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Exercise intolerance and rapid skeletal muscle energetic decline in human age-associated frailty

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Word count: 8578

Subject codes: Frailty, Metabolism, Fatigue, Magnetic Resonance, Exercise Testing

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Conflict of Interest Statement: The authors have declared that no conflict of interest exists. The NIH was the funding source but played no role in study design or data collection/analysis.
Abstract:

**Background:** Physical frailty in older individuals is characterized by subjective symptoms of fatigue and exercise intolerance (EI). Objective abnormalities in skeletal muscle (SM) mitochondrial high-energy phosphate (HEP) metabolism contribute to EI in inherited myopathies, but their presence or link to EI in the frail older adult is unknown.

**Methods:** Three groups of ambulatory, community-dwelling adults with no history of significant coronary disease were studied: frail, older individuals (FO, 81±2.7 years, mean±SEM), non-frail, older individuals (NFO, 79±2.0 years), and healthy middle-aged controls (CONT, 51±2.1 years). Lower extremity SM HEP levels and mitochondrial function were measured with $^{31}$P magnetic resonance (MR) techniques during graded, multistage plantar flexion exercise (PFE). EI was quantified by six-minute walk and peak oxygen consumption during cardiopulmonary testing (peak-VO$_2$).

**Results:** During graded exercise, frail older (FO), non-frail older (NFO), and healthy middle-aged individuals all fatigue at similar SM HEP levels measured by $^{31}$P MR. However, FO fatigued fastest with several-fold higher rates of PFE-induced HEP decline, which correlated closely with shorter exercise duration in the MR scanner and with six-minute walk distance and lower peak oxygen consumption on cardiopulmonary testing (p<0.001 for all). SM mitochondrial oxidative capacity was lower in older individuals and correlated with rapid HEP decline but less closely with EI.

**Conclusions:** Several-fold faster skeletal muscle energetic decline during exercise occurs in frail older individuals and correlates closely with multiple measures of EI. Rapid energetic decline represents an objective, functional measure of SM metabolic changes and a potential new target for mitigating frailty-associated physical limitations.

[word count 249].

**Funding:**
National Institutes of Health R21 AG045634, R01 AG063661, R01 HL61912, and the Johns Hopkins University Claude D. Pepper Older Americans Independence Center P30AG021334. This work was also supported by the Clarence Doodeman Endowment in Cardiology at Johns Hopkins.
Introduction:

Physical frailty in older adults is characterized by self-reported fatigue, weakness, slowness, low activity, and unintentional weight loss(1). Nearly 15% of U.S. adults over the age of 65 are frail and are therefore at increased risk of falls, disability, hospitalization, loss of independence, and cardiovascular death(2, 3). The factors underlying the fatigue and exercise intolerance accompanying physical frailty are poorly understood.

*Fatigue, frailty and exercise intolerance are related but distinct terms with relevance to aging populations.* Fatigue, often defined as the subjective sense of tiredness, is a core criterion of the physical frailty phenotype(1). Fatigue is commonly reported among older individuals, especially among those who have physical disabilities, limited mobility, or difficulty with daily activities, even in the absence of frailty(4, 5). However, the sense of tiredness doesn’t specify the duration or intensity of activity that caused the fatigue. Fatigability, or the symptom of fatigue normalized to the inciting activity duration and intensity, better characterizes this prognostic symptom in older individuals(4, 6-8). Likewise, exercise intolerance is often defined as an impairment in objective measures of volitional activity or exercise performance.

Age-associated changes in skeletal muscle may contribute to the unique entities of fatigue, exercise intolerance and frailty. To the extent that normal aging is characterized by a decline in biologic systems responsible for maintaining homeostasis in response to physiologic stress, the decline in skeletal muscle typically manifests as an age-related fiber atrophy, reduced muscle mass and strength or sarcopenia(9-12), oxidative damage to mitochondrial DNA, and reduced mitochondrial density in some
such age-associated mitochondrial changes could, in theory, contribute to these geriatric functional syndromes.

Muscle exhaustion during exercise is often considered an energy deficient state because isolated skeletal muscle preparations at performance failure have reduced high-energy phosphates (i.e. phosphocreatine (PCr) and ATP) and free energy release from ATP hydrolysis ($\Delta G_{\text{ATP}}$), as well as an accumulation of products of ATP degradation (i.e. inorganic phosphate (Pi), and $H^+$)(7, 8, 15-17). Impaired skeletal muscle energy metabolism contributes to exercise intolerance in people with skeletal myopathies(18, 19), and recent data suggest both an age-related increase in the ATP cost of skeletal muscle contraction and a role of impaired muscle bioenergetics in poorer (or slower) age-related walking speed performance(20, 21). However, the extent to which energetic abnormalities and mitochondrial dysfunction are linked to age-associated fatigability and exercise intolerance in older individuals with the frailty phenotype is not presently known.

Assessment of the potential role of impaired skeletal muscle energetics in fatigability in frail older individuals requires evaluation of skeletal muscle energetic parameters at rest, during exercise performed to performance fatigue, and during recovery. Especially important biochemically are measures of absolute high-energy phosphate concentrations (rather than ratios), rates of ATP synthesis, and rates of change of these energetic parameters during exercise and recovery. Ideally, exercise would include a common range of exercise that can be performed by non-frail and frail older individuals, and would be derived from a small muscle group so as to capture
intrinsic skeletal muscle metabolic abnormalities and thereby diminish the impact of central hemodynamic and cardiac factors.

