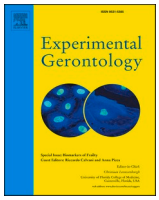
Experimental Gerontology 216 (2026) 113062

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero



Iron dysregulation and mitochondrial dysfunction in aging: A longitudinal study on mobility decline in low- and high-functioning older adults☆

Nevena Stanojevic [a,*], Stephanie Wohlgemuth [a], Rola S. Zeidan [a,b], XiangYang Lou [c], Javier A. Tamargo [a], Bella Bravo [a], Jennifer Newman [d], Anna Picca [e,f], Emanuele Marzetti [f,g], Kevin Wu [h], Christian McLaren [i], Robert Mankowski [a,j], Bhanuprasad Sandesara [k], Aditya Shirali [l], Todd Manini [b], Christiaan Leeuwenburgh [h], Stephen Anton [a,i,**]

[a] University of Florida, Department of Physiology and Aging, Gainesville, FL, USA
[b] University of Florida, Department of Health Outcomes and Biomedical Informatics, Gainesville, FL, USA
[c] University of Florida, Department of Biostatistics, Gainesville, FL, USA
[d] H. Lee Moffitt Cancer Center & Research Institute, Clinical Trials Office, Tampa, FL, USA
[e] Department of Medicine and Surgery, LUM University, Casamassima, Italy
[f] Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy
[g] Department of Geriatrics, Orthopedics and Rheumatology, Università Cattolica del Sacro Cuore, Rome, Italy
[h] University of Florida, Department of Medicine, Division of Geriatric Medicine, College of Medicine, Institute on Aging, Gainesville, FL, USA
[i] Department of Clinical and Health Psychology, College of Public Health and Health Professions, University of Florida, Gainesville, FL, USA
[j] University of Alabama at Birmingham, Department of Medicine, Division of Gerontology, Geriatrics and Palliative Care, Birmingham, AL, USA
[k] Department of Medicine, Division of Geriatric Medicine, University of Florida, Gainesville, FL, USA
[l] Department of Surgery, University of Florida College of Medicine, Gainesville, FL, USA

ARTICLE INFO

Section Editor: Carel Meskers

Keywords:
Mitochondrial dysfunction
iron regulation
Ferritin
Inflammation
Physical function
Aging
Muscle strength

ABSTRACT

Background: Mobility loss in older adults reduces quality of life and increases risks of falls, hospitalizations, and mortality. Low-functioning (LF) older adults experience faster mobility decline than their high-functioning (HF) peers, but the underlying biological mechanisms remain unclear. Although iron accumulation in aging muscle mitochondria has recently been linked to lower physical function, its longitudinal impact on physical function remains understudied.

Methods: Muscle Iron Flow is a prospective, observational study which enrolled LF and HF older adults ($N = 114$; age 75.8 ± 3.2 years, 64.9% female) to examine links between iron dysregulation, mitochondrial function, and physical performance. Assessments include blood biomarkers, physical function tests, and behavioral measures (diet, activity, sleep, medication use), collected at baseline and annually, and muscle biopsies obtained at baseline and year three. This manuscript reports baseline characteristics and measurement procedures only; longitudinal portion is ongoing.

Results: At baseline, HF older adults demonstrated significantly better physical performance than LF adults across all functional tests, including Short Physical Performance Battery (SPPB), 6-minute walk distance, handgrip strength, and knee extensor torque (ps < 0.05). LF participants also had lower hemoglobin levels and higher red cell distribution width (RDW; ps < 0.05).

Discussion: This study is among the first to investigate how biomarkers of iron dysregulation, mitochondrial function, and related ferroptosis and senescence pathways may contribute to changes in physical function in LF and HF older adults. By integrating molecular and functional assessments, the study will inform how disrupted iron handling and mitochondrial health influence mobility trajectories in aging populations.

☆ This article is part of a Special issue entitled: 'Study of Aging and Longevity' published in Experimental Gerontology.

* Corresponding author.
** Correspondence to: S. Anton, University of Florida, Department of Physiology and Aging, Gainesville, FL, USA.
E-mail addresses: nevenastanojevic@ufl.edu (N. Stanojevic), santon@ufl.edu (S. Anton).

https://doi.org/10.1016/j.exger.2026.113062
Received 16 July 2025; Received in revised form 20 January 2026; Accepted 6 February 2026
Available online 9 February 2026

1. Background

Mobility loss is a common feature of aging, significantly impacting independence and quality of life in older adults (Abrams, 2004; Arantes-Oliveira et al., 2003; Drew, 2018). Many older individuals prioritize maintaining mobility over longevity (Manini et al., 2017; Mankowski et al., 2017), yet the increasing life expectancy also raises the risk of age-related diseases that contribute to declines in physical function (O'Neill and Forman, 2020). Currently, 3.2 million Americans aged 65 and older report significant difficulty walking, a number projected to rise to 5.5 million by 2050 (Anton et al., 2015). Impaired mobility is associated with a greater incidence of falls, hospitalizations, and mortality (Callahan et al., 2018; Cawthon et al., 2020; Manini et al., 2018; Marsh et al., 2016). As a result, preserving mobility has become a key focus in clinical and public health initiatives, including the National Institute on Aging (NIA) and National Institutes of Health (NIH) strategic plans for aging research (National Institute on Aging, 2021).

Notably, functionally limited older adults experience an accelerated decline in physical function compared to their higher-functioning peers (Chang et al., 2004; Guralnik et al., 1995; Pavasini et al., 2016). While mobility decline is well-documented, the biological mechanisms driving this process remain incompletely understood. Mitochondrial dysfunction is a major contributor to physical decline and is linked to several hallmarks of aging, including genomic instability, epigenetic alterations, cellular senescence, and deregulated nutrient signaling (Chistiakov et al., 2014; López-Otín et al., 2023; van der Rijt et al., 2020). Mitochondrial dysfunction has been widely studied in this context, as impaired mitochondrial bioenergetics are known to reduce cellular energy production, contributing to muscle weakness and physical decline with age (Manini et al., 2018).

There are a number of potential biological mechanisms that contribute to age-related mitochondrial dysfunction, and a growing area of research focuses on the important role of iron dysregulation. Emerging evidence suggests that disrupted iron regulation, marked by altered iron-handling proteins, contributes to mitochondrial damage and oxidative stress. Such biological changes would be expected to contribute to functional decline, but the impact of cellular iron regulation on changes in physical function in older adults is not well understood.

Accumulation of non-heme iron in muscle tissue, may play a critical role in mitochondrial dysfunction (Xu et al., 2008; Picca et al., 2019a, 2020). Given the mitochondria's reliance on iron for essential processes such as electron transport and energy production, disruptions in iron homeostasis can contribute to oxidative stress, inflammation, and eventually, physical decline (Joseph et al., 2012). Disruptions in cellular and mitochondrial iron transport may contribute to increased mutations and deletions, leading to mitochondrial genome instability and impaired function (Chistiakov et al., 2014; López-Otín et al., 2023; van der Rijt et al., 2020). Inflammatory cytokines, such as IL-6, stimulate hepcidin production, disrupting iron balance by inhibiting the iron export protein ferroportin, which can lead to intracellular iron overload. This, in turn, promotes oxidative stress, inflammation, and cellular senescence. Recent reports point toward links between cellular iron dysregulation and hallmarks of aging. For example, studies in humans and mice indicate that intracellular iron accumulation is a hallmark of senescent cells (Masaldan et al., 2018; Zhang et al., 2021), other reports highlight how chronic low-grade inflammation, mitochondrial dysfunction, and macrophage dysregulation contribute to stem cell aging and impaired tissue regeneration (Tatullo et al., 2024); and iron overload has been shown to drive ferroptosis, an iron-dependent regulated form of cell death, which is emerging as an important mechanism in aging tissues and may contribute to decline in muscle health (Tatullo et al., 2024; Dixon et al., 2012; Picca et al., 2023).

While prior studies identified associations between physical function and biomarkers related to iron regulation and mitochondrial function in older adults, limited research has examined these relationships longitudinally, particularly in lower and higher functioning older adults (Picca et al., 2019a, 2020, 2023; Joseph et al., 2012). To investigate these relationships, the Muscle Iron Flow study was initiated, in which high-functioning (HF) and low-functioning (LF) participants, aged 70–80 years, are followed for a three-year period. While the study is ongoing, the primary aim of this manuscript is to describe the study design and methodology, including the longitudinal tracking of physical and biological outcomes, such as blood biomarkers, muscle biopsies, and functional assessments, over a three-year period in high- and low-functioning older adults.

