JCI insight

CLINICAL MEDICINE

Platelet bioenergetics correlate with muscle energetics and are altered in older adults

Andrea Braganza,[1] Catherine G. Corey,[1] Adam J. Santanasto,[2] Giovanna Distefano,[3], Paul M. Coen,[3] Nancy W. Glynn,[2] Seyed-Mehdi Nouraie,[1,4] Bret H. Goodpaster,[3] Anne B. Newman,[2] and Sruti Shiva[1,5,6]

[1]Vascular Medicine Institute, [2]Department of Epidemiology, [3]Division of Endocrinology, [4]Division of Pulmonary, Allergy, and Critical Care, Department of Medicine, [5]Department of Pharmacology and Chemical Biology, [6]Center for Metabolism and Mitochondrial Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.

BACKGROUND. Physical function decreases with age, and though bioenergetic alterations contribute to this decline, the mechanisms by which mitochondrial function changes with age remain unclear. This is partially because human mitochondrial studies require invasive procedures, such as muscle biopsies, to obtain live tissue with functional mitochondria. However, recent studies demonstrate that blood cells are potentially informative in identifying systemic bioenergetic changes. Here, we hypothesize that human platelet bioenergetics reflect bioenergetics measured in muscle biopsies.

METHODS. Bioenergetics were measured in platelets isolated from younger (18–35 years) and older (86–93 years) adults by extracellular flux analysis. Muscle biopsy respirometry and noninvasive ^{31}P-MRS were also performed in older adults.

RESULTS. Maximal and ATP-linked respiratory rate measured in platelets from older adults correlated significantly with muscle maximal respiration (r = 0.595; P = 0.003) and maximal ATP production (r = 0.643; P = 0.004; by ^{31}P-MRS) in the same individuals. Comparison of platelet bioenergetics in older and younger adults showed lower basal and ATP-linked respiration in older adults. Platelets from older adults also showed enhanced proton leak, which was due to increased protein levels of uncoupling protein 2, and correlated with gate speed (r = 0.58; P = 0.0019). While no significant difference in glycolysis was observed in older compared to younger adults, platelet glycolytic rate correlated with fatigability (r = 0.44; P = 0.016).

CONCLUSION. These data advance the mechanistic understanding of age-related changes in mitochondrial function. Further, they suggest that measuring platelet bioenergetics provides a potential supplement or surrogate for muscle biopsy measurement and may be a valuable tool to study mitochondria in age-related decline of physical function.

FUNDING. NIH grants T32HL110849 and 1R01HL133003-01A1, NIH Career Development Award K01 AG044437, the Hemophilia Center of Western Pennsylvania, and University Pittsburgh Medical Center Stimulating Pittsburgh Research in Geroscience.

Authorship note: AB and CGC contributed equally to this work.

Conflict of interest: The authors have declared that no conflict of interest exists.

Submitted: February 18, 2019
Accepted: May 16, 2019
Published: July 11, 2019.

Reference information: *JCI Insight*. 2019;4(13):e128248. https://doi.org/10.1172/jci.insight.128248.

Introduction

Maintaining physical function is vital to sustaining independence of older adults, and declining strength and increased fatigability are often characteristics of aging that precede cognitive dysfunction and other physical disabilities (1–3). Consistent with this, assessments of physical function, such as gait speed, are strongly predictive of morbidity and mortality (3).

Changes in mitochondrial function have long been associated with age-related functional decline. Data from a number of human populations and animal models demonstrate various alterations in mitochondrial morphology and content across different organs that are thought to underlie molecular mechanisms of aging, including cell senescence, oxidative stress, and chronic inflammation (4–8). However, the effect of

age on mitochondrial function has most prominently been studied in skeletal muscle, in which sarcopenia leads to loss of physical function in older adults. A number of studies have shown a decrease in mitochondrial electron transport chain content, and ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS) studies have demonstrated decreased ATP production and recovery of phosphocreatine after exercise in older adults (9–11). However, other researchers have not observed these changes (6, 12). Thus, the exact manifestations and mechanisms of mitochondrial alteration with age, and whether these mitochondrial changes directly underlie declining physical function, remain unclear.

One barrier to routine measurement of mitochondrial function in large human cohorts is the necessity to obtain sufficient quantities of live tissue. Muscle biopsy studies remain the gold standard to study human mitochondria but are highly expensive and invasive. Alternatively, ^{31}P-MRS or near infrared spectroscopy (NIRS) can be used to noninvasively measure the kinetics of ATP generation or tissue oxygen consumption and perfusion, respectively. However, ^{31}P-MRS methodology requires expensive specialized equipment, access to a magnetic resonance magnet, and expertise in analysis and interpretation. Although NIRS is relatively less costly than ^{31}P-MRS, measurements can be affected by variable amounts of adiposity in human subjects as well as differences in skin pigmentation and blood flow (13, 14).

Recent studies have demonstrated that mitochondrial function in circulating blood cells can reflect tissue mitochondrial energetics (15–17). Further, mitochondrial function in circulating platelets and peripheral blood mononuclear cells (PBMCs) correlates with some clinical parameters and physical function, respectively (15, 18–22). However, it is unclear whether platelets directly reflect mitochondrial function measured in the muscle of older human adults and whether there are measurable changes in platelet bioenergetics in young versus older adults.

Here, we hypothesized that platelet bioenergetics are altered with age, reflect skeletal muscle mitochondrial function measured by respirometry and ^{31}P-MRS, and are associated with clinical parameters of physical function in a population of older adults. We show that platelet bioenergetics in older adults correlate significantly with muscle mitochondrial function in the same cohort. Further, platelets from older adults demonstrate altered bioenergetics. The implications of these data are important for uncovering mitochondrial mechanisms of aging and for the use of platelet bioenergetics to serve as a supplement or potential surrogate to human muscle mitochondrial measurement.

Results

Platelet bioenergetic parameters reflect muscle mitochondrial function measured by muscle respirometry and ^{31}P-MRS. We first determined whether human platelet bioenergetics reflect mitochondrial function measured in muscle samples from the same individuals. We isolated platelets from individuals in the Health, Aging and Body Composition (Health ABC) cohort, which consists of older adults (88 ± 2 years; n = 32; Table 1). In these intact platelets, we assessed cellular oxygen consumption rate (OCR) by Seahorse extracellular flux (XF) analysis and calculated mitochondrial OCR by correcting for the measured nonmitochondrial OCR as previously described (18). A subset of these subjects also underwent ^{31}P-MRS to noninvasively measure skeletal muscle ATP kinetics and provided skeletal muscle biopsies for respirometry (Figure 1). Table 2 shows the muscle bioenergetic data for all subjects with measurements by ^{31}P-MRS and respiration in skeletal muscle fibers from biopsy. In the subset of subjects with platelet measurements and concomitant muscle measurements, we assessed the association between platelet and muscle bioenergetics to determine whether platelet bioenergetics reflect muscle mitochondrial function. Platelet basal OCR showed an association with muscle ATP synthesis measured by ^{31}P-MRS (r = 0.420; P = 0.032; n = 26; Table 3 and Figure 2A). This correlation was statistically significant when platelet ATP-linked OCR (calculated as basal OCR minus proton leak) was used instead of basal OCR (r = 0.643; P = 0.004; Figure 2B). Platelet proton leak, maximal respiratory capacity, and basal glycolysis did not show a significant association with ATP synthesis measured by ^{31}P-MRS (Table 3).