Here we set out to test the hypothesis that skeletal muscle mitochondrial oxidative capacity is reduced and that high-energy phosphate decline during exercise is faster in frail older individuals than in middle-aged or age-matched, non-frail individuals. We used an energetic plantar flexion exercise (PFE) fatigability test with concomitant noninvasive in vivo phosphorus magnetic resonance spectroscopy ($^{31}$P MRS) measures of skeletal muscle high-energy phosphates, Pi, intracellular pH, and mitochondrial maximal oxidative capacity. Three groups were studied; community-dwelling older non-frail individuals (NFO), older individuals with a clinical phenotype of frailty (FO), and healthy middle-aged participants who served as controls (CONT). Finally, to determine the “real world” relevance of energetic observations, we related the energetic and exercise intolerance exhibited in the MRI scanner with walking and whole-body exercise capacity. The findings are consistent with the hypothesis that reduced skeletal muscle energy metabolism is closely related to exercise intolerance in age-associated frailty and that an energetic myopathy contributes to the age-associated physical frailty phenotype.

**Results:**

**Patient Characteristics**

There were no differences in age or body mass index (BMI) between the FO and NFO groups, although both older groups had higher BMI than the middle-aged individuals (Table 1). As expected, objective exercise intolerance was most marked in FO
individuals as evidenced by the shortest six-minute walk distance (6MW), while 6MW was shorter in the NFO individuals than in the CONT group (Table 1, p<0.01). Respiratory exchange ratios (RER) were similar among the groups and indicated adequate effort during cardiopulmonary exercise testing (CPET). Likewise, FO individuals had significantly lower bicycle peak oxygen consumption (peak-VO₂) compared to both the controls and NFO individuals (Table 1, p <0.01).

**Plantar Flexion Exercise Performance and Test of Global Function**

FO individuals had the most profound exercise intolerance during graded plantar flexion exercise (PFE) performed in the MRI as evidenced by the shortest mean exercise time and lowest total work performed in comparison to both the CONT and NFO individuals (Figure 1A-B). FO individuals performed only one-third of the total work performed by their NFO counterparts. Importantly, metrics of PFE performance, including exercise duration and total work significantly correlated with established measures of global functional capacity, including 6MW and bicycle peak-VO₂ (Figure 1C-F). Thus, FO had the poorest performance inside and outside of the MRI, as expected, and, importantly, PFE performance in the MRI scanner paralleled conventional global functional assessments in this cohort.

**Skeletal Muscle Energetics at Rest and at Fatigue**

Representative ³¹P MRS spectra and the time course of energetic data are shown for two individuals in Figure 2. Skeletal muscle metabolite concentrations, including creatine phosphate (PCr) and ATP, were similar under resting conditions in all three groups (Figure 2,3). ATP synthesis rates also did not differ significantly among the three
groups at rest (Figure 3). Dynamic Pi started at similarly low concentrations at rest and accumulated during PFE to equivalent concentrations at fatigue among the three groups, with a similar intracellular acidosis as well amongst all three cohorts (Figures 2,3). Likewise, there was a progressive, parallel decline in PCr during PFE with preservation of ATP in all three cohorts. Notably, at fatigue, all three groups had similar reductions in mean PCr, intracellular pH, and ΔG, along with similar Pi and ADP accumulation (Figure 3). Thus, healthy middle-aged, non-frail older, and frail older individuals had a similar skeletal muscle energetic profile, reflecting a similar skeletal muscle resting reserve and common energetic limit at performance fatigue, in spite of differences in exercise duration and the total work performed.

*Rate of Skeletal Muscle High-energy Phosphate Depletion during Exercise and Functional Capacity*

Since FO individuals fatigued faster (Figure 1), but at similar high-energy phosphate concentrations (Figure 3), the mean rate of phosphocreatine depletion during exercise normalized to the work of activity performed was ten-fold higher in FO than the mean rate in CONT, and four-fold higher than the mean rate observed in age-matched, NFO individuals (FO: 78.4±29.9 μmol/g/kJ; NFO 18.2±7.0 μmol/g/kJ, p<0.02; CONT 7.5±1.2 μmol/g/kJ, p<0.005, Figure 4A). The average rate of PCr decline was also determined over just the initial stages of low intensity exercise performed by nearly all participants both to directly compare groups at the same workloads and to minimize differences in cardiac output among the three groups that may be present at peak exercise. If peak cardiac output is limiting, it would impact rates averaged over the entire exercise
interval. The initial rate of PCr decline in the first four minutes was still fastest in FO individuals compared to that in CONT (p <0.001) and NFO individuals (p<0.05, Figure 4B). Even when not normalized to work performed, FO individuals still have several fold faster rates of energetic depletion during the first stage of exercise when all subjects lifted the same weight (CONT p<0.005, NFO p<0.05, Supplement Figure 9).

There was a strong inverse correlation between the rate of PCr decline during exercise and PFE duration (Spearman, r= (-) 0.9252, p<0.0001, Figure 4C) as well as total PFE work performed (r= (-) 0.934, p<0.0001, Figure 4D). In addition, the rate of PCr decline during PFE in the MRI correlated inversely with 6MW performance (r= (-) 0.6256, p<0.0001, Figure 4E) and peak-VO₂ at CPET (r= (-) 0.5762, p <0.001, Figure 4F). These findings indicate that the rate of skeletal muscle high-energy phosphate depletion was several-fold faster in FO than in NFO and CONT individuals, the rapid depletion in FO began early during even low-level exercise, and that the rates of high-energy phosphate decline correlated in all with diminished performance during PFE in the MRI scanner and with reduced established functional parameters measured outside of the MRI scanner. These observations are all consistent with the premise that rapid skeletal muscle energy depletion contributes to exercise intolerance in FO individuals.