To investigate these relationships, the Muscle Iron Flow study was initiated, in which high-functioning (HF) and low-functioning (LF) participants, aged 70–80 years, are followed for a three-year period. The primary aim of this manuscript is to describe the study design and methodology, including the longitudinal tracking of physical and biological outcomes, such as blood biomarkers, muscle biopsies, and functional assessments, over a three-year period in high- and low-functioning older adults. Although full longitudinal outcomes are not yet available, the Muscle Iron Flow study was designed with the expectation that LF participants would exhibit greater impairments across these measures compared to HF participants at baseline. Specific hypothesis such as elevated hepcidin, increased muscle iron accumulation, altered expression of iron-handling proteins (e.g., ferritin, transferrin receptor), and mitochondrial dysfunction are expected to predict with slower walking speed. Longitudinally, it is expected that elevated hepcidin levels, increased muscle non-heme iron accumulation, and greater mitochondrial dysfunction would predict a faster decline in walking speed and physical function.

2. Methods

The Muscle Iron Flow study is ongoing. Recruitment and baseline visits were completed in December 2024, and participants are expected to complete all follow-up visits by 2027.

2.1. Study design

The study was designed to follow participants for three years, with annual assessments of functional and biological outcomes. The first aim (cross-sectional) of this study is to determine if LF participants exhibited higher circulating hepcidin levels, greater muscle iron content, and more pronounced mitochondrial dysfunction compared to HF participants, and to explore how these factors relate to physical function and strength performance. The second aim (longitudinal) is to evaluate whether longitudinal changes in iron regulation and mitochondrial function are associated with the rate of decline in walking speed and physical performance. The third aim is exploratory and focuses on assessing whether mitochondrial dysfunction mediates the relationship between iron dysregulation and functional decline.

Biological data, including hepcidin, cytokines, and other markers, were collected through blood draws at baseline and will be repeated annually during follow-up visits (FV-1, FV-2, FV-3). Additionally, muscle biopsies were performed at baseline and will be repeated after three years to assess muscle iron content and mitochondrial function. Physical function was evaluated using walking performance assessments, muscle strength tests, and behavioral measures, with assessments conducted at baseline and repeated annually (FV-1 to FV-3). The physical function assessments include the short physical performance battery (SPPB), Six-Minute Walk Test (6MWT), and muscle strength evaluations. Changes in health behaviors, such as activity levels, dietary intake and sleep are measured using both objective and subjective measures. This comprehensive approach provides insights into the relationship between changes in biological measures and physical function over time.

2.2. Recruitment

Participants were recruited through targeted recruitment strategies including direct mailings, flyers, newspaper classified ads, publication print ads, community luncheons, health fairs, clinic referrals and social media advertisements (through a digital marketing group company BUMP) (*BUMP*, n.d.). Participants were categorized as LF or HF based on their SPPB scores at the screening visit, with a score of 9 or below identifying LF individuals and scores from 11 to 12 classifying HF peers. Participants with an SPPB score of 10 were excluded to create a clear distinction between HF and LF participants. Recruiting community-dwelling older adults for muscle biopsy procedures was inherently challenging due to medical comorbidities, procedure-related concerns, and the overall burden of participation, which contributed to slower enrollment. Inclusion/exclusion criteria are listed in Table 1.

2.3. Participants

This study has enrolled 114 participants aged 70 to 80 years and has entered the follow-up stage. After 9 withdrawals, the study is following 105 participants, 44 with an SPPB score of ≤9 (LF group) and 61 an SPPB score of 11–12 (HF group).

The cut-point of SPPB ≤9 was selected to define older adults as LF because previous longitudinal studies have shown that individuals with SPPB scores in this range have substantially higher rates of disability over four-year follow-up compared to individuals with SPPB scores of 11 or 12 (Guralnik et al., 1995, 2000). To explore potential effects of race and ethnicity, we recruited 12 minority participants (approximately 11% of the sample). The demographic characteristics of the study participants are presented in Table 2, divided by high-functioning (HF) and low-functioning (LF) groups.

There were no differences in baseline demographic characteristics between the high and low functioning older adults. Baseline data are presented to characterize the cohort for future longitudinal analyses.

2.3.1. Screening visit

Individuals who appeared to be eligible based on a phone screening were invited to an in-person screening visit. During this visit, potential participants received detailed information on the study's purposes and methods and were asked to give their informed consent. The screening visit included a medical history and medication review to confirm eligibility, as well as a demographic and contact information collection. A study physician conducted a physical exam (at the screening or baseline visit) to assess participants' health status and suitability to participate in this study. A self-reported physical activity questionnaire, the Community Healthy Activities Model Program for Seniors (CHAMPS) (Stewart et al., 2001), was collected to assess activity levels and confirm they were not engaging in 150 min of physical activity per week. SPPB was done to determine eligibility and assign participants to the HF/LF groups. Finally, a global cognitive-function assessment (Mini-Mental State Examination; MMSE) (Folstein et al., 1975) test was completed to assess eligibility and cognitive function. If all criteria were

Table 1
Eligibility criteria for enrollment.

Inclusion criteria
• 70–80 years old • SPPB ≤9 (Low Functioning Group) or 11–12 (High Functioning Group) • Physically inactive lifestyle (i.e., <150 min/week of moderate-intensity aerobic exercise) and no active participation in a formal exercise program within the past 3 months (i.e., brisk walks are considered formal exercise, but leisurely walks are not) • Ability to walk one block (400 m) • Mini-Mental State Examination (MMSE) score ≥ 24 • Willingness to undergo all testing procedures

Exclusion criteria
• Failure to provide informed consent • Use of walking assistance devices such as cane, crutches, walkers • Active treatment for cancer, stroke (<6 months), peripheral vascular disease, coronary artery disease, myocardial infarction (<6 months), congestive heart failure (stage III or IV), valvular heart disease, COPD • Allergy to lidocaine • SPPB score of 10 • Major psychiatric disease • Malabsorption (e.g., celiac disease) • Major gastrointestinal disorders (e.g., peptic ulcer, chronic gastritis/duodenitis, inflammatory bowel disease) • Chronic inflammatory disorders (e.g., autoimmune disease, chronic infections) • Splenectomy • Hemolytic anemia, severe anemia, liver or renal disease within the past 3 years • Diabetes (self-report) or fasting glucose >126 mg/dL • Severe osteoarthritis, fracture in upper or lower extremity (<6 months), upper or lower extremity amputation • Neuromuscular disorders including ALS, Parkinson's disease, muscular dystrophy, multiple sclerosis • Excessive alcohol use (i.e., >14 drinks/week) or alcohol abuse (i.e., >5 drinks/day for males or >4 drinks/day for females) • Other substance abuse within the past 3 years • Smoking history in the past 3 years • Current use of anabolic hormones (i.e., growth hormones or testosterone) or anticoagulants • Unwilling or study physician's advice not to stop antiplatelet drug 5 to 7 days before and 2 to 3 days after the muscle biopsy • Participating in another clinical trial or has received an investigational product within 30 days prior to screening/enrollment

Temporary exclusion criteria
• Recent bacterial/viral infection (<3 weeks) • Acute febrile illness in the past 2 months • Resting heart rate > 120 bpm • High blood pressure (i.e., ≥180/100 mmHg) at screening visit • Major surgery or hip/knee replacement (<6 months)

Table 2
Baseline demographic characteristics of high- vs. low-functioning older adults.