Muscle biopsies were also performed on a subgroup of the Health ABC cohort and muscle fiber respirometry was assessed. Comparison of platelet OCR to muscle fiber respiration in the same individuals (n = 23) showed that platelet maximal OCR correlated significantly with muscle maximal respiration (r = 0.595; P = 0.003; Table 4 and Figure 2C). In addition, platelet proton leak was significantly associated with muscle state 4 respiration (respiration in the presence of substrates for complex I but no ADP), a parameter of respiration driven by proton leak (r = 0.620; P = 0.002; Figure 2D). Platelet ATP-linked respiration showed a trend to correlation with muscle state 3 respiration (respiration in the presence of substrates and ADP), but this did not reach statistical significance after correction for multiple comparisons (r = 0.568; P = 0.005; Table 4).

Table 1. Demographics of the young and older adults

Characteristic	Young adults (*n* = 32)	Older adults (*n* = 32)
Age, years	26 ± 5	88 ± 2
Age range, years	18–35	86–93
Female	20 (62.5)	20 (62.5)
White race	20 (62.5)	20 (62.5)
Black race	12 (37.5)	12 (37.5)
BMI, kg/m^2	27 ± 4.8	27 ± 3.9

Values in the table denote mean ± SD or *n* (%).

Platelets from older adults show greater proton leak and less ATP-linked respiration than platelets from young individuals. Given that platelet respiration reflected muscle respiration, we next compared basal glycolytic rate and mitochondrial OCR in intact platelets isolated from the cohort of older adults (88 ± 2 years; n = 32) with platelets isolated from a younger cohort (26 ± 5 years; n = 32). Demographics for young and older adult subjects are shown in Table 1. Basal OCR was lower in platelets from older adults compared with younger adults (107.4 ± 5.29 vs. 123.6 ± 6.083 pmolO_2/min/5 × 10^7 platelets; P = 0.047; Figure 3A). We next inhibited ATP synthesis with oligomycin to measure OCR not linked to ATP production, which is traditionally attributed to proton leak across the inner mitochondrial membrane. Proton leak was significantly higher in older adults compared with the young adults (39.78 ± 2.70 vs. 30.34 ± 2.32 pmolO_2/min/5 × 10^7 platelets; P = 0.010; Figure 3B). ATP-linked OCR (or efficient respiration), calculated as the difference between basal OCR and proton leak, was significantly lower in older adults compared with young adults (74.9 ± 4.61 vs. 112 ± 7.58 pmol O_2/min/5 × 10^7 platelets; P = 0.001; Figure 3C). However, there was no significant difference in the maximal capacity of respiration between the 2 groups (177.4 ± 15.01 vs. 214.5 ± 15.57 pmolO_2/min/5 × 10^7 platelets; P = 0.091; Figure 3D).

To determine whether glycolysis was increased in the older adults, we next calculated the basal glycolytic rate by measuring the extracellular acidification rate (ECAR) of intact platelets, which could be inhibited by the glycolytic inhibitor 2-deoxyglucose (2-DG). There was no significant difference in basal glycolytic rate between the older and young adults (5.85 ± 0.77 vs. 6.19 ± 0.39 measured pH/min/5 × 10^7 platelets; P = 0.69; Figure 3E).

Platelets from older adults show lower enzymatic activity of the electron transport chain complexes and higher uncoupling protein 2 expression. To determine whether changes in mitochondrial proteins potentially underlie increased proton leak and lower ATP-linked respiration in older adults, we measured the protein levels and enzymatic activity of the platelet mitochondrial electron transport chain complexes. Within the electron transport chain, lower levels of complex III protein were observed in the older adults compared with the young adults, while there was no significant change in the protein levels of complexes I, II, IV, and V (Figure 4, A–B). Measurement of the individual enzymatic activity of each electron transport complex showed that the enzymatic activities of complexes II, III, and V were significantly lower in the older adults compared with the young adults (Figure 4C). When associations were tested between complex activities and platelet bioenergetic parameters, no significant correlation was found (Table 5).

Uncoupling proteins (UCPs) are present in the inner mitochondrial membrane and allow the entry of protons into the mitochondrial matrix, resulting in both the generation of heat and attenuation of oxidant production (23, 24). To determine whether the increased proton leak observed in older adults was due to upregulation of UCPs, we measured the protein abundance of UCP2. Protein levels of UCP2 were significantly greater in the platelets from the older adults than young adults (Figure 4, D and E). Further, the protein level of UCP2 showed a significant positive correlation with proton leak in the older adults (r = 0.632; P = 0.001; Figure 4F).

Table 2. Muscle bioenergetic data

	Range	Mean	SD	*n*
ATP_{max} by ^{31}P-MRS	0.25–0.66	0.47	0.10	26
State 4 (pmol/s × mg DW)	11.0–57.7	26.8	9.5	23
State 3 (pmol/s × mg DW)	113–396	234	71.6	23
Maximal (pmol/s × mg DW)	122–423	271	88.9	23

DW, dry weight.