Maximal Mitochondrial Oxidative Capacity and Functional Capacity

Rapid high-energy phosphate decline during exercise could be due to increased ATP consumption and/or to decreased mitochondrial ATP production. To evaluate the latter, maximal mitochondrial oxidative capacity was measured and FO individuals had longer average post-exercise PCr recovery times (FO: 57.9 ± 3.4 s, CONT: 40 ± 4.2 s, p<0.02,
Figure 5A) and nearly half of the maximal oxidative capacity of CONT (FO, 0.37 ± 0.03 µmol/g/s; CONT, 0.71± 0.08 µmol/g/s, p<0.005; Figure 5B). NFO individuals had an intermediate PCr recovery time (NFO: 52.5 ± 5.4 s) and oxidative capacity (NFO: 0.48 ± 0.05 µmol/g/s; Figure 5A-B) that differed significantly from CONT, but not from FO. Collectively, maximal oxidative capacity inversely correlated with the logarithm of the rate of PCr decline (r=-0.4046, p<0.02, Figure 5C). The maximal oxidative capacity was directly correlated with the functional parameters of PFE time (p<0.05) in the MRI, with 6MW (p<0.005), and with peak-VO₂ (p<0.02, Figure 5D-F) with the caveats that the range of oxidative capacities was much reduced in FO vs. CONT and the relationships were not as tight as those with the rate of PCr decline (Figure 4C vs Figure 5F). Rapid PCr decline during exercise was inversely associated with mitochondrial function (Figure 5C). Thus, mitochondrial function was significantly reduced in older individuals, trended lowest in frail older individuals, and correlated inversely with rapid energetic decline during exercise.

**Skeletal Muscle Mass and Intramuscular Fatty Replacement**

To determine whether sarcopenia accounts for the plantar-flexion exercise intolerance observed in this FO cohort, we measured lower extremity muscle cross-sectional area by MRI and there was no difference among the three groups (Figure 6B). Because muscle lipid accumulation occurs in frail and prefrail individuals(22), we determined whether skeletal muscle lipid accumulation occurs with frailty, and if so, whether it is associated with the observed energetic and functional changes. FO individuals had three times the mean intramuscular fat fraction of CONT (FO, 16.2% ± 2.9%; CONT,
5.5% ± 0.6%, p<0.001; Figure 6C). NFO individuals exhibited intermediate fat fraction (NFO, 11.5% ± 3.3%; Figure 6C) which was not significantly different from CONT (p=0.0503, ns), but significantly less than in FO individuals (p<0.02). There was a direct correlation between muscle fat fraction and BMI (Supplement Figure 1), and an inverse correlation between muscle fat fraction and PFE time, even with the highest fat fraction outliers omitted (Figure 6D, Supplement 2). However, BMI did not correlate with exercise tolerance during PFE (Supplement 3). Muscle fat fraction directly correlated with average PCr decline (Spearman, r=0.4759, p<0.02; Figure 6E and Supplement 4) and was inversely correlated with maximal oxidative capacity (Spearman, r=-0.5185, p <0.02; Figure 6F and Supplement 5), even with outliers omitted. Thus, FO individuals with the highest intramuscular fat exhibited the most rapid rates of PCr decline and the most impaired mitochondrial function.

Discussion:
Although the physical frailty phenotype in older individuals is an independent predictor of disability, hospitalizations, loss of independence, and cardiovascular death(1, 3), the factors underlying this vulnerability and the accompanying fatigue and exercise intolerance in this population are incompletely understood. This study used an energetic 31P MRS fatigability test in older frail and non-frail individuals with a range of exercise tolerances to provide insight into the hypothesis that skeletal muscle mitochondrial and energetic abnormalities occur with aging, accelerate with frailty, and are closely related to exercise intolerance, slowness, and increased fatigability. There are four novel findings in this report. First, skeletal muscle high-energy phosphate
stores do not differ at rest or at performance fatigue in older frail or non-frail older individuals as compared to middle-aged individuals, consistent with a common energetic limit for fatigue regardless of frailty status and age. Second, the mean rate of skeletal muscle high-energy phosphate decline during exercise was four- to ten-fold-faster in frail older than in non-frail older and middle-aged individuals, respectively, and was very closely associated with exercise intolerance exhibited both during plantar flexion exercise in the MRI scanner and with walking and whole-body exercise capacity. Third, the rapid skeletal muscle high-energy phosphate decline during exercise in frail older individuals cannot be explained by a reduction in the primary muscle phosphagen reaction, creatine kinase. However, rapid skeletal muscle high-energy phosphate depletion was inversely related to maximal mitochondrial oxidative capacity among all study subjects, and was reduced by nearly fifty percent in older as compared to middle-age individuals. Fourth, skeletal muscle fat content was increased several-fold in frail older individuals and was associated with rapid energetic decline during exercise, shortened exercise duration, and reduced mitochondrial oxidative capacity. These observations are consistent with the hypothesis that exercise intolerance in older frail individuals is closely related to rapid skeletal muscle energetic decline during exercise, reduced mitochondrial energy metabolism with aging, and marked skeletal muscle fatty infiltration, and that these findings may occur in some frail individuals in the absence of muscle atrophy.