Variable	Category	Total (N = 114) N (%) Mean ± SD	High (N = 64) N (%) Mean ± SD	Low (N = 50) N (%) Mean ± SD	P-value
Age	Mean ± SD	75.78 ± 3.17	76.10 ± 3.33	75.53 ± 3.04	0.4103
Sex	Male	40 (35.1%)	13 (26.0%)	27 (42.2%)	0.0792
	Female	74 (64.9%)	37 (74.0%)	37 (57.8%)	
Race	Caucasian/ White	103 (90.4%)	42 (84.0%)	61 (95.3%)	0.1261
	African American or Black	6 (5.3%)	5 (10.0%)	1 (1.6%)	
	Asian	3 (2.6%)	2 (4.0%)	1 (1.6%)	
	American Indian or Alaskan Native	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Native Hawaiian or other Pacific Islander	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	Others	2 (1.8%)	1 (2.0%)	1 (1.6%)	
	Refuse to answer	0 (0.0%)	0 (0.0%)	0 (0.00%)	
Ethnicity	Hispanic or Latino	2 (1.8%)	1 (2.0%)	1 (1.6%)	0.9999
	Not Hispanic or Latino	109 (95.6%)	48 (96.0%)	61 (95.3%)	
	Refuse to answer	3 (2.6%)	1 (2.0%)	2 (3.1%)	
Education	No formal education (00)	0 (0.0%)	0 (0.0%)	0 (0.00%)	0.3263
	Elementary school (K-08)	0 (0.0%)	0 (0.0%)	0 (0.00%)	
	High school/ equivalent (09–12)	12 (10.5%)	6 (12.0%)	6 (9.4%)	
	Technical/ vocational (13–14)	12 (10.5%)	8 (16.0%)	4 (6.3%)	
	College (13–16)	53 (46.5%)	24 (48.0%)	29 (45.3%)	
	Post Graduate	31 (27.2%)	10 (20.0%)	10 (20.0%)	
	Others	6 (5.3%)	2 (4.0%)	2 (4.0%)	
	Refuse to Answer	0 (0.0%)	0 (0.0%)	0 (0.0%)	

met, participants were scheduled to return to the Research Center for a baseline visit. A total of 214 individuals were screened in-person for the study. Of these, 93 participants did not meet the eligibility criteria, and 7 were withdrawn due to noncompliance or health-related concerns. The most common reasons for exclusion included an SPPB score of 10 (n = 34), engaging in more than 150 min of physical activity per week (n = 27), and the use of blood-thinning medications or other health-related exclusions (n = 8). Ultimately, 114 participants were successfully enrolled.

2.3.2. Baseline visits

Eligible participants were invited to undergo two baseline visits (Baseline 1 and Baseline Biopsy) that included functional testing, a muscle biopsy, and a blood draw. To reduce participant burden, the physical function tests (baseline 1 visit) and muscle biopsy (baseline 2 visit) were not performed at the same baseline visit and were scheduled at least five days apart.

2.3.2.1. Baseline visit 1. At baseline visit 1, participants underwent a blood draw for a complete blood count (CBC) and a comprehensive metabolic panel (CMP). Additional biomarkers detailed below were also collected and stored for further analysis. Participants were also asked to provide an updated list of their medications and to report any adverse events that may have occurred since their last visit. They completed the Pepper Assessment Tool for Disability (PAT-D) (Rejeski et al., 2008), which is a self-reported questionnaire that is used to provide an estimate on participant's functioning difficulty.

The physical function tests at this visit included the SPPB, the 6MWT, the Grip strength test and the Biodex Test (knee extension peak torque).

Additionally, participants were asked to wear an Apple™ smartwatch with the activated Real-time Online Assessment and Mobility Monitor (ROAMM) (Clark et al., 2019) app during daytime to collect their activity levels as well as the Actigraph device during the night to collect sleep quality data. They received instructions on how to use the Apple™ smartwatch and navigate the ROAMM app as well as how to use the Actigraph device. Participants were asked to wear the Apple™ smartwatch and Actigraph device for approximately 14 days at one of the baseline visits. At the conclusion of the Baseline 1 visit, participants were scheduled for the baseline muscle biopsy, which occurs between 5 days and 12 weeks later. Prior to the biopsy visit, participants were given detailed preparation instructions, which included discontinuing antiplatelet medications 5 to 7 days before the procedure, avoiding strenuous exercise for 48 h prior, and fasting for 12 h.

2.3.2.2. Baseline biopsy visit. At the baseline biopsy visit, participants were asked to complete the Iron Intake Frequency Food Questionnaire (IRONIC-FFQ) (Głąbska et al., 2017) and Diet History Questionnaire III (DHQ-III) (*EGRP/DCCPS/NCI/NIH*, n.d.) as well as the Pittsburgh Sleep Quality Questionnaire (PSQI) (Buysse et al., 1989). The muscle biopsy was performed by a trained physician. A biopsy was obtained from the vastus lateralis muscle on the dominant thigh (using a percutaneous biopsy needle under local anesthesia). Participants were instructed to avoid antiplatelet medications for 2 to 3 days following the biopsy to reduce the risk of bleeding. The study conducted 107 baseline biopsy visits. Of these, 2 participants withdrew from the study following the procedure. Additionally, 5 participants required a repeat biopsy due to the failure to obtain muscle tissue during the initial procedure. A total of 105 participants progressed to the annual follow-up stage (see Fig. 1 – Enrollment Summary).

2.3.3. Annual follow-up visits (FV1, FV2, FV3)

After the conclusion of the baseline visits, participants will be followed for an approximate three-year period. During this time, they will be contacted on a quarterly basis to monitor their well-being. All communication between the participant and the study team will be documented in the participants' records.

Additionally, annual follow-up visits will occur approximately one year (± 3 months) after each participant's baseline visit to allow for scheduling flexibility while maintaining a consistent follow-up interval. At the follow-up visits, all measures taken at the baseline 1 visit will be reassessed (see Table 3. Schedule of Assessments as well Fig. 2 – Study Timeline).

2.3.4. Third-year biopsy visit

After the third-year follow-up visit (FV3), participants will be asked to come in for a third-year biopsy visit. This visit will follow the same structure as the baseline biopsy visit.

2.4. Outcome measures

2.4.1. Physical function measures (baseline and annual follow-up visits)

A comprehensive battery of measures is used to assess participants' physical function, including validated measures of functional performance, muscle strength, and walking speed. These measures are conducted at baseline and during follow-up visits. The tests include SPPB, 6MWT, Biodex test of Knee Extension Peak Torque, and Grip Strength test.

Table 3
Schedule of assessments.

Visit type	Phone screen	Screening visit	Baseline visit 1	Baseline biopsy	FV1	FV2	FV3	FV3 biopsy
Eligibility								
Telephone interview screen	•							
Informed consent		•						
Medical history & medications, MMSE		•						
Short Physical Performance Battery (SPPB)		•						
CHAMPS questionnaire		•						
Vitals (blood pressure, heart rate)		•						
Demographic and contact information		•						
Physical exam		•						
Biological measures								
Medication update			•		•	•	•	
Fating blood draw (CMC, CMP, biomarkers)			•		•	•	•	
Biospecimen collection (muscle biopsy)				•				•
Physical measures								
SPPB			•		•	•	•	
6 minute walk			•		•	•	•	
Grip strength			•		•	•	•	
Biodex test – knee extension peak torque			•		•	•	•	
Actigraph/Apple™ Watch			•		•	•	•	
Health behavior questionnaires								
Diet History Questionnaire III (DHQ III)				•	•	•	•	
IRONIC-FFQ (Iron Intake Questionnaire)				•	•	•	•	
Pittsburgh Sleep Quality Index (PSQI)				•	•	•	•	
CHAMPS Questionnaire		•			•	•	•	
Pepper Assessment Tool for Disability (PAT-D)			•		•	•	•	

2.4.1.1. Short Physical Performance Battery (SPPB). The SPPB is used to evaluate the performance of lower extremities in older adults. It consists of three components: a timed 4-meter walk to assess gait speed, a repeated chair stand test to measure lower body strength, and a balance test that includes standing side-by-side, semi-tandem, and full-tandem positions. Each component is scored on a scale from 0 to 4, with higher scores indicating better performance. The scores are summed up to give a total score ranging from 0 to 12, which reflects the overall physical performance. Higher total scores indicate greater mobility and lower risk of disability. Vice versa, a lower SPPB score is associated with increased risk of mobility disability, falls, hospitalization, and mortality in older adults. A total score below 10 typically indicates reduced physical function, with scores below 6 suggesting significant mobility impairment that may require intervention or monitoring. The SPPB test had been proved to be reliable in determining whether a participant was higher or lower functioning in terms of mobility, as well as predicting disability in older populations (Guralnik et al., 1994).