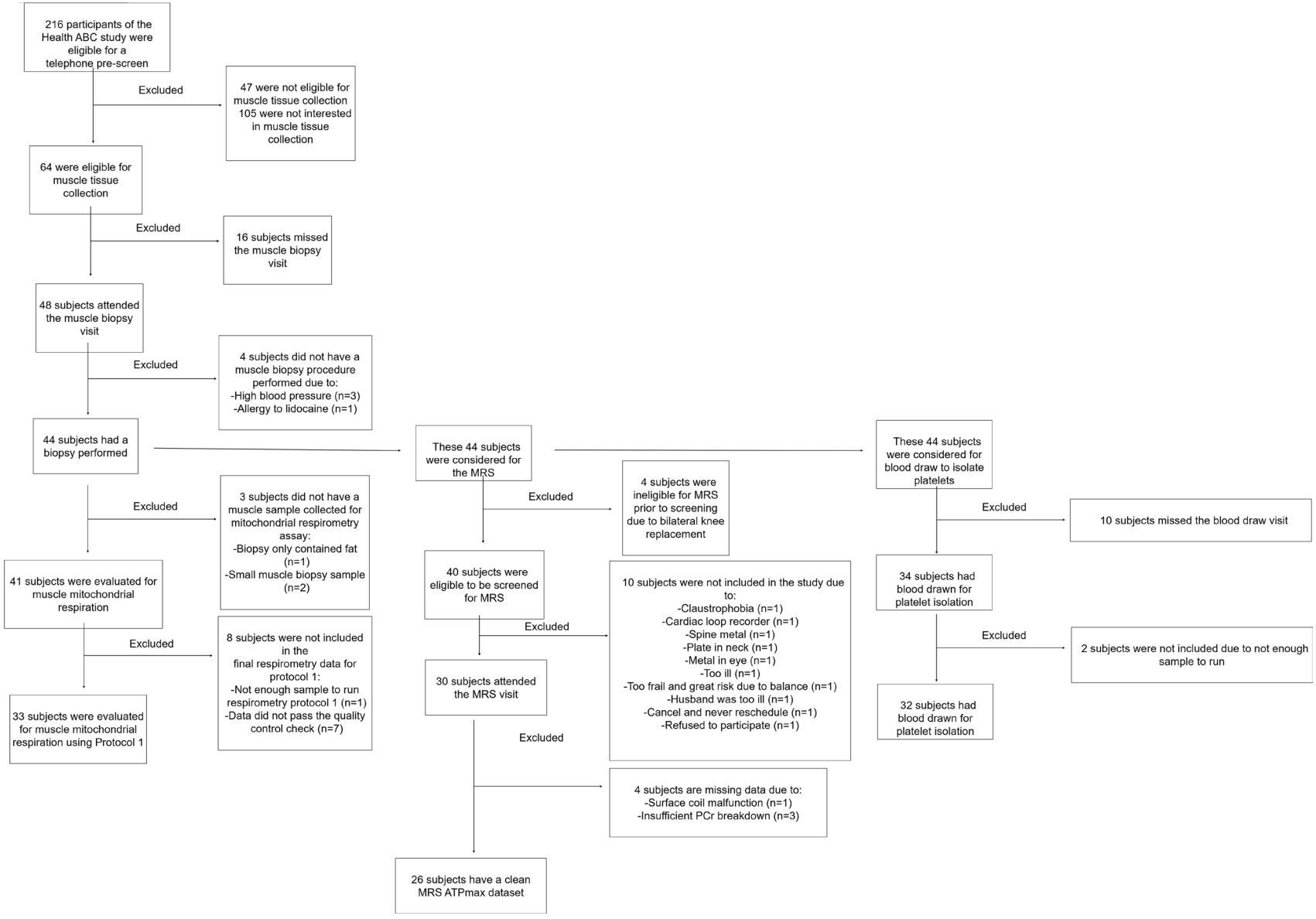

Figure 1. Subject enrollment and completion of the study endpoints. Of 216 Health ABC subjects who were eligible for phone screen for muscle biopsy studies, biopsies were ultimately obtained from 44 subjects. Of these biopsies, 33 were ultimately used. The 44 subjects who underwent biopsy were screened for eligibility for ^{31}P-MRS and platelet studies. The flowchart shows reasons for exclusion of subjects such that ^{31}P-MRS was obtained on 26 subjects and platelets isolated from 32 subjects. PCr, phosphocreatine.

Table 3. Pearson's correlations between platelet bioenergetics and ATP_{max} measured by ^{31}P-MRS

	r	*P*
Basal	**0.420**	**0.032**
ATP linked	**0.643**	**0.004**[A]
Proton leak	−0.037	0.854
Maximal	0.169	0.397
Basal glycolysis	−0.058	0.781

[A]$P < 0.01$ (nominal α value for 5 comparisons); $n = 26$. Bold font denotes $P < 0.05$.

Platelet bioenergetic parameters correlate with parameters of physical function and fatigability. Because platelet respiration correlated with parameters of muscle respiration, we next assessed whether platelet bioenergetics in the older adults correlated with parameters of physical function and fatigability. These associations were tested in 28 subjects because 5 individuals did not complete the physical function tests or fill out the fatigability questionnaire. There was a positive correlation between platelet basal glycolytic rate and physical fatigability score ($r = 0.451$; $P = 0.016$; Figure 5A). This correlation was stronger after controlling for sex or race (Table 6). Additionally, increased proton leak was significantly associated with faster gait speed ($r = 0.58$; $P = 0.0019$; Figure 5B), and this relationship became more significant when controlled for BMI, sex, age, or race (Table 7). In contrast, no significant associations were found between platelet basal or maximal respiration and parameters of physical function (Tables 8 and 9).

Discussion

This study demonstrates that (a) platelet bioenergetic parameters correlate significantly with muscle mitochondrial function in the same cohort; (b) compared with young adults, platelets from older adults show an alteration in mitochondrial function characterized by higher proton leak, which is likely due to upregulation of UCP2; and (c) in the older cohort, greater platelet glycolysis and higher proton leak were associated with higher perceived physical fatigability and faster gait speed, respectively.

Although this is the first report to our knowledge of bioenergetic measurements in platelets from healthy older versus young adults, the alterations in bioenergetics observed in platelets are consistent with changes observed in other tissues. For example, several studies in human skeletal muscle show decreased activity

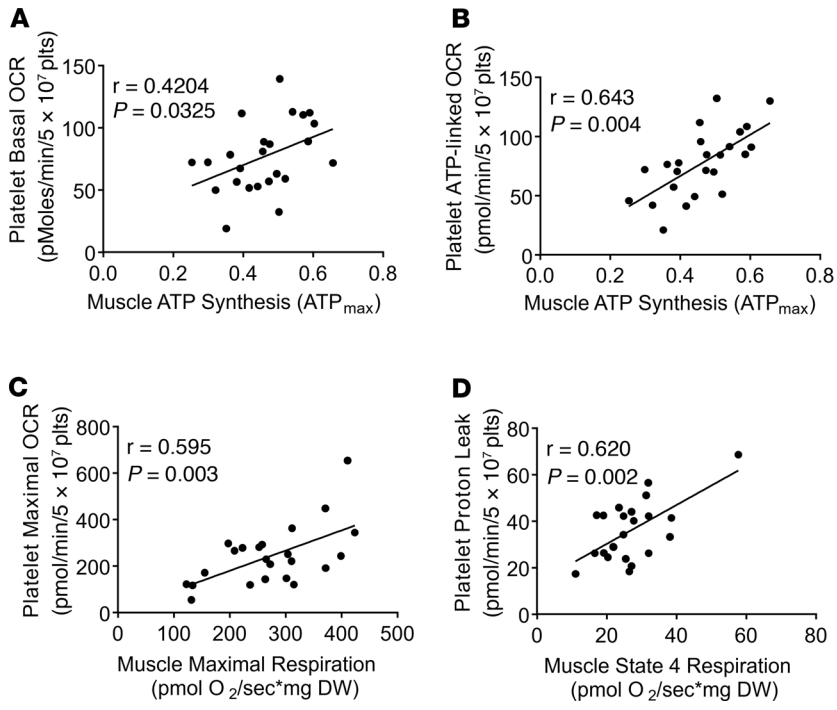

Figure 2. Platelet bioenergetic parameters correlate with muscle mitochondrial measurements in older adults. Bioenergetic parameters measured in platelets isolated from older adults were tested for associations with muscle mitochondrial measurements in the same individuals. (**A**) Correlation between platelet basal OCR (pmol/min/5 × 10^7 plts) and muscle ATP synthesis (ATP_{max}) measured by ^{31}P-MRS. $n = 26$. (**B**) Correlation between platelet ATP-linked respiration (pmol/min/5 × 10^7 plts) and muscle ATP synthesis (ATP_{max}) measured by ^{31}P-MRS. $n = 26$. (**C**) Correlation between maximal OCR in platelets versus muscle. $n = 23$. (**D**) Correlation between non–ATP-linked respiration rate (proton leak) in platelets and state 4 respiration rate in muscle. $n = 23$. All correlations are Pearson's *r*. For **A** and **B**, $P < 0.01$ is significant after multiple-comparisons correction. For **C** and **D**, $P < 0.004$ is significant after correction for multiple comparisons.