In isolated preparations of skeletal muscle, fatigue is related to high-energy phosphate depletion, reduced Gibbs free energy release from ATP hydrolysis ($\Delta G_{-ATP}$), and the accumulation of inorganic phosphate (Pi) and H$^+$ from ATP degradation(7, 16,
Decreased skeletal muscle energy metabolism is thus a plausible contributor to fatigability in age-associated frailty since normal muscle function is inherently dependent on ATP and bioavailable energetic stores(7, 24). In fact, there is a precedent for skeletal muscle energy deprivation and impaired ATP metabolism to occur and contribute to muscle fatigue and weakness in muscular dystrophies and metabolic myopathies(18, 19, 25-27). An animal model of age-associated frailty, the aged homozygous IL-10 knockout mouse, has in vivo skeletal muscle reductions at rest in high-energy phosphates, ATP synthesis rates, and ΔG_{ATP} at rest(28). Our current study is arguably the first to directly measure absolute concentrations of skeletal muscle ATP and high energy phosphates in older individuals with frailty at rest and during exercise-induced energy decline performed to fatigue. Our study does not depend on the assumption that ATP concentrations are constant across age groups.

We observed that frail older individuals experience subjective fatigue at lower workloads and at shorter exercise times but at the same skeletal muscle concentrations of PCr, Pi, and ATP as well as pH, as non-frail older and healthy-middle aged individuals (Figure 4). The observation that performance fatigue occurs at a common energetic level in all individuals studied does not prove, but is consistent with, the hypothesis that skeletal muscle energetic depletion and/or catabolite accumulation is a determinate of fatigue. If non-energetic factors primarily determined the time of fatigue, then performance fatigue could occur at variable high-energy phosphate concentrations. This was not observed. This common energetic limit coupled with the observation that high-energy phosphates decline faster in frail individuals with high fatigability (i.e. reach fatigue at lower workloads and less activity duration), indicates that performance fatigue
in frail individuals is very closely associated with more rapid skeletal muscle high-energy phosphate depletion (Figure 4C).

The faster skeletal muscle energetic decline observed in frail older people at matched workloads (Fig 4B) could be due to reduced high-energy phosphate production and/or increased high-energy phosphate consumption. Energetic decline during exercise is buffered by muscle phosphagen transfer reactions such as the creatine kinase reaction, which serves as the primary muscle energy reservoir during exercise by rapidly and reversibly converting PCr and ADP to creatine and ATP(29). Activities of daily living that can be impacted by the frailty phenotype include standing, initiation of walking, and grip strength; all processes that utilize ATP from phosphagen reactions and strongly predict disability in older populations(30-35). An in vivo study of age-related frailty in mice showed reduced skeletal muscle ATP flux through creatine kinase at rest(28) and lower skeletal muscle creatine kinase activity with aging(36). However, our finding that in vivo skeletal muscle ATP synthesis from creatine kinase is not reduced with frailty indicates that reduced skeletal muscle creatine kinase is not the cause of exercise intolerance in these older frail individuals.

Unlike phosphagen reactions, which can provide ATP during brief episodes for burst activities, most sustained muscle ATP generation occurs in the mitochondria by oxidative phosphorylation(11). Mitochondrial function can directly impact skeletal muscle performance, and deleterious oxidative damage to mitochondrial DNA is thought to decrease mitochondrial content and function with increasing age(11, 37, 38). Previous studies have shown that aerobic capacity is lower in older inactive individuals than in comparison to their younger inactive counterparts(12), and mitochondrial-
coupling efficiency is also lower in older, than younger adults(39). Furthermore, older individuals with higher fatigability on treadmill exercise tests have lower capacity for oxidative phosphorylation than older individuals with lower fatigability(40). Lower mitochondrial capacity is also associated with slower walking speeds and lower muscle strength in older individuals(41, 42). Other studies suggest that mitochondrial density and function in older individuals are related to their activity level(43, 44), and mitochondrial function is associated with walking performance in older, active adults, but not in sedentary older adults.(45) Chronic exercise training, and thus increased physical activity, may modify mitochondrial dysfunction(46, 47). Furthermore, skeletal muscle phosphocreatine recovery is delayed and mitochondrial respiratory complex protein and activity reduced in older pre-frail individuals as compared to active older adults (48). These prior studies are consistent with the hypothesis that impaired mitochondrial function occurs in older individuals and is associated with reduced functional capacity and lower muscle strength. We report here that skeletal muscle maximal oxidative capacity is reduced in older individuals, trends lower in those with frailty, and is linked to rapid high-energy phosphate decline and exercise intolerance in older individuals. However, another novel observation here is that the relationship between exercise intolerance and rapid energetic decline (Figure 4C) exists and is much stronger than the relationship between exercise intolerance and reduced mitochondrial function (Figure 5F).