2.4.1.2. Six-Minute Walk Test (6MWT). The 6MWT is used to assess functional exercise capacity in various populations, including older adults. Participants are asked to walk as far as possible in the span of 6 min, while in a temperature controlled, safe research environment. Distance, walking speed, fatigue levels and heart rate following completion of the test are measured. This test is valued for its simplicity and correlation with overall health and functional status. Its validity and reliability has been confirmed by studies, making it a critical tool in both clinical and research settings (Meys et al., 2023).

2.4.1.3. Biodex test (knee extension peak torque). The Biodex knee extension peak torque test is conducted to measure the maximum strength of the quadriceps muscles. This test uses an isokinetic dynamometer to evaluate peak torque during knee extension at various speeds. The dynamometer is set to 90°/s, and participants are asked to perform knee extension and flexion concentric repetitions, which are administered by trained and certified research staff. Maximal muscle strength is summarized as the peak torque achieved in Nm. A muscle endurance index is calculated as the decline in peak torque following a 50-repetition muscle endurance test. The Biodex test is widely used in both clinical and research settings to assess muscle function and the effects of interventions on muscle strength. Research supports its reliability and validity, demonstrating the Biodex system's accuracy and consistency in measuring isokinetic strength (Drouin et al., 2004).

2.4.1.4. Grip strength test. The grip strength test is used to measure upper body strength and is correlated with total body strength (Porto et al., 2019; Wang et al., 2019). During the test, the participant is seated with their elbow bent at a 90-degree angle, their forearm in a neutral position, and their wrist slightly extended. They are instructed to squeeze the dynamometer with maximum effort for a few seconds. Participants complete the test twice with both hands. Grip strength provides an index of upper body strength.

Only completed baseline procedures are described; longitudinal outcome interpretation is not included.

2.4.2. Biological measures (baseline and three-year follow-up)

Skeletal muscle biopsy is obtained from the vastus lateralis muscle by percutaneous biopsy needle. Up to 500 mg (typically between 150 and 500 mg) of muscle tissue is collected and immediately prepared for analysis, as described below.

2.4.2.1. High-resolution respirometry. High-resolution respirometry, using the Oxygraph-2k (Oroboros, Austria), is performed for a detailed analysis of mitochondrial respiratory function in freshly collected muscle samples, as described in (Mankowski et al., 2023), with slight modifications. Specific substrate-uncoupler-inhibitor titration protocols, as previously reported (Mankowski et al., 2023; Joseph et al., 2022), assessed mitochondrial complex- and substrate-specific respiratory capacities. Data is acquired and analyzed using DatLab vs. 7.4 software (Oroboros) (Mankowski et al., 2023; Joseph et al., 2022). To ensure the reliability and reproducibility of results, instruments are calibrated before each use, and mitochondrial membrane integrity is tested as a quality-control step (cytochrome *c* test). Assessing mitochondrial

respiratory function in muscle biopsies directly indicates the energy-producing capacity of mitochondria and potential impairments associated with aging and functional decline (Figueiredo et al., 2009).

2.4.2.2. Quantification of mitochondrial DNA (mtDNA) 4977 bp deletion and mtDNA copy number. The relative abundance of mtDNA 4977 bp deletion is determined by quantitative real-time polymerase chain reaction (qRT-PCR) via SYBR Green chemistry on a CFX96 Touch™ PCR Detection System (Bio-Rad Laboratories, Hercules, CA), as described previously (Fuke et al., 2008). Measuring these markers of mitochondrial DNA integrity helps determine the extent of mitochondrial damage and biogenesis capacity, both of which are implicated in muscle aging and dysfunction (Joseph et al., 2012).

2.4.2.3. Assessment of single-strand break, double-strand break, and abasic sites. The abundance of differential DNA damage within specific regions of the mt genome (ND1/2, ND4/5, COII/ATPase 6/8, and D-loop) is determined using a qRT-PCR-based assay (Furda et al., 2014). Quantifying DNA damage within specific mitochondrial genome regions provides insights into the oxidative stress and genomic instability that may contribute to mitochondrial dysfunction and subsequent mobility loss (Shokolenko et al., 2009).

2.4.2.4. Determination of non-heme iron and free iron levels. In addition to total non-heme iron content in muscle samples, non-heme iron content in skeletal muscle mitochondria is measured as previously described and adapted to a 96-well format (Jung et al., 2008; Seo et al., 2008; Rebouche et al., 2004). Standard mass spectrometry analysis is used to quantify iron as the primary measure, as well as copper and zinc as secondary measures (Dixon et al., 2012; Xu et al., 2012; Picca et al., 2019b). Free iron content in formalin-fixed and paraffin-embedded muscle samples is visualized histologically, as previously described (Hofer et al., 2008). This method detects ferric iron in tissues through the reaction of iron with acid ferrocyanide, producing a blue color (Prussian blue reaction). Measuring total and mitochondrial non-heme iron, along with free iron visualization, directly assesses iron accumulation in muscle tissue and within mitochondria, a key aspect of the hypothesized iron dysregulation contributing to mitochondrial damage (Seo et al., 2008).

2.4.2.5. Protein analysis (TfR-1, Fn, frataxin, and mitoferrin) using immunodetection. Protein content of transferrin receptor protein 1 (TfR-1), ferroportin (Fn), frataxin, ferritin, and mitoferrin is measured in tissue lysates from muscle samples using either automated, capillary-based immunoassay with a Jess system (ProteinSimple, San Jose, CA), or traditional Western blotting (Mankowski et al., 2023), with commercially available primary and secondary antibodies. Analyzing the protein levels of key iron-handling proteins provide insights into the cellular mechanisms of iron uptake, storage, and mitochondrial iron import, and how these are altered with aging and functional status (Ali et al., 2022).

2.4.2.6. Circulating markers. Circulating levels of hepcidin, ferritin, soluble transferrin receptor, total iron-binding capacity, and iron are measured in plasma or serum using commercially available ELISA kits. Cytokines are measured using ELISA or Luminex technology (MILLIPLEX® Analyzer 3.1 xPONENT System (Luminex® 200) and MILLIPLEX® Analyst software; Merck Millipore, Burlington, MA). Plate processing and data collection are performed per the manufacturers' instructions. Absorbance is read on a Synergy HT Multi-Detection microplate reader (Agilent/BioTek, Winooski, VT). These markers reflect systemic iron regulation, while cytokine measurements assess the inflammatory environment, both of which can influence muscle health and function (Kotze et al., 2009).

From participants' CBC and CMP panels, data on hemoglobin, hematocrit, red blood cell indices, serum iron, ferritin, transferrin saturation, glucose, liver and kidney function markers, and electrolyte levels are collected to evaluate systemic health, iron regulation, and potential metabolic or organ dysfunction related to mitochondrial health and physical performance in aging.

2.4.3. Behavioral measures

Changes in health behaviors, including dietary intake, physical activity levels, and medication use, which could influence physical function in older adults, are assessed using both objective and subjective measures, at baseline and each annual visits. These measures are used as covariates in all statistical analyses.

2.4.3.1. Dietary intake

2.4.3.1.1. Diet History Questionnaire III (DHQ-III). The DHQ-III is a web-based dietary questionnaire developed by the National Cancer Institute for assessing dietary intake (*EGRP/DCCPS/NCI/NIH*, n.d.). The DHQ-III is a robust, validated tool that can be self- or interviewer-administered and include questions on frequency and portion size for 135 food items and 26 dietary supplement questions. Nutrient and food group estimates in the database are derived primarily from the United States Department of Agriculture (USDA) Food and Nutrient Database for Dietary Studies, the USDA Food Patterns Equivalents Database, and the Nutrition Data System for Research (Nutrition Coordinating Center, University of Minnesota). The DHQ-III provides output for 219 nutrients, dietary constituents, and food groups (*EGRP/DCCPS/NCI/NIH*, n.d.). This questionnaire is administered via the DHQ-III online platform at baseline and each follow-up visit. It is user-friendly and has been validated against other food frequency questionnaires (Subar et al., 2001). Understanding the overall dietary intake of participants is important as nutrient deficiencies or excesses can impact energy levels, muscle health, and overall physiological function, potentially influencing mobility decline (Shlisky et al., 2017).