Table 4. Pearson's correlations between platelet bioenergetics and muscle energetics

	Basal		ATP linked		Proton leak		Maximal	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
State 4	0.290	0.170	0.156	0.171	**0.620**	**0.002**[A]	0.361	0.073
State 3	0.516	0.012	**0.568**	**0.005**	0.103	0.639	**0.453**	**0.030**
Maximal	0.344	0.108	0.297	0.168	0.262	0.228	**0.595**	**0.003**[A]

[A]$P < 0.004$ (nominal α value for 12 comparisons); $n = 23$. Bold font denotes $P < 0.05$.

and content of citrate synthase and electron transport in older adults (10–12, 25). Additionally, studies in permeabilized human platelets showed a weak correlation between decreased complex II activity and age in individuals between the ages of 12 and 60 (26, 27). Notably, despite lowered levels of electron transport complex enzymatic activity, our data showed no difference in maximal respiratory capacity between the platelets from older and younger cohorts. Additionally, no correlation was observed between electron transport chain activity and platelet OCR, consistent with prior publications (28). This is likely due to the fact that with the exception of complex V, mitochondrial electron transport complexes in the platelet are expressed in excess of what is required to maintain respiration (28). Thus, when uncoupled maximal respiration is measured (in which complex V activity is uncoupled from the rest of the electron transport chain), the decrease in respiration observed basally is no longer present. However, one may speculate that although mitochondrial enzymes are in excess of what is necessary for respiration, under conditions of stress that damage these enzymes, older adults may be more susceptible to respiratory dysfunction compared with young adults given that enzymatic activities are already lower in older adults. Further studies that expose young and older platelets to oxidants or cell stressors are required to test this hypothesis. In addition to complex activity levels, the source of substrate and availability govern maximal respiration. For example, we have previously shown that in subjects with pulmonary arterial hypertension, maximal respiratory capacity is increased despite no change in the majority of the electron transport complexes, and this was due to a substrate switch from glucose to fatty acid oxidation (20). A limitation of the current study is that we did not measure substrate utility or availability in the platelets. More in-depth metabolomics studies are required to test this concept.

We did not directly measure platelet ATP production in this study; however, it would be expected that the older adults would generate less ATP than the young adults by oxidative phosphorylation because ATP-linked respiration is decreased in platelets from older adults. This decrease in ATP-linked respiration is predominantly due to an increase in proton leak, which correlates with increased protein levels of UCP2. Although it is well established that UCPs decrease the efficiency of ATP production, several studies also suggest that low levels of mitochondrial uncoupling are beneficial to the cell (29, 30). UCP1 is crucial to thermogenesis in brown adipose tissue (23). However, much less is known about UCP2, though all UCPs have been shown to be upregulated by reactive oxygen species and once expressed dissipate the high membrane potential of the mitochondrial inner membrane, which can decrease oxidant production by the electron transport chain (31–34). In this regard, the upregulation of UCP2 may serve as an adaptive response to mitigate oxidant production, which is known to increase with age. This beneficial effect of UCP2 expression may also be involved in the mechanisms that associate increased proton leak in our study with increased gait speed.

We observed no significant increase of glycolysis in the older adults compared to the young adults despite a decrease in ATP-linked OCR. Although absolute glycolytic rate may not be different between the older and young adults, it is still possible that basal glycolysis may increase with age in the same individual. Longitudinal studies examining glycolytic rate are required to investigate this further. Notably, we did observe a significant correlation between glycolytic rate and fatigability score. It is interesting to speculate that this association is indicative of a shift from oxidative phosphorylation to the less efficient process of glycolysis, which may lead to an ATP deficit and contribute to fatigue. However, direct measurement of ATP production is required to definitively determine whether glycolysis is mechanistically linked to or causative of perceived physical fatigability.

Molina and colleagues previously reported that maximal respiration of PBMCs (consisting of lymphocytes and monocytes) correlate with gait speed as well as an expanded short physical performance battery in older adults (21, 22). We did not observe any significant correlation between platelet ATP production and physical function. This could be due to differences in functionality of the participants as shown by Santanasto et al. (35). Alternatively, this could be due to a difference in demographics of the participants

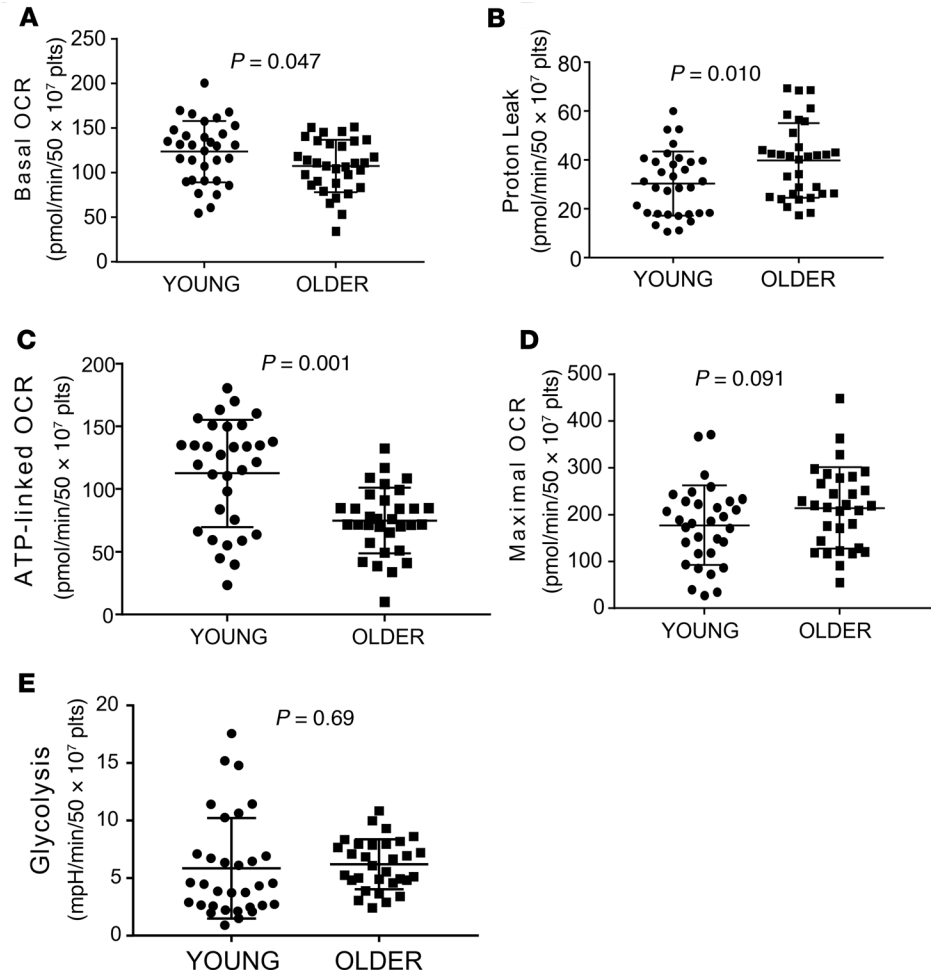

Figure 3. Platelet basal respiration is decreased and proton leak is increased in older adults. Bioenergetic parameters were measured by XF analysis in platelets isolated from young (n = 32) and older adults (n = 32). (**A**) Basal OCR was measured in intact platelets in the absence of any treatment. (**B**) Proton leak was measured in the presence of the ATP synthase inhibitor oligomycin. (**C**) ATP-linked OCR was calculated for each subject by subtracting proton leak from basal OCR. (**D**) Maximal OCR was measured in the presence of the protonophore carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) and represents the maximal capacity of respiration. (**E**) Basal glycolytic rate is the rate of extracellular acidification of platelets that is sensitive to treatment with the glycolytic inhibitor 2-DG. Each dot represents an individual subject, and the lines denote the mean ± SD. Significance was calculated by unpaired Student's t test. $P < 0.05$ was considered significant.

in the 2 studies. For example, the mean age of our study participants was greater (88 ± 2 years) compared to theirs (68 ± 4 years; refs. 21, 22). However, it is more likely due to biological differences in the cell types used (PBMCs versus platelets). Indeed, Chacko and colleagues have defined differences in the bioenergetic profiles of intact platelets versus other leukocytes (36), and it is further plausible that the aging process differentially affects each circulating cell type. This suggests that perhaps an index composed of bioenergetic measurements in both PBMCs and platelets may offer increased opportunity to more precisely assess bioenergetic health and its relation to physical function in aging.