We observed a three-fold increase in intramuscular fat in older frail individuals as compared to that of healthy, middle aged subjects (p<0.001) and thirty percent more fat in comparison to age-matched non-frail (p<0.02) individuals. This is consistent with data
previously reported in which frail older individuals were noted to have increased intramuscular adipose tissue by thigh MRI in comparison to non-frail, age- and BMI-matched peers(49). Also noted in that study was increased interleukin-6 (IL-6) muscle mRNA and IL-6 protein content in frail individuals with a significant association between intramuscular fat and IL-6 mRNA and IL-6 protein expression(49). IL-6 muscle mRNA also correlated with 6MW performance(49). The current work adds to that literature by not only confirming increased muscle fat in older frail individuals, but by demonstrating for the first time that muscle fat accumulation and rapid energetic decline are closely associated and, in turn, closely linked to exercise intolerance and increased fatigability. Furthermore, this work demonstrates that skeletal muscle maximal oxidative capacity is inversely associated with intramuscular fat content. Taken together, all of these observations are consistent with a working metabolic framework of age-related frailty whereby the skeletal muscle exhibits normal high energy phosphate stores at rest, but very rapid energetic depletion to a common energetic limit at fatigue that is closely associated with profound exercise intolerance and increased fatigability. The rapid skeletal muscle energetic decline is associated with significantly reduced mitochondrial oxidative capacity and markedly increased muscle fat accumulation. While our data do not answer the question of whether mitochondrial abnormalities cause fatty accumulation or vice versa, it seems possible, if not likely, that fatty replacement of skeletal muscle and the previously described pro-inflammatory state(50), contribute to impaired skeletal muscle high-energy phosphate metabolism possibly through paracrine effects(51-53) and, in turn, increased fatigability in age-related frailty.
**Limitations:** This study recruited a relatively small number of subjects and we did not study individuals with the most extreme clinical manifestations of frailty since ambulation was required for participation in our protocol. Despite the modest sample size and absence of individuals with extreme manifestations of frailty, the study was sufficient to detect highly significant skeletal muscle energetic abnormalities and relationships between energetic abnormalities and reduced functional performance. Please note that we cannot at this time ascribe the faster rate of high energy phosphate depletion exclusively to impaired mitochondrial function in the frail subjects. For example, we did not study the extent to which abnormalities in macro- or microcirculatory oxygen delivery with aging\(^{54, 55}\) could impair mitochondrial function during exercise and contribute to the rapid exercise-induced energetic decline. We were not able to obtain dynamic lower extremity macrocirculatory blood flow measurements during exercise due to significant artifacts and limitations of MRI without contrast agent administration. However, we did not observe a significant difference in resting peak blood flow between the frail and non-frail older individuals (Supplement Figure 6). Thus, although PFE involves relatively small muscle groups not likely limited by peak cardiac output, especially during early low-level exercise, additional studies are needed to probe the possibility that abnormal blood flow, dynamic shunting with exercise, or microcirculatory abnormalities contribute to the rapid energetic decline in frail older individuals. This study investigated only the muscles responsible for plantar flexion while prior work suggests that age-associated changes in mitochondrial capacity differ among muscle groups\(^{43}\). We believe it would be important to study other muscle groups in the future but emphasize that plantar flexion is important for ambulation and several activities of daily living and that these findings correlated both
with 6MW and whole-body peak-VO₂ during bicycle exercise. We also recognize that catabolite accumulation (inorganic phosphate and H⁺) could contribute significantly to exercise-induced skeletal muscle fatigue in addition to rapid high-energy phosphate decline. So although one interpretation of the rapid energetic decline with a common energetic limit is that frail older individuals “run out of fuel faster” and that is closely related to them stopping exercise earlier, another is that the frail accumulate catabolites faster and these cause them to stop earlier. Because high-energy metabolite decline and catabolite accumulation are intimately linked in vivo, in vitro systems would likely have to be explored in the future to distinguish their relative contribution to skeletal muscle fatigue. Although tissue biopsies could provide more molecular insights and would have been of interest, they were not obtained due to the invasive nature and reluctance of many older subjects to undergo a surgical procedure. The ³¹P MR detection of rapid skeletal muscle energetic decline during exercise offers an objective, noninvasive, metric for quantifying metabolic changes associated with physical frailty and may be used in the future to test metabolic treatment strategies. However, additional studies are needed to determine whether these metabolic abnormalities are themselves related to increased risk of disability and reduced resilience, in which case, they may eventually complement the well-established but partly subjective frailty criteria.

Conclusions: Physically frail older individuals have profound exercise intolerance but similar skeletal muscle energy metabolites at rest as non-frail older individuals, both in high-energy phosphate concentrations and ATP synthesis rates via the creatine kinase reaction, i.e. they begin exercise with equivalent energetic fuel. Frail individuals, however, exhibit rapid depletion of high-energy phosphates, which declines to a level
common to that of non-frail older and middle-aged individuals during exercise, and the rapid rate of energy decline correlates closely with objective metrics of exercise intolerance and decreased global functional capacity. Maximal mitochondrial oxidative capacity is reduced in older individuals and is associated with rapid energetic decline and exercise performance. In addition, intramuscular fat content is increased in older frail individuals and is also associated with rapid exercise-induced energetic decline. Although physical frailty in the aging population is prevalent and an independent predictor of falls, morbidity, and cardiovascular mortality, its underlying factors are poorly understood. These observations identify rapid, exercise-induced skeletal muscle energetic decline in frailty and its close relation to decreased physical function and ambulation. Our study supports recognition of frailty, at least in part, as a skeletal muscle metabolic myopathy of aging, offers a noninvasive energetic fatigability test to objectively quantify these changes, and suggests skeletal muscle energetic abnormalities as new therapeutic targets to possibly mitigate this debilitating frailty phenotype.