2.4.3.1.2. Iron Intake Calculation-Food Frequency Questionnaire (Ironic-FFQ). The IRONIC-FFQ is a dietary assessment tool specifically designed to evaluate iron intake from various food sources (Głąbska et al., 2017). It captures detailed information on the frequency and portion sizes of iron-rich foods consumed over a typical week. This tool is administered to supplement the dietary intake data collected with the DHQ-III, providing a more comprehensive analysis of the iron-specific intakes of participants in the study. The IRONIC-FFQ has only been validated in young Polish women (Głąbska et al., 2017), thus, needs validation in diverse populations. Participants are asked to complete this questionnaire at baseline and follow-up visits. Given the study's focus on iron dysregulation, this detailed assessment of iron intake allows us to explore the relationship between dietary iron and changes in mobility and mitochondrial function over time (Zeidan et al., 2024).

2.4.3.2. Physical activity measures

2.4.3.2.1. Real-Time, Online Assessment and Mobility Monitor (ROAMM). The Real-Time, Online Assessment, and Mobility Monitor (ROAMM) mobile-based platform is utilized to collect data from Apple™ smartwatches. The ROAMM app captures GPS data to assess mobility patterns, including excursion size (the maximum distance a participant travels away from home) and average excursion span (the average daily distance between recorded locations away from home) (Clark et al., 2019). This GPS data is automatically transferred to a web-based visualization platform for analysis. The smartwatch works in tandem with the app to provide real-world data on how participants navigate their environment, offering insight into their mobility behavior and potential decline over time on participants' activity levels for up to 14 days. The smartwatch's sensors and user interface quantified activity patterns, walking behavior, daily heart rates, and community mobility (Clark et al., 2019). ROAMM \ objectively measured participants' daily movement patterns, providing key information on how they move

through their environment and identifying subtle changes in their physical activity that could be linked to iron levels and mitochondrial health.

2.4.3.2.2. Community Healthy Activities Model Program for Seniors (CHAMPS). Physical activity is also assessed using the CHAMPS, which evaluates the weekly frequency and duration of various physical activities typically undertaken by older adults (Stewart et al., 2001). The questionnaire was designed to improve recall accuracy and bias from socially desirable responses by incorporating non-exercise activities such as socializing and hobbies, which are excluded from the physical activity scores. The questionnaire could be either self-administered or conducted by an interviewer. Studies have demonstrated its validity and high test-retest reliability (Hekler et al., 2012). Assessing the frequency and duration of various physical activities provides a broader understanding of participants' activity levels and how different types of engagement might influence the trajectory of mobility decline in relation to our key biomarkers (Aggio et al., 2019).

2.4.3.2.3. Pepper Assessment Tool for Disability (PAT-D). The PAT-D is a brief 19-item measure of functional difficulty that targets both discrete tasks and social behaviors. Questions assess mobility, activities of daily living (ADL), and instrumental activities of daily living (IADL) (Rejeski et al., 2008). All factors have been validated as reliable tools for distinguishing between higher- and lower-functioning adults (Rejeski et al., 2013). This questionnaire allows us to capture participants' perceived limitations in mobility and daily activities and examine its association with iron dysregulation and mitochondrial function.

2.4.3.3. Sleep measures

2.4.3.3.1. The Pittsburgh Sleep Quality Index (PSQI). The PSQI is a self-reported questionnaire designed to assess sleep quality and disturbances over a one-month period (Buysse et al., 1989). It comprises of 19 items that generate seven component scores, which are summed up to produce a global score reflecting overall sleep quality. The questionnaire was given to participants at baseline and will be given at each follow-up visit. The validity and reliability of the PSQI have been well-established, making it a valuable and comprehensive tool for sleep assessment (Manzar et al., 2018; Al Maqbali et al., 2020). Given the known impact of sleep quality on energy levels, muscle recovery, and overall health, the PSQI helped us control for sleep disturbances as a potential confounder or contributing factor to mobility decline (Sun et al., 2020).

2.4.3.3.2. Actigraph. An Actigraph device is used to track movement over time, recording it as activity counts. This compact, wristwatch-sized device continuously records activity and rest movements multiple times per second. Its core component, the accelerometer, serves as the primary sensor, measuring movement waves, including vibrations and the velocity of movements (Cleveland Clinic, n.d.). The device also analyzes the frequency and duration of physical activity bouts and categorizes them as light, moderate, and vigorous intensities. Actigraphy has become an important tool in both clinical and research settings, providing an objective assessment of sleep patterns and daytime activity (Kheirkhahan et al., 2016). In this study, Actigraph devices are used to monitor participants' sleep activity, and participants are asked to maintain a self-reported sleep diary alongside the device to enhance the accuracy of sleep assessments.

2.4.3.4. Medication inventory. Participants are asked to bring all their medication (prescription and over-the-counter) to their baseline and annual visits. For each medication, the name, dose, and formulation are documented. Reported medication use is categorized for subsequent data analyses into three categories: (1) Medications that potentially accelerate functional decline, (2) Medications that potentially slow functional decline, and (3) Medications that might influence skeletal muscle function. This 'medication inventory' method of drug assessment has been validated in older adults (Psaty et al., 1992). It is important to carefully record medication use as some medicines can affect muscle strength, fatigue, and overall physical ability, which may interfere with the biological processes we are studying (Janssen et al., 2020).

3. Statistical analyses

Baseline data are reported in this manuscript to provide an overview of the enrolled participants, however, analyses of longitudinal outcomes will be the focus of future reports. The analyzed outcomes included measures related to iron regulation, mitochondrial function, and physical performance. Specifically, circulating hepcidin levels (CHL), ferroportin level (MID1), mitochondrial iron level (MID2), TfR-1 (MID3), muscle ferritin levels (MID4), mtDNA deletions/damage (MtD1), mitochondrial respiration (MtD2), SPPB, muscle strength, and 6MWT were measured at baseline and the follow-up visits. In addition, each participant's age, sex, race/ethnicity, comorbidity index, dietary intake, physical activity, long-term medication use, and sleep measures were considered as covariates. When significant effects for sex and/or race are identified, stratified analyses or subgroup analyses were conducted. Algorithms from the Cardiovascular Health Study and the Women's Health and Aging Study were used to identify and verify comorbidities. These algorithms integrate data from medical histories, physician comments, patient reports, laboratory values, and medications (Psaty et al., 1992). Comorbidities adjusted in the analyses included ischemic heart disease, stroke, heart failure, diabetes mellitus, and pulmonary disease. Associations of iron regulatory proteins and mitochondrial dysfunction measures with musculoskeletal conditions such as knee arthritis, hip arthritis, spinal disk disease, and spinal stenosis were also examined. If strong associations are found, these comorbidities will be included as covariates in subsequent analyses.

Prior to conducting inferential analyses, data was examined for potential outliers and aberrant measurements (e.g., values exceeding 3-times the 3 interquartile range). Questionable observations were cross-checked via laboratory logs and corrected if errors are found. Measurements deemed erroneous and uncorrectable was treated as missing data during analysis. Data was also checked for conformity with the distributional assumptions of the proposed analyses. Descriptive statistics were provided for all outcome measures and covariates by group at each visit including mean and standard deviation (SD) for quantitative variables and count and frequency for categorical variables. Wilcoxon rank-sum and Fisher's exact tests was used to compare the differences between HF and LF groups, respectively, when necessary.

3.1. Analysis plan for aim 1 (cross-sectional)

Two-sample *t*-tests or their nonparametric equivalent, the Wilcoxon rank-sum test, were used to compare the LF and HF older adults on measures related to iron regulation and mitochondrial function (CHL, MID1-MID4, MtD1-MtD2) at baseline, depending on the conformity to distributional assumptions. A general linear model was fitted to assess the association between these measures and walking speed from the 6MWT or muscle strength, adjusting for covariates (age, sex, race, comorbidity index, dietary intake, physical activity, long-term medication use, and sleep). In total, 14 hypotheses were tested for this aim (two tests for each iron-regulation and mitochondrial function measure), and the Holm-Bonferroni method will be used to control the family-wise error rate at $p < 0.05$.