Here we demonstrate that parameters of platelet oxidative phosphorylation correlate with similar measures in skeletal muscle by respirometry and ^{31}P-MRS. Notably, Molina and colleagues have shown in nonhuman primates that platelet and leukocyte respiratory capacity correlates with glucose metabolism measured noninvasively by ^{18}F-fluorodeoxyglucose PET imaging (16). Our study corroborates the utility of circulating cells as a potential proxy for noninvasive imaging methods and extends this concept to the use of ^{31}P-MRS in humans. Molina and colleagues also demonstrated in nonhuman primates that platelet maximal respiratory capacity correlates significantly with both maximal and state 3 res-

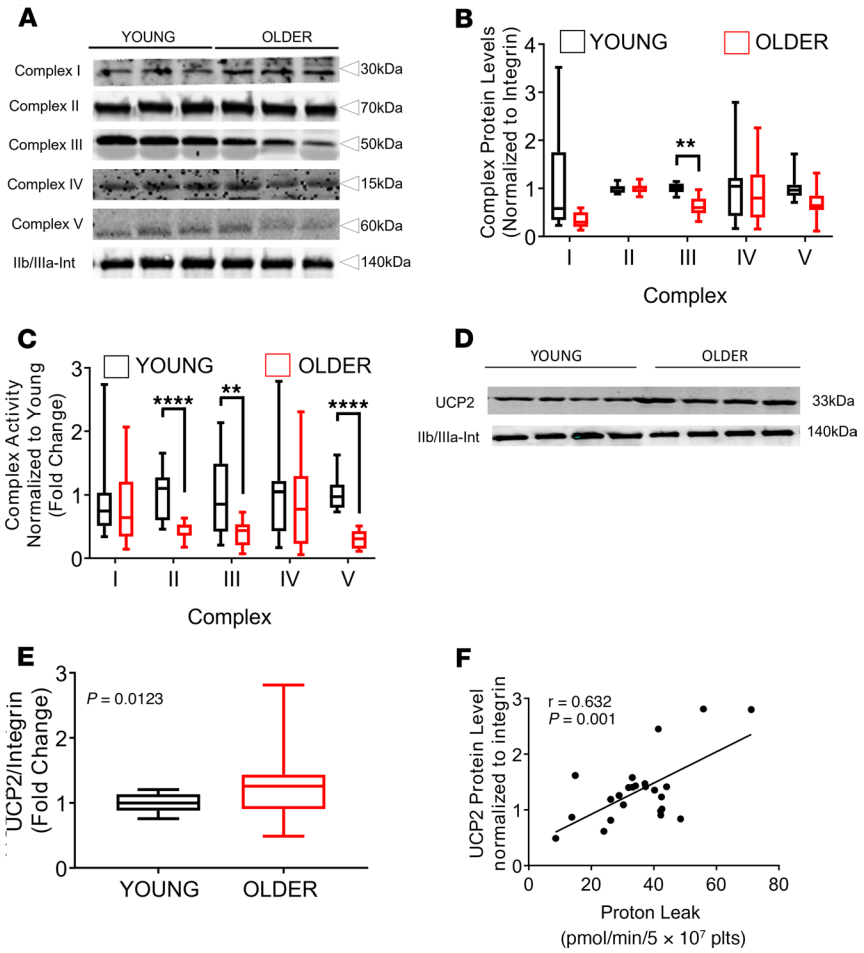

Figure 4. Platelet electron transport chain activity is decreased and UCP2 expression is increased in older adults. To determine whether changes in mitochondrial proteins (electron transport complexes and UCP2) are present in older adults versus young adults, the protein levels and activity of these proteins were measured. Western blots were performed in platelets from young and older adults to measure protein levels of electron transport complexes I, II, III, IV, and V. (**A**) Representative Western blots (representative of 20 total samples) are shown along with (**B**) quantitation of all 20 Western blots normalized to βII/αIII integrin protein levels. (**C**) Enzymatic activity of individual complexes I, II, III, IV, and V expressed as a fold change of the younger adults. (**D**) Representative Western blot (representative of 24 similar blots) showing UCP2 level in young versus older adults relative to integrin αII/βIII. (**E**) Fold change in the expression level of UCP2 (normalized to integrin) in young versus older adult platelets. Data shown in panels **B**, **C**, and **E** are box-and-whiskers plots in which the whiskers represent the range of the data, the length of the box represents the interquartile range, and the line represents the median of the data set. $^{**}P < 0.01$, and $^{****}P < 0.0001$ as determined by unpaired Student's *t* test. (**F**) Pearson's correlation of UCP2 level with platelet proton leak in older adults. $n = 24$. Pearson's $r = 0.632$, and $P = 0.001$.

piration of permeabilized skeletal muscle fibers (15). The results presented here again are consistent with previous results and extend this observation to humans. Although measurement of muscle biopsies and assessment by ^{31}P-MRS remain the gold standard in terms of methodology for the investigation of mitochondrial function, these techniques are complicated by their invasiveness (biopsy) and expense (^{31}P-MRS). Our data, consistent with prior findings by Molina and colleagues (15, 16), are compelling from a methodological standpoint in that they suggest bioenergetic measurement from a simple, less invasive, and less expensive blood draw may serve as a powerful supplement or even surrogate for the measurement of mitochondrial function from muscle biopsies or by ^{31}P-MRS. This would allow for the measurement of bioenergetics repeatedly over a longitudinal study, particularly in aged populations undergoing muscle loss in which repeated muscle biopsies are not an option.

In conclusion, we provide evidence that platelet mitochondrial function is altered with age and plate-

Table 5. Pearson's correlations between complex activities and platelet bioenergetic parameters

	Basal		Proton leak		Maximal	
	r	*P*	*r*	*P*	*r*	*P*
Complex I	0.06	0.72	0.01	0.99	0.04	0.83
Complex II	0.19	0.36	0.36	0.06	0.23	0.21
Complex III	0.12	0.55	0.05	0.79	0.42	0.06
Complex IV	0.36	0.05	0.03	0.80	0.31	0.10
Complex V	0.06	0.76	−0.05	0.80	0.01	0.61

All correlations are for $n = 20$ subjects.