Methods:

Subjects:
Informed consent after explanation of the study protocol was obtained from all participants. The Johns Hopkins Institutional Review Board (IRB) approved all human studies. Twenty-three ambulatory, community-dwelling, older individuals without a history of significant or limiting co-morbidities (Supplement Table 1) were referred to this study from a research registry of older adults. All older subjects referred had their physical frailty status determined using a well-validated aggregate of five criteria that
includes grip strength, walking speed, weight loss, fatigue, and physical activity measures (1, 56) (Supplement Table 2). Eleven participants (81±2.7 years, mean±SEM) met ≥3 of 5 physical frailty criteria, and were considered frail (frail older, FO). Twelve participants (79±2.0 years) comprised a “non-frail” older group that included eleven robust older adults with a score of 0 and one older adult with a score of 1. When recruiting from the research registry, FO were first enrolled followed by age- and sex-matched NFO individuals to form two similar sized cohorts. Eleven healthy, middle-aged participants (age 51±2.1 years) without a history of diabetes mellitus, hypertension, heart disease, or vascular disease served as controls (CONT), and were reported in part previously (57). Individuals were excluded if they were unable to ambulate or exercise, unable to lie flat or complete the MR study, or had implanted devices (i.e. pacemakers, defibrillators) or other hardware contraindicated for MRI. Older participants had the choice to complete all of the studies on the same day or within one week.

**Study Protocol:**
Subjects underwent $^{31}$P MRS at rest, dynamic $^{31}$P MRS during graded multi-stage PFE to exhaustion, and during post-exercise recovery for assessment of skeletal muscle energetics in a 3T MRI system (Philips Healthcare, The Netherlands), using previously described methods (57). Subjects were seated with their shoulders on a 25.5-cm (10 inch) incline during image acquisition and exercise. The dominant foot was secured to a custom-built MR-compatible plantar-flexion weighted foot pedal to minimize exercise contributions from other muscles. The calf muscles (gastrocnemius, soleus) were centered on a custom-built $^{31}$P MRS coil. Scout images were confirmed by scout MRI.
Under resting conditions, all participants underwent conventional MRI of the lower-extremity for baseline evaluation of calf muscle area and fat content. Muscle composition was ascertained using spin-spin relaxation T2-weighted MRI imaging, in which fat fraction results were measured. As previously described, T2-weighted images (FOV = 220 x 220 x 175 mm$^3$, resolution = 0.78 x 0.78 x 10 mm$^3$, 16 slices, pulse repetition time (TR) = 2141 ms, echo time (TE) = 100 ms) were acquired before and after plantar flexion exercise(57). Popliteal artery blood flow was also measured prior to exercise in older participants using MRI velocity-mapping(58).

Prior to exercise, resting-state skeletal muscle $^{31}$P MRS was performed to measure: (i) the absolute high-energy phosphate and Pi concentrations; and the unidirectional ATP synthesis rates through (ii) the creatine-kinase (CK) reaction using the ‘TRiST’ method(59), and (iii) the ATP→Pi reaction, as previously described(57). After baseline measures, PFE was initiated and plantar flexion was performed every second on a foot pedal connected by a pulley to a weight that was increased at the start of each 120-second exercise stage, as previously described(57). Participants were coached before and during plantar flexion exercise by a research nurse present in the MRI scanner room and the foot pedal excursion was noted. One-second time cues for plantar flexion were provided by a metronome sound. The first stage commenced with a 0.9 kg weight. A 0.9 kg weight was added at the second stage and 1.8 kg was added at each subsequent stage. Dynamic $^{31}$P MRS data were acquired every two seconds, starting 120 seconds prior to PFE (baseline), continuing throughout exercise and post-exercise recovery. During PFE, noting displacement of the weight once per second, total work was calculated (in Joules) by the sum of energy for each stage, which was
calculated by force (kg*m/s^2) * distance (m), (i.e. Force (weight pounds * 0.453kg/lbs. *9.81m/s^2) * distance (distance_inches *2.54 cm/in ÷100 cm). Exercise was terminated when subjects said they were unable to continue exercise at the prescribed rate of once per second.

Heart rate and blood pressure were measured at each exercise stage using a fingertip pulse oximeter or ECG device, and an automated blood pressure cuff. Subjective fatigue was recorded during each exercise stage using an 11-point (0-10) BORG rating scale of perceived exertion for both leg and total body fatigue (60).

Participants also underwent functional assessments of exercise tolerance using six-minute walk (6MW) and bicycle cardiopulmonary stress tests. Supervised 6MW testing commenced after a quiet 10-minute resting period in a 60-foot section of level hallway free of pedestrian traffic, with clear markers signifying the beginning and end. Distance walked and BORG symptoms were noted at the completion of six minutes. Cardiopulmonary bicycle exercise testing (CPET) was performed with gas-exchange analysis using a standard cycle ergometer protocol with a 25-watt graded intensity increase every 3 minutes. Subjects exercised to peak fatigue with a target respiratory exchange ratio (RER) >1.1 and a rated perceived exertion (RPE) >18 for adequate effort. Vital signs, ECG, and BORG symptoms were monitored at each exercise stage and throughout recovery. Oxygen consumption was measured with each breath and averaged over 15 second intervals. Peak oxygen consumption was taken as the average of the two highest values of oxygen uptake during the last minute of exercise (57). The 6MW was performed on all participants, except one healthy volunteer. Peak-
VO$_2$ during CPET was measured in all participants except one frail individual and one healthy volunteer.

**Image Analysis:**

Acquired T2-weighted fat images of central slices of calf muscles were processed using Matlab (Mathworks, Natick, MA) and segmented manually to remove subcutaneous fat and bones. To compare images among the subject groups, T2-weighted images were normalized by subcutaneous fat signal intensity as previously described (57). Popliteal blood flow was calculated from cine images from the same segment of vessel through each phase of the cardiac cycle using Matlab software.

