this ADP titration method showed that muscle mitochondrial ADP insensitivity and mitochondrial reactive oxygen species (ROS) production increases with age [42]. In another study, aged mice also exhibited higher ADP insensitivity and ROS production than young mice which was linked to muscle fatigue [43]. Potential causes of age-associated insensitivity to ADP remain unknown but could be connected to

age-related changes in OXPPOS components, ADP and substrate transport, and oxidative stress damage. Our outcomes, which were adjusted by age, showed that frail older adults have lower submaximal OXPPOS_{CI}, suggesting that ADP insensitivity along with its potential effectors contribute to frailty.

Interestingly, the lower odds of frailty in submaximal OXPPOS_{CI} (POR = 1.35) compared to Max OXPPOS_{CI+CII} (POR = 1.65), suggest a role of OXPPOS regulation in frailty. Maximal OXPPOS_{CI+CII} was achieved with the addition of succinate, the excessive oxidation of which is known to suppress FAO. Among all the measures of muscle mitochondrial respiration that was lower in frail older adults, our data show that decrements in maximal OXPPOS_{CI+CII} were related to the greatest likelihood of frailty (POR = 1.65). Succinate metabolism results in higher oxygen consumption without a commensurate increase in proton shuttling to the intermembrane space compared to complex I-linked substrates. We speculate that the high odds of frailty predicted by this measure may be due to dysregulation of mitochondrial membrane potential, or related changes in mitochondrial dynamics, calcium homeostasis, or activation of the permeability transition pore (mPTP) [44, 45]. This was supported by the observation that Max ETS_{CI+CII} was not as highly predictive of frailty (POR = 1.31), a respiratory state that is measured after the addition of FCCP, which dissipates membrane potential. Succinate is also known to increase ROS production through succinate-driven reverse electron flow or transfer (RET), a process dependent on substrate availability [46]. RET remains narrowly understood with some studies showing that it contributes to disease pathology and basic physiology of mitochondrial function [47, 48]. More research is needed to resolve the most likely mechanisms responsible for lower muscle mitochondrial energetics in frail individuals. Inclusion of oxidative stress measures in future human frailty studies could also help reveal if impaired ADP sensitivity or heightened RET problematically increases ROS production—which has long been suspected to progress frailty [49].

Phenotypic frailty and *in vivo* muscle mitochondrial energetics (³¹P MRS)

Numerous studies have used *in vivo* measures of muscle mitochondrial energetics by ³¹P MRS to study

frailty in humans, an approach which does not require a muscle biopsy. In a study of active older adults, those with sarcopenia had reduced ATP/ADP at rest and lower ATP/PCR during exercise when compared to non-sarcopenic lean adults [50]. In another study of active older adults, prefrail participants had decreased ATP_{max} and lower protein expression of muscle mitochondrial respiratory complexes compared to non-frail participants [17]. ATP_{max} provides an estimate of the maximum ATP production of the mitochondria based on the rate PCr recovery following a short exercise bout. In this study, we posit that the less robust relationship between *in vivo* ATP_{max} and frailty status (POR = 1.30) than observed for maximum oxygen consumption (e.g., Max OXPPOS_{CI+CII} POR = 1.65, Max OXPPOS_{CI+CII+FAO} POR = 1.40) could be that these differences in ATP_{max} across groups are an underestimate of the actual difference. The estimate of ATP_{max} assumed that ATP and PCr concentrations are constant across study participants. There is some evidence that these concentrations may differ at rest and during exercise in older adults [51, 52], and a recent study showed that the rate of substrate decline, not substrate concentrations, differed during exercise in older adults with frailty compared to those without [9]. A lower ATP or PCr concentration in frail individuals would lead to greater differences in the calculated ATP_{max} for a given PCr recovery rate [53]. The more modest relationship between *in vivo* ATP_{max} and frailty than observed for the respiration measures may indicate a few things. Perhaps when compared to other aspects of mitochondrial biology that are implicated in the ATP_{max} assessment, such as redox homeostasis, intermediate metabolite regulation, or calcium metabolism, the activity of the ETS captured by respirometry assessment seems to be more important. The direct comparison of these aspects of muscle mitochondrial biology and how they may differentially relate to frailty warrants further study.