3.2. Analysis plan for aim 2 (longitudinal)

For the primary analysis, general linear mixed effect models were fitted to compare LF and HF older adults on changes in iron dysregu-

lation and mitochondrial dysfunction over time. Specifically, if X_{ikj} represents the k-th measurement ($k = 1, 2, \ldots, 7$, indexing the seven measures of iron deregulation and Mt. dysfunction) for individual i at time j ($j = 0, 1, 2$, and 3 for baseline, FV-1, FV-2, and FV-3, respectively), then the fixed effect portion of the model was:

$$E(X_{ikj}) = \beta_{k0} + \beta_{k1}Group_i + \beta_{k2j} + \beta_{k3j}Group_i + \sum\nolimits_s \beta_{k4s}Z_{is} + \sum\nolimits_s \beta_{k5js}Z_{is} + \sum\nolimits_s \beta_{k6s}Z_{ijs},$$

where βs are the model parameters; β_{k1} is the group effect and $Group_i$, either 0 or 1, is a dummy variable indicating the group to which individual i belonged; β_{k2j} represents the effect of time j, with the constraint $\beta_{k20} = 0$; β_{k3j} is the interaction between group and time j, with the constraint $\beta_{k30} = 0$; Z_{i1} to Z_{i12} are baseline covariates for individual i including age, gender, race, comorbidity index, dietary intake, physical activity, three indicator variables for medication use (use of medications that might accelerate functional decline, use of medications that might slow functional decline, and use of medications that might influence skeletal muscle), and sleep duration, efficiency, and quality; β_{k4s} and β_{k5js} is the effect and interaction with time j of the corresponding baseline covariate, respectively ($s = 1, 2, \ldots, 12$), with the constraint $\beta_{k50s} = 0$; Z_{ij5} to Z_{ij12} are covariate changes from baseline to time j for individual i including dietary intake, physical activity, indicator variables for medication use, and sleep measures; and β_{k6s} is the effect of the corresponding covariate change. The group main effect, β_{k1}, and the group*time interaction effects, β_{k3j}, measures the difference between two groups, corresponding to the difference in intercepts of two regression lines, and the difference in the change rate between two groups, corresponding to the difference in slopes of two regression lines, respectively. We conducted statistical tests of the fixed effects β_{k3j}, to determine whether LF participants have greater impairment changes over time in biological and functional measures. As in Aim 1, the Holm-Bonferroni method was used to adjust for a total of seven pre-planned multiple tests. To further assess whether the circulating hepcidin levels, muscle iron deregulation, and Mt. dysfunction and their longitudinal changes are associated with the clinical outcome(s) such as slower walking speed and lower physical function, the linear mixed model for repeated measures was used to assess effects of iron deregulation and mitochondrial dysfunction on annual decline in walking speed and muscle strength. Let y_{ij} be the walking speed or muscle strength for individual i at time j, the full fixed effect portion of the model was:

$$E(y_{ij}) = \alpha_0 + \alpha_1 Group_i + \alpha_{2j} + \alpha_{3j}Group_i + \sum\nolimits_k \alpha_{4k}X_{ikj} + \sum\nolimits_k \alpha_{5kj}X_{ik0} + \sum\nolimits_s \alpha_{6s}Z_{is} + \sum\nolimits_s \alpha_{7js}Z_{is} + \sum\nolimits_s \alpha_{8s}Z_{ijs},$$

where αs are the model parameters; α_1 is the group effect; α_{2j} is the effect of time j, with the constraint $\alpha_{20} = 0$; α_{3j} is the interaction between group and time j, with the constraint $\alpha_{30} = 0$; X_{i1j} to X_{i7j} stands for iron regulation and mitochondrial function (CHL, MID1-MID4, MtD1-MtD2) at time j (MID1-MID4 and MtD1-MtD2 available only in biopsies at $j = 0$ and 3) for individual i; α_{4k} and α_{5kj} are the corresponding main effect and interaction with time j, with the constraint $\alpha_{5k0} = 0$; Z_{i1} to Z_{i12} are baseline covariates for individual i, and α_{6s} and α_{7js} are the main effect and interaction effect of the corresponding baseline covariate, with the constraint $\alpha_{70s} = 0$; and Z_{ij5} to Z_{ij12} are covariate changes at time j for individual i and α_{8s} is the effect of change in covariate. The model included random subject effect to capture the within-subject correlation between repeated measures. Our statistical inference focused on fixed effects α_{41} to α_{47} and α_{51j} to α_{57j}, the main effect of the variables (i.e., CHL, MID1-MID4, MtD1-MtD2) and their interactions with time, which represent effects of iron deregulation and mitochondrial dysfunction on slope of decline in walking speed or muscle strength under adjustment for group (LF vs. HF) and other covariates listed above. The overall test was conducted to determine the existence of the joint effects, followed by individual tests corresponding to each measure. Also, we conducted statistical tests of the fixed effects α_{3j}, which represent the difference between LF and HF older adults in physical function decline. Furthermore, as an exploratory analysis, we tested whether the effects of iron deregulation (CHL, MID1-MID4) on physical-function decline operate through adverse changes in Mt. function by modeling mitochondrial function (MtD1-MtD2) as mediator variables to help understand and explain the mechanism.

Given the multi-year follow-up and the advanced age of the cohort, some attrition and missing longitudinal data are anticipated. Recruiting community-dwelling older adults for muscle biopsy procedures is also inherently challenging due to medical comorbidities, procedure-related concerns, and the overall burden of participation, all of which contributed to data missing in a longitudinal study. We plan to make use of all available data in the analysis. Missing data patterns and the underlying missingness mechanisms will be examined. Mixed-effects models estimated via maximum likelihood and statistical imputation procedures can appropriately handle missing data under an assumption of ignorable missingness (i.e., missing at random). To account for potential non-ignorable dropout and missingness (i.e., missing not at random), we will employ pattern-mixture models, complemented by sensitivity analyses using prespecified parameters to evaluate the robustness of the results.

3.3. Sample size calculation

The statistical power is calculated at the family-wise significance level of 0.05, assuming LF and HF groups had 50 and 64 participants, with and without an attrition rate of 30% over the 3 years of follow-up. For Aim 1, statistical power is computed using a two-sample t-test based on the empirical parameter (standardized difference, also known as Cohen's d) derived from prior muscle biopsy studies in older adults conducted by our group, which examined iron dysregulation, mitochondrial function, and age-related skeletal muscle changes (Picca et al., 2019a, 2020, 2023; Joseph et al., 2012). For Aim 2, the minimum detectable coefficient of determination in a multiple regression and the minimum detectable difference in the standardized regression slope (change rate) between the LF and HF groups will be computed for a power of 80% under various scenarios of within-subject correlation in a repeated measures design. Since no empirical parameters such as the difference in rate of decline, its estimation variance, and within-subject correlation are available from a longitudinal study, these estimates are based on assumptions.

The power analysis suggests that the study design provided ample power ($\geq$ 95.39%) to detect similar effect sizes in Aim 1. For Aim 2, the minimum detectable effect size for a power of 80% is $R^2 = 0.145$ and ES (d) = 0.739, which is considered medium-to-large effect sizes based on the literature. Since there is no prior knowledge of the effect sizes of mediators or available data to estimate them, a formal power calculation is not conducted for the exploratory mediation analysis.

Composite scores from self-reported measures of diet, activity, sleep, and medication use, as well as a summary index of each objective measure, are used as covariates in the statistical models to reduce dimensionality and prevent inflation of model complexity. Only covariates with established biological relevance or strong theoretical justification were included in the models to minimize overfitting and improve the stability and interpretability given the sample size. The inclusion of informative covariates generally reduced noise and led to higher statistical power. Therefore, the calculations represent a

Table 4
Baseline physical function measures in high- and low-functioning older adults.