Absolute concentrations (µmol/g wet weight) were measured using a previously validated external reference method (61). The unidirectional rate of ATP synthesis from both the creatine kinase reaction (PCr to ATP) and from inorganic phosphate (Pi to ATP), were obtained by measuring the PCr or Pi MRS signals in spectra acquired with γ-ATP saturated relative to a control saturation scan, as detailed previously (57, 59). Spectra acquired during PFE were analyzed using the ‘AMARES’ tool of the ‘jMRUI’ software package (62). High-energy phosphates during exercise were obtained by averaging the last ten spectra at rest and during each subsequent exercise stage before fitting in AMARES. Cytosolic adenosine diphosphate (ADP) concentration was calculated assuming that 15% of the total creatine was unphosphorylated at rest and an equilibrium constant of $K_{eq}=1.66\times10^9$ (63, 64). Gibbs free energy was then calculated using the cytosolic ADP, Pi, and ATP concentrations (57, 65). The individual post-exercise recovery time for PCr was determined by fitting a mono-exponential function to post-exercise PCr after the patient reported exhaustion and stopped exercising (57, 66,
Mitochondrial function, as estimated by maximal oxidative capacity, was calculated using Michaelis-Menten kinetics, as previously described (57, 68).

Statistics:
The Shapiro-Wilk test was used to test whether data were normally distributed. One-way analysis of variance corrected for multiple comparisons, was used to test for differences among the three cohorts in normally distributed variables. The Kruskal-Wallis test corrected for multiple comparisons and Mann-Whitney two-tailed pairwise testing was used to test group differences in non-normally distributed data. Spearman correlation tests were used to calculate correlation coefficients. A p-value less than 0.05 was considered significant for all statistical tests. Statistical analysis was performed using GraphPad Prism version 8 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Study Approval
The Johns Hopkins Institutional Review Board (Baltimore, MD) approved this study and protocol involving human subjects. All participants were given a detailed explanation of the study protocol, all questions were answered, and each provided informed, written consent prior to enrollment.

Author contributions:
MS, GG, JDW, and RGW designed the study; MS wrote the MRS acquisition software; SCL, KW, MS, and YZ collected and analyzed the MRS/MRI data; SCL and TJS performed blinded analyses to test reproducibility of MRS, SCL and QX performed the statistical analysis; JDW referred participants; SCL, AS, and RGW supervised exercise

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tests; SCL and RGW drafted the manuscript; and SCL, PAB, JDW, GG and RGW edited the manuscript.

**Disclosures:** None

**References:**


**Table 1:** Participant Demographics. Data are means±SEM. 6MW indicates 6-minute walk distance; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin type II receptor blocker; ASA, aspirin; BMI, body mass index; Peak VO2, normalized peak oxygen consumption on cardiopulmonary exercise test; and RER, respiratory exchange ratio. *p<0.05 vs Non-Frail Older; ¥ p<0.02 vs Non-Frail Older; l p<0.05 vs Control; δ p<0.02 vs Control.