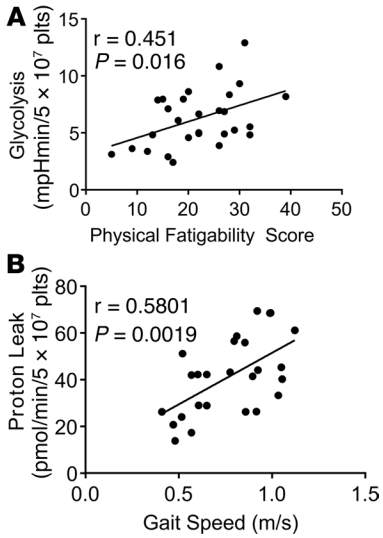

Figure 5. Parameters of platelet bioenergetics correlate with parameters of physical function and fatigability. Parameters of physical function and fatigability were measured as described in the Methods in the cohort of older adults. Bioenergetic parameters were measured in intact platelets isolated from the same cohort. Platelet bioenergetic parameters were tested for an association with parameters of physical function and fatigability measured. (**A**) Pearson's correlation between platelet basal glycolytic rate and perceived physical fatigability score in 27 subjects. Pearson's $r = 0.451$; $P = 0.016$ ($P < 0.016$ considered statistically significant after Bonferroni's correction for multiple comparisons; Table 6). (**B**) Pearson's correlation between platelet proton leak and gait speed measured in 27 subjects. Pearson's $r = 0.5801$; $P = 0.0019$ ($P < 0.016$ considered statistically significant after Bonferroni's correction for multiple comparisons; Table 7).

let bioenergetic parameters correlate with markers of physical function, perceived fatigability, as well as muscle mitochondrial function. These data suggest that measurement of platelet bioenergetics may serve as a powerful translational tool to study the mechanistic links between mitochondrial function and physical decline with age. Moreover, the use of platelet bioenergetics as a surrogate for muscle biopsies or ^{31}P-MRS may serve as a powerful clinical tool, enabling the design of large longitudinal studies in which mitochondrial measurements can be made more frequently to understand the role of the mitochondrion in aging and to monitor therapies to improve mitochondrial bioenergetics.

Methods

Materials. All chemicals were obtained from MilliporeSigma unless otherwise noted.

Study population. The study population consisted of 2 groups — young and older adults. The young adults consisted of 32 individuals (ages 18 to 35) recruited at the University of Pittsburgh via protocol 08110422 approved by the University of Pittsburgh Institutional Review Board (IRB). Young adults were recruited by advertisement, and inclusion criteria included being 18 years of age or older with no history of anemia, vascular disease, or any other diagnosed disease. Pregnant or lactating women were excluded. Older adults were a subset of the national Health ABC prospective cohort (37, 38). Health ABC enrolled 3075 Black (41.7%) and White men and women (51.5%) aged 70 to 79 years between March 1997 and April 1998, who resided in the Memphis, Tennessee, and Pittsburgh, Pennsylvania, areas. Eligibility criteria included no self-reported difficulty walking a quarter mile, climbing 10 steps, or performing activities of daily living; no reported use of a walking aid; and no active cancer treatment. Exclusion criteria included

Table 6. Pearson's and partial correlations between platelet glycolytic rate and physical function parameters

	Gait speed		Physical fatigue score		400-meter walk	
	r	*P*	*r*	*P*	*r*	*P*
Pearson's	−0.154	0.49	**0.441**	**0.016**	0.171	0.431
Partial						
BMI	−0.204	0.32	**0.43**	**0.0312**	0.274	0.13
Sex	−0.215	0.29	**0.51**	**0.0103**[A]	0.22	0.23
Race	−0.243	0.22	**0.45**	**0.0065**[A]	0.236	0.21
Age	−0.255	0.31	**0.48**	**0.0212**	0.22	0.23

[A]$P < 0.016$ (nominal α value for 3 comparisons); $n = 27$. Bold font denotes $P < 0.05$.

Table 7. Pearson's and partial correlations between platelet proton leak and physical function parameters

	Gait speed		Physical fatigue score		400-meter walk	
	r	*P*	*r*	*P*	*r*	*P*
Pearson's	**0.5803**	**0.0019[A]**	0.1419	0.5507	0.1511	0.4491
Partial						
BMI	**0.63**	**0.0002[A]**	0.24	0.43	0.23	0.23
Sex	**0.57**	**0.0007[A]**	0.26	0.37	0.28	0.38
Race	**0.58**	**0.0006[A]**	0.23	0.29	0.21	0.41
Age	**0.58**	**0.0006[A]**	0.29	0.31	0.17	0.37

[A]$P < 0.016$ (nominal α value for 3 comparisons); $n = 27$. Bold font denotes $P < 0.05$.

cognitive impairment and inability to communicate. A complete description of the eligibility criteria of the Health ABC study can be found in Goodpaster et al. (37). The older cohort in the current study was a subset of the Health ABC cohort that included 32 participants who were aged 86 to 93 at the time of the study, who were from Pittsburgh, Pennsylvania, and who completed a muscle biopsy as part of Health ABC study. In addition, the Health ABC participants for this ancillary study had to safely complete a ^{31}P-MRS measurement. The study was approved by the University of Pittsburgh IRB (IRB960212), and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. See Table 1 for demographics of young and older subjects and Figure 1 for more information on subject enrollment and completion of endpoints.

Determination of ATP kinetics by ^{31}P-MRS. Following an acute bout of knee extensor exercise, in vivo maximal mitochondrial ATP production (ATP_{max}) was determined using ^{31}P-MRS. To quantify rates of mitochondrial ATP production, PCr recovery after exercise was used. Here, participants were free of unsafe metal or other implants, were free of bilateral joint replacements, and were able to lie in a supine position for 1 hour. The exercise protocol was performed in a magnetic resonance imaging magnet (3T TIM Trio, Siemens Medical Solutions) where participants laid supine with the right knee elevated at approximately 30°. Straps were placed over the legs, and a 2.5-inch surface RF coil tuned to ^{31}P was placed over the quadriceps. Signal was collected by a hemisphere defined by the coil radius (1.25 inches). Participants kicked repeatedly as hard and as fast as they could for 2 bouts (30 and 36 seconds), each followed by a 6-minute rest. Phosphorus spectra were collected using a standard one pulse experiment to determine levels of PCr, ATP, Pi, and pH by integration using Varian VNMR 6.1C software (Varian Medical Systems) throughout exercise and recovery.

Platelet isolation. Platelets were isolated from peripheral blood samples obtained by standard venipuncture without a tourniquet, to avoid platelet activation as previously described (18, 20). Briefly, prostaglandin I_2 (1 μg/mL) was added to whole blood (to further prevent artifactual activation) before it was centrifuged (150 *g*, 10 minutes) to isolate platelet-rich plasma. A subsequent centrifugation step (1500 *g*, 10 minutes) yielded isolated platelets, which were washed with erythrocyte lysis buffer and resuspended in modified Tyrode's buffer

Table 8. Pearson's and partial correlations between platelet basal OCR and physical function parameters

	Gait speed		Physical fatigue score		400-meter walk	
	r	*P*	*r*	*P*	*r*	*P*
Pearson's	−0.091	0.67	−0.271	0.15	−0.06	0.07
Partial						
BMI	−0.112	0.51	0.035	0.85	−0.125	0.08
Sex	−0.0977	0.43	0.032	0.86	−0.121	0.09
Race	−0.0961	0.11	0.093	0.62	−0.133	0.11
Age−0.121	0.13	0.037	0.74	−0.141	0.07	

No correlations were significant as determined by $P < 0.016$ (nominal α value for 3 comparisons); $n = 27$.

Table 9. Pearson's and partial correlations between platelet maximal OCR and physical function parameters

	Gait speed		Physical fatigue score		400-meter walk	
	r	*P*	*r*	*P*	*r*	*P*
Pearson's	−0.232	0.27	−0.2714	0.14	0.04	0.82
Partial						
BMI	−0.243	0.12	−0.294	0.109	0.143	0.71
Sex	−0.251	0.09	−0.286	0.111	0.131	0.64
Race	−0.312	0.11	−0.277	0.101	0.129	0.69
Age	−0.333	0.08	−0.315	0.09	0.155	0.55

No correlations were significant as determined by $P < 0.016$ (nominal α value for 3 comparisons); $n = 27$.