Measures	Overall (N = 114)	High (N = 64)	Low (N = 50)	P-value[a]
Physical performance				
SPPB	N	64	50	0.0052
	Mean ± SD	11.02 ± 1.02	8.56 ± 1.84	
6MWT distance walked (m)	N	64	50	<0.0001
	Mean ± SD	436.05 ± 63.60	347.47 ± 81.93	
Handgrip strength				
Dominant hand grip strength (kg)	N	61	49	0.0053
	Mean ± SD	33.38 ± 12.02	26.96 ± 10.50	
Non-dominant hand grip strength (kg)	N	60	48	0.0030
	Mean ± SD	31.85 ± 11.08	25.46 ± 9.98	
Biodex measures (dominant knee)[b]				
Peak torque (N-m) AWY (extension)	N	42	29	0.0049
	Mean ± SD	76.24 ± 27.90	58.97 ± 26.07	
Avg power (W) AWY (extension)	N	42	29	0.0075
	Mean ± SD	49.50 ± 17.04	38.43 ± 18.55	
Peak torque (N-m) TWD (flexion)	N	42	29	0.0283
	Mean ± SD	44.01 ± 17.00	35.49 ± 13.37	
Avg power (W) TWD (flexion)	N	42	29	0.0014
	Mean ± SD	20.84 ± 11.92	12.08 ± 8.59	

Abbreviations: SPPB, Short Physical Performance Battery; 6MWT, 6 Minute Walk Test; m, meters.
[a] The p-value was calculated using Wilcoxon's rank sum test.
[b] Missing values are due to a temporary malfunction of the Biodex machine over a period of several months.

conservative estimate of power when covariate adjustment is applicable. In the worst-case scenario, where the covariates have no contribution to the outcome variation (a contribution of 0.0), the power results with and without covariate adjustment are virtually the same.

This study aimed to examine how changes in CHL, ferroportin, mitochondrial iron levels, and mitochondrial function influenced physical performance, such as walking speed and muscle strength, over a 3-year follow-up period in older adults (see Fig. 3). Statistical analyses included general linear mixed models to evaluate changes in these biomarkers and their impact on physical function, while adjusting for covariates such as age, gender, race, comorbidities, dietary intake, physical activity, medication use, and sleep. Group-time interactions were assessed to evaluate differences between LF and HF participants, with particular attention to changes in walking speed and muscle strength.

The study data informed power calculations for future clinical trials and refined methods for assessing the impact of iron dysregulation and mitochondrial dysfunction on functional decline in older adults.

4. Results

4.1. Baseline characteristics

At baseline, participants had a mean age of 75.78 ± 3.17 years, with no significant differences between groups (Table 2). The majority of participants were female and Caucasian/White, with no significant differences in sex, race, or ethnicity between HF and LF groups. Education level was also comparable between groups. Overall, the groups were demographically similar, supporting the validity of subsequent comparisons in functional and biological measures.

4.2. Baseline physical function

As presented in Table 4, significant differences at baseline were observed between the HF and LF groups across all assessed domains, including SPPB scores, 6MWT, Knee Extension Peak Torque, and Grip Strength for both dominant and non-dominant hands, which aligns with our initial predictions. Across all functional measures, the LF participants exhibited significantly worse physical performance compared to the HF group. Fig. 4 displays the differences between LF and HF older adults on the walking speed at baseline. These baseline differences reflect the functional distinctions defined by SPPB classification and provide the foundation for future longitudinal analyses.

4.3. Baseline biological measures

The baseline clinical laboratory measures of the study participants divided between high-functioning and low-functioning groups is summarized in Table 5. As originally predicted, at baseline, significant differences were observed in total bilirubin levels, hemoglobin levels, and red cell distribution width (RDW), with the low-functioning group generally exhibiting less favorable values. Other parameters, including creatinine, albumin, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC), showed no statistically significant differences between the groups. These routine clinical measures provide a broad assessment of systemic health, allowing us to identify potential comorbidities, monitor overall well-being, and assess how baseline health status relates to iron regulation, mitochondrial health, and physical performance in the two functional groups.

4.4. Summary of baseline findings

Together, the baseline demographic, functional, and biological characteristics describe a well-phenotyped cohort of older adults entering the Muscle Iron Flow Study. These baseline data form the foundation for evaluating longitudinal relationships among cellular iron regulation, mitochondrial function, and physical performance once follow-up is completed.

5. Discussion

Mobility decline in older adults is well documented, however, the longitudinal relationships between iron dysregulation, mitochondrial dysfunction, and physical performance remain underexplored, especially between low- and high-functioning individuals. To our knowledge, the Muscle Iron Flow study is the first observational, longitudinal study to explore the relationship between iron dysregulation, mitochondrial function, and physical performance in LF and HF older adults. This study uniquely examines the effects of these biological measures, which have not been assessed longitudinally in both high- and low-functioning older participants. By recruiting relatively healthy older adults aged 70 to 80 years, the study enables extended follow-up, allowing for a detailed exploration of functional and biological changes over time.

Although this manuscript includes descriptive statistics on baseline physical and biological measures, it is not a data-driven outcomes paper. The intention is to provide a detailed overview of the study protocol, including the rationale, design, and analytic framework. The baseline data serve to contextualize the enrolled cohort and set the stage for

Table 5
Clinical measures at baseline.

Variable	Category/statistics	Overall (N = 114)	High (N = 64)	Low (N = 50)	P-value[a]
Creatinine	N	109	60	49	0.9976
	Mean ± SD	0.92 ± 0.22	0.92 ± 0.26	0.90 ± 0.17	
Albumin	N	109	60	49	0.8390
	Mean ± SD	4.30 ± 0.24	4.31 ± 0.22	4.30 ± 0.26	
Total bilirubin	N	109	60	49	0.9827
	Mean ± SD	0.66 ± 0.28	0.66 ± 0.27	0.66 ± 0.29	
Hemoglobin	N	107	60	47	0.0100
	Mean ± SD	13.49 ± 1.27	13.79 ± 1.22	13.12 ± 1.25	
MCV[1]	N	107	60	47	0.1843
	Mean ± SD	89.83 ± 5.90	90.43 ± 5.28	89.05 ± 6.59	
MCHC[2]	N	107	60	47	0.3346
	Mean ± SD	33.71 ± 0.98	33.74 ± 1.03	33.67 ± 0.92	
RDW[3]	N	107	60	47	0.0271
	Mean ± SD	13.88 ± 0.97	13.68 ± 0.75	14.15 ± 1.14	

Abbreviations: MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.
[a] The p-value was calculated using Wilcoxon's rank sum test.

future longitudinal analyses. Baseline assessments revealed significant differences between HF and LF groups, with LF participants exhibiting markedly lower physical performance per the study protocol. Older participants from the LF group, defined by lower SPPB scores, also demonstrated significantly shorter 6-minute walk distances, reduced peak torque, and weaker grip strength in both dominant and non-dominant hands. Additionally, LF participants had lower hemoglobin levels and higher red cell distribution width (RDW), suggesting hematological differences that may contribute to diminished physical capacity. Interestingly, studies show a positive association between RDW and the risk of stroke, morbidity and mortality across age groups, especially those who are critically ill (Said et al., 2017; Felker et al., 2007; Allen et al., 2010; Lee et al., 2013; Ani and Ovbiagele, 2009; Perlstein et al., 2009; Lorente et al., 2014).

Despite comparable levels of creatinine, albumin, MCV, and MCHC between groups, the observed disparities in functional and strength measures underscore the distinct physiological profiles of HF and LF individuals. These findings validate the classification criteria and provide a strong foundation for investigating how iron dysregulation and mitochondrial dysfunction contribute to physical decline over time – factors that have not been previously examined alongside physical function. Although these baseline patterns cannot determine causality, they provide essential anchors for characterizing within-person change and for testing whether alterations in iron handling, mitochondrial capacity, or inflammatory tone predict functional decline over the three-year follow-up.

While our baseline findings highlight significant differences in function between our pre-defined functional groups, understanding the longitudinal contribution of iron and mitochondrial factors requires comparison with existing research. To our knowledge, there is one prominent study, the Study of Muscle, Mobility, and Aging (SOMMA) (Cummings et al., 2023), that investigates the biological factors contributing to mobility decline in older adults. SOMMA integrates a broad spectrum of assessments, including muscle biopsies, RNA sequencing, and cardiopulmonary fitness tests, to explore the relationships between muscle mass, energy generation, and mobility loss (Cummings et al., 2023). While SOMMA provides valuable insights into the general biological pathways of aging, it does not focus specifically on the molecular mechanisms of iron dysregulation or mitochondrial dysfunction, nor does it distinguish between LF and HF older adults. In contrast, the Muscle Iron Flow study is specifically designed to explore how iron metabolism and mitochondrial health influence functional decline. It targets key biomarkers such as hepcidin, ferroportin, and mitochondrial DNA integrity. Additionally, unlike SOMMA, which does not control for exercise and includes a much broader age range, the Muscle Iron Flow study specifically focuses on a more homogenous group of older adults aged 70 to 80 and accounts for exercise, ensuring a more precise understanding of how iron dysregulation and mitochondrial dysfunction contribute to mobility decline.