<table>
<thead>
<tr>
<th>Descriptors</th>
<th>Healthy Middle-Aged (CONT) (n=11)</th>
<th>Non-Frail Older (NFO) (n=12)</th>
<th>Frail Older (FO) (n=11)</th>
<th>ANOVA p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>50.5±2.1</td>
<td>78.8±2.0 δ</td>
<td>80.5±2.7 δ</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>6 (55)</td>
<td>8 (73)</td>
<td>7 (64)</td>
<td></td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1 (9)</td>
<td>0 (0)</td>
<td>4 (36)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>8 (73)</td>
<td>12 (100)</td>
<td>7 (64)</td>
<td></td>
</tr>
<tr>
<td>Smoker, former (%)</td>
<td>1 (9)</td>
<td>3 (25)</td>
<td>7 (64)</td>
<td></td>
</tr>
<tr>
<td>Smoker, active (%)</td>
<td>0 (0)</td>
<td>1 (8)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.7 ± 1.0</td>
<td>25.0±0.8</td>
<td>28.8±2.1 l</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Weight (lb.)</td>
<td>154 ± 9.1</td>
<td>155.4±7.4</td>
<td>174.3±14.4</td>
<td>p = ns</td>
</tr>
<tr>
<td>Height (in.)</td>
<td>68.5 ± 1.4</td>
<td>66.1± 1.2</td>
<td>65.3±1.6</td>
<td>p = ns</td>
</tr>
<tr>
<td>ACEI/ARB, n (%)</td>
<td>0 (0)</td>
<td>2 (17)</td>
<td>4 (36)</td>
<td></td>
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<tr>
<td>B-Blockers, n (%)</td>
<td>0 (0)</td>
<td>2 (17)</td>
<td>3 (27)</td>
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<tr>
<td>Diuretics, n (%)</td>
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<td>0 (0)</td>
<td>4 (36)</td>
<td></td>
</tr>
<tr>
<td>ASA, n (%)</td>
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<td>1 (8)</td>
<td>4 (36)</td>
<td></td>
</tr>
<tr>
<td>Statin, n (%)</td>
<td>1 (9)</td>
<td>2 (17)</td>
<td>6 (55)</td>
<td></td>
</tr>
<tr>
<td>%MPHR Achieved</td>
<td>113.3 ± 1.9</td>
<td>111.4±4.1</td>
<td>100.5±4.4</td>
<td>p = ns</td>
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<tr>
<td>6MW (m)</td>
<td>622 ± 33</td>
<td>443±34 δ</td>
<td>310±38 ¥δ</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Peak VO2 (ml/kg/min)</td>
<td>34.5 ± 3.2</td>
<td>19.1±1.3 δ</td>
<td>13.5±1.2 ¥δ</td>
<td>p &lt; 0.0001</td>
</tr>
<tr>
<td>Absolute Peak VO2 (ml/min)</td>
<td>2428 ±277</td>
<td>1379 ± 123 δ</td>
<td>1062 ± 94 δ</td>
<td>p &lt; 0.0002</td>
</tr>
<tr>
<td>RER</td>
<td>1.08 ± 0.03</td>
<td>1.02 ± 0.02</td>
<td>1.0 ± 0.0</td>
<td>p = ns</td>
</tr>
</tbody>
</table>
**Figure 1:** (A) Plantar Flexion exercise (PFE) time, (B) total PFE work, and (C-F) correlations between indices of PFE performance (PFE time and PFE work) and established global functional indices (6MW and peak VO$_2$ at CPET). Control (CONT, n=11, black triangles), non-frail older (NFO, n=12, dark gray circles), and frail older (FO, n=11, open squares) individuals. Data are individual points and means±SEM. Statistical tests were (A) ANOVA with multiple comparisons tests, (B) Kruskal-Wallis with Mann-Whitney U tests, and (C-F) Spearman correlations. * p < 0.05, ** p <0.02, *** p <0.005, **** p <0.001.
Figure 2: Representative $^{31}$P MR spectra at baseline (A) and at fatigue (B) of a healthy-middle aged subject. Time course of skeletal muscle high-energy phosphate metabolites: PCr (dark blue) and ATP (light blue) and inorganic phosphate (red) before, during, and after exercise in a healthy middle-aged subject (C) and frail older subject (D). The rate of PCr decline during exercise is several-fold faster in the frail older subject.
Figure 3: Skeletal muscle energetic parameters (PCr, ATP, Pi, ΔG:\textsubscript{ATP}, ADP, pH) during resting conditions (A-F) and at performance fatigue (G-L). There were no significant differences in any of these metabolic parameters at rest or at performance fatigue among the three groups. CONT (n=11, black triangles), NFO (n=12, dark gray circles), FO (n=11, open squares). Skeletal muscle unidirectional ATP synthesis rates from PCr through CK at rest (M, CONT, n=11, black triangles, NFO, n=12, dark gray circles, FO, n=8, open squares) and of ATP synthesis rates from Pi at rest (N, CONT, n=11, black triangles, NFO, n=9, dark gray circles, FO, n=8, open squares) did not differ among the three groups. Data are individual data points and means±SEM. Statistical tests were ANOVA and Kruskal-Wallis ANOVA.
Figure 4: Both the average rate of PCr decline during all of PFE (A) and the initial rate of PCr decline during the first four minutes of PFE (B) were significantly faster in frail older individuals (open squares) than in non-frail older (dark gray circles) and healthy middle-age participants (black triangles). (C) Short exercise time was strongly associated with rapid energetic decline in that there was an inverse correlation between PFE time and the rate of PCr decline (p<0.0001). Total work performed during PFE (D), six-minute walk distance (E), and peak VO₂ (F), all correlated inversely and significantly with the rate of PCr decline during exercise. CONT (n=11, black triangles), NFO (n=12, dark gray circles), FO (n=11, open squares). Data are individuals points and means±SEM. Statistical tests were Kruskal-Wallis ANOVA with Mann-Whitney U tests and Spearman correlations. * p < 0.05, ** p <0.02, ***p <0.005, **** p <0.001.
Figure 5: Recovery time of creatine phosphate (PCr) after plantar flexion exercise (PFE) is longer (A), and maximum oxidative capacity is reduced (B) in frail older individuals. The rate of PCr decline during PFE correlates with reduced maximum oxidative capacity (C, p<0.02). Correlations between 6MW and maximum oxidative capacity (D), between peak VO₂ and maximum oxidative capacity (E), and between PFE time and maximum oxidative capacity (F). Data are individual points and means±SEM. CONT (n=11, black triangles), NFO (n=12, dark gray circles), FO (n=11, open squares). Statistical tests were (A) Kruskal-Wallis with Mann-Whitney U tests, (B) ANOVA with multiple comparisons tests, (C-F) Spearman correlations. * p < 0.05, ** p <0.02, *** p <0.005, **** p <0.001.
**Figure 6:** (A) Magnetic resonance images showing fat distribution (white signal) in the calf of a healthy middle-aged individual (left), in a non-frail older (middle) and in a frail older individual (right). Total cross-sectional muscle areas across the three groups did not differ (B), however muscle fat content was significantly elevated in frail older individuals (C). (D) Correlation between PFE time and muscle fat fraction % (without outliers). (E) Correlation between average PCr decline and muscle fat fraction % (without outliers). (F) Correlation between maximal oxidative capacity and muscle fat fraction % (without outliers). See supplement for all comparison figures with/without outliers. Muscle fat fraction was considered an outlier if intramuscular fat content exceeded 42%, which included two participants (one frail, one non-frail). Data are individual points and means±SEM. CONT (n=10 black triangles), NFO (n=11 dark gray circles), FO (n=10 open squares). Statistical tests used were Spearman correlations. The correlations were statistically significant at p<0.05 level with or without outliers. * p < 0.05, **p <0.02, ***p <0.005, ****p <0.001.
Graphical Abstract:

Rapid Skeletal Muscle Energetic Decline During Exercise in Frail Elderly

Healthy Middle Age: Slow Energetic Decline During Exercise

Frail Older: Rapid Energetic Decline During Exercise

Spearman r, -0.9252
p<0.0001

Avg. PCr Decline
[μmol/g wet wt/kJ]