(20 mmol/L HEPES, 128 mmol/L NaCl, 12 mmol/L bicarbonate, 0.4 mmol/L NaH_2PO_2, 5 mmol/L glucose, 1 mmol/L $MgCl_2$, 2.8 mmol/L KCl, pH 7.4). The purity of the isolated platelet sample was determined by measurement of CD41a expression using flow cytometry as previously described (18, 20).

Platelet bioenergetics measurements. OCR and ECAR were measured in freshly isolated platelets (5×10^7 platelets/well) within 2 hours of blood draw by XF analysis (XF24, Agilent Seahorse Technologies) as previously described (18). After measurement of basal OCR, 2.5 μmol/L oligomycin A, 0.7 μmol/L FCCP (to measure maximal OCR), and 15 μmol/L rotenone were consecutively added. Mitochondrial OCR was calculated by subtracting the rotenone-insensitive rate from the basal, proton leak, and maximal OCR. ATP-linked OCR was calculated by subtracting the proton leak from basal OCR. Basal glycolytic rate was calculated by determining the basal ECAR that was sensitive to 2-DG (100 mmol/L). The assay was performed in unbuffered Dulbecco's modified Eagle medium supplemented with 25 mmol/L glucose, 1 mM pyruvate, and 2 mmol/L glutamine. All rates were normalized to platelet number.

Western blots. Mitochondrial protein expression was measured by Western blot as previously described (18, 20). Antibodies for complex II (MS204) and complex V (MS502) were purchased from Mitoscience; complex I (ab14711), complex III (ab14745), and complex IV (ab14744) from Abcam; and citrate synthase (sc30538) and UCP2 (sc-6525) from Santa Cruz Biotechnology. Blots were imaged with a LI-COR Odyssey imaging system and analyzed using LI-COR Odyssey infrared imaging software version 3.0. Blots were reprobed with integrin αIIβ antibody (sc-166599, Santa Cruz Biotechnology) for normalization.

Electron transport chain complex activity. Enzymatic activity of complexes I, II, III, IV, and V and citrate synthase were performed by spectrophotometric kinetic assays as previously described (18, 20).

Muscle biopsy and permeabilized fiber bundle preparation. Skeletal muscle biopsy procedures occurred within 6 months of the blood draw for platelet measurements and fatigability questionnaire administration. Participants fasted overnight and did not engage in physical exercise for 48 hours before the biopsy. Percutaneous muscle biopsy samples were obtained under local anesthesia (2% buffered lidocaine) at the University of Pittsburgh's Clinical Translational Research Center and immediately prepped for mitochondrial respiration measurements as previously described (39, 40).

Skeletal muscle mitochondrial respiration. After permeabilization, the muscle fiber bundle was placed into the respirometer chamber of an Oxygraph 2K (Oroboros) and the assay run as previously described (39, 40). Once a stable baseline was acquired, 5 mmol/L pyruvate, 2 mmol/L malate, and 10 mmol/L glutamate were added to measure state 4 respiration (oxygen consumption driven by proton leak). ADP (54 mmol/L) was added to elicit complex I–supported oxidative phosphorylation. Then, 10 mmol/L succinate was added to evaluate complex I– and complex II–supported state 3 respiration. Finally, 2 μmol/L FCCP was added to determine maximal uncoupled respiratory capacity. Cytochrome *c* was used to check the quality of the muscle fiber preparation and assess the integrity of the outer mitochondrial membrane. Steady state oxygen flux for each respiratory state was determined and normalized to dried bundle weight.

Measurements of physical function and perceived fatigability. The Long Distance Corridor Walk (LDCW), an endurance walking test, was administered to the Health ABC cohort as previously described (41). The participants were asked to walk 10 laps around traffic cones placed 20 meters apart in a dedicated corridor for a total of 400 meters while wearing a portable oxygen consumption device (COSMED K4b2). They were given a 2-minute warm-up period where they were instructed to cover as much ground as possible and then asked to perform the LDCW as quickly as possible at a pace that could be maintained. Time to walk 400 meters

was recorded and used to calculate gait speed (total meters walked/total time in seconds). Perceived physical fatigability was also measured in the Health ABC cohort using the Pittsburgh Fatigability Scale (PFS) (42). The PFS was self-administered within 2 hours of the blood draw and within 6 months of the skeletal muscle biopsy. This 10-item questionnaire assesses whole-body tiredness as a function of duration and intensity of activity. Each item is scored from 0, indicating no fatigue, to 5, indicating extreme fatigue. The 10 items are summed, with PFS scores ranging from 0 to 50, with higher score indicating higher fatigability. Of the 32 total subjects, 5 subjects did not complete the walk or fill out the fatigability questionnaire.

Statistics. Comparisons of older versus young adults were made using unpaired 2-tailed Student's *t* test, but comparison of ECAR was made using a nonparametric Mann-Whitney test because of nonnormal distribution (evaluated by the Shapiro-Wilk and D'Agostino-Pearson normality tests). Using data from the older group, Pearson's correlations were used to determine the relationship among variables of platelet bioenergetics, muscle energetics, and physical function parameters. A 2-tailed *P* value was calculated, and Bonferroni's posttest for multiple comparisons was used to determine the appropriate α value for each set of comparisons. *P* values below 0.05 are highlighted in each table. Statistical significance is determined by the α value calculated for each individual experiment and is denoted in the respective table. *P* values of less than 0.05 were considered significant. All statistics and analyses were calculated using IBM SPSS (v22), and figures were generated using Prism 7 (GraphPad Software Inc.). Values reported in the text are mean ± SEM.

Study approval. The study population consisted of 2 groups: young and older adults. The young adults were recruited at the University of Pittsburgh via protocol 08110422, approved by the University of Pittsburgh IRB. Older adults were a subset of the national Health ABC prospective cohort (37, 38) and were enrolled as part of an ancillary study approved by the University of Pittsburgh IRB (IRB960212). Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki.

Author contributions

AB and CGC contributed by conducting the experiments, acquiring and analyzing the data, and writing the manuscript. AJS and GD contributed by conducting the experiments and acquiring and analyzing the data. PMC, NWG, BHG, and ABN contributed by designing experiments and providing the reagents as well as editing the manuscript. SS contributed by designing the research studies, analyzing the data, providing the reagents, and writing the manuscript. SMN advised on and made the statistical calculations.

Acknowledgments

This work was supported by T32HL110849 to AB, Career Development Award K01 AG044437 to PMC, and NIH grant 1R01HL133003-01A1 to SS, as well as by the Hemophilia Center of Western Pennsylvania (to SS) and the University Pittsburgh Medical Center Stimulating Pittsburgh Research in Geroscience fellowship (to AB).

Address correspondence to: Sruti Shiva, BST 1240E, 200 Lothrop Street, University of Pittsburgh, Pittsburgh, Pennsylvania 15216, USA. Phone: 412.383.5854; Email: sss43@pitt.edu.

GD, PMC, and BHG's present address is: Translational Research Institute for Metabolism and Diabetes, Florida Hospital, Sanford Burnham, Orlando, Florida, USA.

1. Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *N Engl J Med*. 1995;332(9):556–561.
2. Hardy SE, Perera S, Roumani YF, Chandler JM, Studenski SA. Improvement in usual gait speed predicts better survival in older adults. *J Am Geriatr Soc*. 2007;55(11):1727–1734.
3. Studenski S, et al. Gait speed and survival in older adults. *JAMA*. 2011;305(1):50–58.
4. Sun N, Youle RJ, Finkel T. The mitochondrial basis of aging. *Mol Cell*. 2016;61(5):654–666.
5. Chabi B, Ljubicic V, Menzies KJ, Huang JH, Saleem A, Hood DA. Mitochondrial function and apoptotic susceptibility in aging skeletal muscle. *Aging Cell*. 2008;7(1):2–12.
6. Distefano G, et al. Chronological age does not influence ex-vivo mitochondrial respiration and quality control in skeletal muscle. *J Gerontol A Biol Sci Med Sci*. 2017;72(4):535–542.
7. Lyons CN, Mathieu-Costello O, Moyes CD. Regulation of skeletal muscle mitochondrial content during aging. *J Gerontol A Biol Sci Med Sci*. 2006;61(1):3–13.
8. Santanasto AJ, et al. Skeletal muscle mitochondrial function and fatigability in older adults. *J Gerontol A Biol Sci Med Sci*. 2015;70(11):1379–1385.

9. Conley KE, Jubrias SA, Esselman PC. Oxidative capacity and ageing in human muscle. *J Physiol (Lond)*. 2000;526 Pt 1:203–210.
10. Cooper JM, Mann VM, Schapira AH. Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: effect of ageing. *J Neurol Sci*. 1992;113(1):91–98.
11. Trounce I, Byrne E, Marzuki S. Decline in skeletal muscle mitochondrial respiratory chain function: possible factor in ageing. *Lancet*. 1989;1(8639):637–639.
12. Hepple RT. Mitochondrial involvement and impact in aging skeletal muscle. *Front Aging Neurosci*. 2014;6:211.
13. Homma S, Fukunaga T, Kagaya A. Influence of adipose tissue thickness on near infrared spectroscopic signal in the measurement of human muscle. *J Biomed Opt*. 1996;1(4):418–424.
14. van Beekvelt MC, Borghuis MS, van Engelen BG, Wevers RA, Colier WN. Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. *Clin Sci*. 2001;101(1):21–28.
15. Tyrrell DJ, Bharadwaj MS, Jorgensen MJ, Register TC, Molina AJ. Blood cell respirometry is associated with skeletal and cardiac muscle bioenergetics: Implications for a minimally invasive biomarker of mitochondrial health. *Redox Biol*. 2016;10:65–77.
16. Tyrrell DJ, et al. Blood-based bioenergetic profiling reflects differences in brain bioenergetics and metabolism. *Oxid Med Cell Longev*. 2017;2017:7317251.
17. Winnica D, et al. Bioenergetic differences in the airway epithelium of lean *versus* obese asthmatics are driven by nitric oxide and reflected in circulating platelets [published online ahead of print March 6, 2019]. *Antioxid Redox Signal*. https://doi.org/10.1089/ars.2018.7627.
18. Cardenes N, et al. Platelet bioenergetic screen in sickle cell patients reveals mitochondrial complex V inhibition, which contributes to platelet activation. *Blood*. 2014;123(18):2864–2872.
19. Chacko BK, et al. The Bioenergetic Health Index: a new concept in mitochondrial translational research. *Clin Sci*. 2014;127(6):367–373.
20. Nguyen QL, et al. Platelets from pulmonary hypertension patients show increased mitochondrial reserve capacity. *JCI Insight*. 2017;2(5):e91415.
21. Tyrrell DJ, Bharadwaj MS, Van Horn CG, Kritchevsky SB, Nicklas BJ, Molina AJ. Respirometric profiling of muscle mitochondria and blood cells are associated with differences in gait speed among community-dwelling older adults. *J Gerontol A Biol Sci Med Sci*. 2015;70(11):1394–1399.
22. Tyrrell DJ, Bharadwaj MS, Van Horn CG, Marsh AP, Nicklas BJ, Molina AJ. Blood-cell bioenergetics are associated with physical function and inflammation in overweight/obese older adults. *Exp Gerontol*. 2015;70:84–91.
23. Chouchani ET, Kazak L, Spiegelman BM. Mitochondrial reactive oxygen species and adipose tissue thermogenesis: Bridging physiology and mechanisms. *J Biol Chem*. 2017;292(41):16810–16816.
24. Tian XY, Ma S, Tse G, Wong WT, Huang Y. Uncoupling protein 2 in cardiovascular health and disease. *Front Physiol*. 2018;9:1060.
25. Porter C, et al. Mitochondrial respiratory capacity and coupling control decline with age in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2015;309(3):E224–E232.
26. Sjövall F, et al. Mitochondrial respiration in human viable platelets--methodology and influence of gender, age and storage. *Mitochondrion*. 2013;13(1):7–14.
27. Lenaz G, et al. Mitochondrial complex I defects in aging. *Mol Cell Biochem*. 1997;174(1-2):329–333.
28. Chacko BK, et al. Mitochondria in precision medicine; linking bioenergetics and metabolomics in platelets. *Redox Biol*. 2019;22:101165.
29. Bellanti F, et al. Many faces of mitochondrial uncoupling during age: damage or defense? *J Gerontol A Biol Sci Med Sci*. 2013;68(8):892–902.
30. Mookerjee SA, Divakaruni AS, Jastroch M, Brand MD. Mitochondrial uncoupling and lifespan. *Mech Ageing Dev*. 2010;131(7-8):463–472.
31. Jezek P, Garlid KD. Mammalian mitochondrial uncoupling proteins. *Int J Biochem Cell Biol*. 1998;30(11):1163–1168.
32. Kowaltowski AJ, Costa AD, Vercesi AE. Activation of the potato plant uncoupling mitochondrial protein inhibits reactive oxygen species generation by the respiratory chain. *FEBS Lett*. 1998;425(2):213–216.
33. Parton LE, et al. Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. *Nature*. 2007;449(7159):228–232.
34. Echtay KS, et al. Superoxide activates mitochondrial uncoupling proteins. *Nature*. 2002;415(6867):96–99.
35. Santanasto AJ, et al. Body composition remodeling and mortality: the health aging and body composition study. *J Gerontol A Biol Sci Med Sci*. 2017;72(4):513–519.
36. Chacko BK, et al. Methods for defining distinct bioenergetic profiles in platelets, lymphocytes, monocytes, and neutrophils, and the oxidative burst from human blood. *Lab Invest*. 2013;93(6):690–700.
37. Goodpaster BH, et al. Attenuation of skeletal muscle and strength in the elderly: The Health ABC Study. *J Appl Physiol*. 2001;90(6):2157–2165.
38. Newman AB, et al. Strength and muscle quality in a well-functioning cohort of older adults: the Health, Aging and Body Composition Study. *J Am Geriatr Soc*. 2003;51(3):323–330.
39. Coen PM, et al. Skeletal muscle mitochondrial energetics are associated with maximal aerobic capacity and walking speed in older adults. *J Gerontol A Biol Sci Med Sci*. 2013;68(4):447–455.
40. Coen PM, et al. Exercise and weight loss improve muscle mitochondrial respiration, lipid partitioning, and insulin sensitivity after gastric bypass surgery. *Diabetes*. 2015;64(11):3737–3750.
41. Simonsick EM, Montgomery PS, Newman AB, Bauer DC, Harris T. Measuring fitness in healthy older adults: the Health ABC Long Distance Corridor Walk. *J Am Geriatr Soc*. 2001;49(11):1544–1548.
42. Glynn NW, et al. The Pittsburgh Fatigability scale for older adults: development and validation. *J Am Geriatr Soc*. 2015;63(1):130–135.