Our study reveals the importance of understanding how different physical frailty phenotype components likely have varying relationships with different underlying biological drivers. This is a critical insight that has, to date, remained narrowly explored and yet is important if molecular therapeutic targets are to be identified to treat frailty. Important to note, a caveat to this cross-sectional analysis, including how frailty components were operationalized, is that it suggests that lower physical activity, a frailty

component, precedes frailty development. However, it should be acknowledged that some of these associations are potentially bidirectional—for example, studies have shown that physical activity levels explain the differences of muscle mitochondrial energetics in older adults [10, 54]. Aside from muscle mitochondrial energetics, the three components—shrinking, weakness, and perhaps exhaustion—are most likely explained by other biological factors involved in these components of frailty. Reductions in mitochondrial content [17, 18], denervation and myofiber cross-sectional area [18], reduction in genes involved in fatty acid oxidation [17, 33], and reactive oxygen species emission [18] have all been identified as potential contributors to the frailty phenotype.

Skeletal muscle mitochondrial content has been correlated with mitochondrial oxidative capacity in other studies [55, 56], but we did not measure mitochondrial content in the present study. Therefore, the associations between mitochondrial energetics and frailty could be conceivably attributed to mitochondrial content, intrinsic mitochondrial functional properties, or a combination of both. Despite this limitation, the key metric most relevant for cellular bioenergetics is the total oxidative capacity, which was the outcome measured in this analysis. Other studies have also used creatine kinase (CK) energetic clamps to account for the physiological flux of substrate-product by titrating the extra-mitochondrial ATP/ADP ratio while measuring mitochondrial respiration *in vitro* [57, 58]. Energetic clamp approaches to measure respiration at subsaturated ADP levels may provide a closer analog to *in vivo* ATP_{max} measurements, which represent ATP production under conditions that better represent steady-state energy demand. Our data show weak associations of *in vivo* ATP_{max} with frailty which may be better resolved in future studies by including similar CK energetic clamps in the *ex vivo* respiration measures. These analyses focused on the physical frailty phenotype, and we acknowledge that the definitions of frailty are varied and include other physiological and cognitive reserves across multiple organ systems beyond the muscle. Many studies have used unintentional weight loss or lower body mass index (BMI) in frailty scores, but data on unintentional weight loss was absent here; therefore, after exploring alternative definitions for shrinking, low muscle mass was elected for its stronger correlations with physical performance assessments (e.g.,

MAT-sf) along with the rationale that it would most closely approximate sarcopenia—the original target of the “shrinking” component [2]. The analyses adjusted for multimorbidity burden but not prescription medications which could pose a limitation to our results. Future studies should explore the specific impact of different medications on mitochondrial respiration which would enable controlling for this in future analyses. We also recognize the limitation that the SOMMA cohort is predominantly non-Hispanic white, and the study’s inclusion criteria limit generalizability of our results. Nevertheless, the cohort is extremely well-phenotyped demographically and clinically. The SOMMA cohort enabled including both *in vivo* and *ex vivo* measures of muscle mitochondrial energetics, and participants expressed a wide range of physical and muscle functional outcomes.

In conclusion, we showed that poorer muscle mitochondrial energetics had a significant association with worse phenotypic frailty. Functional metrics in future frailty definitions may better reflect stress responses (e.g., recovery from surgery or illness). We anticipate that the efforts underway to better define vulnerability to stress seen in frail older adults by measuring resilience could also strengthen the relevance of measured biological drivers. Future work should include longitudinal assessments that would enable testing if changes in muscle mitochondrial energetics predict the onset or progression in frailty in older adults, which if it does would provide grounds for targeting the improvement of muscle mitochondrial energetics in older adults to delay frailty. While the other aspects of muscle mitochondria we mentioned (i.e., fiber type, reactive oxygen species emission) may be influential in precipitating frailty, we showed here that lower muscle mitochondrial energetics in older adults could be important in the etiology of frailty in aging.

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Author contribution Using Contributor Roles Taxonomy (CRediT), TM led the writing team for the original draft; HNB and TLB were co-analysts who led formal analyses. Key conceptualization to this study involved ABN, SBK, PMCa, and TLB. HNB managed data curation. PMCa, PAK, SRB, DJM, and SVR provided the most critical writing review and edits that led to the improvement of the manuscript. DJM, PAK, DG, and PMCo all contributed with scientific validation and verification while DEF, FGST, and SRB proofread multiple drafts of

the manuscript. SRC, PMCa, ABN, SBK, and RTH enabled the study with either funding acquisition, project administration, supervision, and/or conceptualization to the study. All authors reviewed and approved of this manuscript.

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Data availability This manuscript analysis dataset is available on request at (<https://sommaonline.ucsf.edu/>).

Declarations

Conflict of interest SRC and PMCa are consultants to Bioage Labs. PMCa is a consultant to and owns stock in MyoCorps. All other authors report no potential competing or conflicts of interests.

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