Emerging literature suggests that iron overload, mitochondrial dysfunction, and impaired antioxidant defenses converge on pathways, such as ferroptosis and cellular senescence, both of which have been implicated in age-related muscle decline (López-Otín et al., 2023; Dixon et al., 2012; Mavaddatiyan et al., 2025). Recent studies demonstrate that ferroptosis contributes to functional loss across multiple tissues, while mitochondrial stress can accelerate senescence in muscle progenitor cells (Cheng et al., 2024; Wu et al., 2024; Miwa et al., 2022). Additionally, inflammaging, chronic, low-grade inflammation associated with aging, may amplify these pathways, linking iron dysregulation with impaired muscle regeneration (Tatullo et al., 2024). Experimental approaches, including mitochondria-targeted therapies, extracellular vesicle–based mitochondrial rescue, and bioengineering advancements, such as soft exoskeletons, further highlight the translational relevance of studying iron–mitochondria interactions in older adults (Picca et al., 2019b). These mechanistic frameworks provide a compelling rationale for the longitudinal analyses planned in the Muscle Iron Flow Study. Stratification by functional status enables more targeted analyses of biological and physical differences between groups. Furthermore, our integration of novel tools, such as high-resolution respirometry – allowing for detailed analysis of mitochondrial respiratory capacity – and wearable technology with the AppleTM Watch ROAMM App for continuous, objective activity monitoring, alongside comprehensive longitudinal data, positions this study as a novel contribution to understanding age-related muscle deterioration.

The study's statistical framework further strengthens its design. Cross-sectional analyses compared baseline biomarkers between LF and HF groups using two-sample *t*-tests or Wilcoxon rank-sum tests, adjusted for factors such as age, sex, comorbidities, diet, physical activity, and sleep. Longitudinal analyses will use general linear mixed models to track biomarker changes and assess group-time interactions, providing detailed insights into the rates of functional decline. The Holm-Bonferroni correction will address multiple comparisons, and the study is well-powered (≥96.9%) to detect medium-to-large effect sizes, ensuring robust findings despite a projected 30% attrition rate over three years.

5.1. Strength and limitations

The study design addresses key gaps in aging research by providing a multidimensional perspective on functional decline, though several limitations need to be acknowledged. Although muscle biopsies offer critical insights into molecular pathways, their invasiveness may increase attrition and reduce generalizability. Additionally, reliance on self-reported measures for dietary intake and physical activity introduces a risk of bias, though objective tools, such as the Actigraph and ROAMM app, can help mitigate these challenges.

Despite these limitations, the comprehensive baseline data provides a valuable foundation for tracking functional and biological changes over time. This allows for a more nuanced understanding of mobility decline in aging populations. Ultimately, the study's innovative methodology, comprehensive data integration, and rigorous statistical framework position it to provide a significant contribution to the field.

5.2. Future directions

Findings from the Muscle Iron Flow study may guide future mechanistic studies and interventions targeting aging-related mobility loss. Dietary strategies, such as time-restricted eating (TRE) or iron-modulating diets, could be explored for their effects on iron regulation and mitochondrial health. Similarly, clinical trials could examine tailored exercise programs to enhance mitochondrial function and mitigate mobility decline. Combining dietary and physical activity interventions may yield complementary benefits for improving physical function. Studies have revealed an effect for fasting on blood iron levels (Nguyen et al., 2017; Wojciak, 2014), yet the exact mechanism through which this happens remains poorly understood. Additionally, pharmacological improved approaches targeting iron chelation, hepcidin antagonism, or mitochondrial pathways may be developed based on future findings from this study.

Beyond behavioral and pharmacological strategies, emerging experimental therapies targeting mitochondrial function and iron homeostasis offer additional translational possibilities. Mitochondria-targeted puerarin micelles have been shown to reduce apoptosis in cardiomyocytes (Li et al., 2019), illustrating the potential for pharmacologic strategies that enhance mitochondrial resilience under conditions of iron- and oxidative stress–related dysfunction. Complementary advances include mitochondria-targeted fluorescent sensors for reactive species, such as peroxynitrite, which expand the toolkit for developing precision biomarkers in aging populations (Li et al., 2023). Furthermore, extracellular vesicle–based approaches have demonstrated the ability to rescue mitochondrial dysfunction in aging stem cells (Deng et al., 2024), highlighting the potential for regenerative, cell-free therapies.

Advances in gerontechnology, including soft exoskeletons and bioengineered mobility-assist devices, have also shown promise in enhancing gait stability and functional independence in older adults (Chen et al., 2024), and a better understanding of biological pathways, such as iron regulation and mitochondrial resilience, may lead to interventions that complement or optimize these emerging technologies. As the longitudinal results of the Muscle Iron Flow study become available, these emerging therapeutic modalities may help inform targeted strategies aimed at improving mitochondrial health and mobility outcomes in older adults.

6. Conclusion

In conclusion, this manuscript provides the foundational baseline characterization of the Muscle Iron Flow cohort, demonstrating clear functional and biological differences, while showing balanced demographic characteristics, such as age, sex, race, and ethnicity between high- and low-functioning older adults. These baseline findings establish the physiological landscape from which longitudinal changes will be interpreted. Once follow-up data are collected, the study will have the capacity to determine how iron regulation, mitochondrial function, and related molecular pathways contribute to mobility decline. Ultimately, this work has the potential to identify biological targets for future interventions aimed at preserving physical function and extending health span in older adults.

Glossary

LF	Low-functioning
HF	High-functioning
NIA	National Institute on Aging
NIH	National Institutes of Health
SOMMA	Study of Muscle, Mobility, and Aging
FV	Follow-up visits
SPPB	Short Physical Performance Battery
6MWT	Six Minute Walk Test
MMSE	Mini-Mental State Examination
CHAMPS	Community Healthy Activities Model Program for Seniors
CBC	Complete blood count
CMP	Comprehensive metabolic panel
PAT-D	Pepper Assessment Tool for Disability
ROAMM	Real-time Online Assessment and Mobility Monitor
IRONIC-FFQ	Iron Intake Frequency Food Questionnaire
DHQ-III	Diet History Questionnaire III
PSQI	Pittsburgh Sleep Quality Questionnaire
Biodex Test	Knee extension peak torque
mtDNA	Mitochondrial DNA
qRT-PCR	Quantitative real-time polymerase chain reaction
TfR-1	Transferrin receptor protein 1
Fn	Ferroportin
RDW	Red cell distribution width
MCV	Mean corpuscular volume
MCHC	Mean corpuscular hemoglobin concentration
USDA	United States Department of Agriculture
CHAMPS	Community Healthy Activities Model Program for Seniors
PAT-D	Pepper Assessment Tool for Disability
IADL	Instrumental Activities of Daily Living
ADL	Activities of daily living
PSQI	Pittsburgh Sleep Quality Questionnaire
CHL	Circulating hepcidin levels
MID1	Ferroportin level
MID2	Mt. iron level
MID3	TfR-1
MID4	Muscle ferritin levels
MtD1	mtDNA deletions/damage
MtD2	Mitochondrial respiration
SD	Standard deviation

CRediT authorship contribution statement

Nevena Stanojevic: Writing – review & editing, Writing – original draft, Visualization. **Stephanie Wohlgemuth:** Writing – review & editing, Writing – original draft. **Rola S. Zeidan:** Writing – review & editing. **XiangYang Lou:** Writing – review & editing, Data curation. **Javier A. Tamargo:** Writing – review & editing. **Bella Bravo:** Writing – review & editing. **Jennifer Newman:** Writing – review & editing. **Anna Picca:** Writing – review & editing, Visualization. **Emanuele Marzetti:** Writing – review & editing. **Kevin Wu:** Writing – review & editing.

Christian McLaren: Writing – review & editing. **Robert Mankowski:** Writing – review & editing, Investigation, Conceptualization. **Bhanuprasad Sandesara:** Conceptualization, Project administration, Resources. **Aditya Shirali:** Project administration, Resources. **Todd Manini:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization. **Christiaan Leeuwenburgh:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Stephen Anton:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethical approval

All study procedures were approved by the University of Florida Institutional Review Board (IRB#202200753) and conducted according to good clinical practice. Written informed consent was obtained from all participants.

Funding

This study was supported by the National Institutes of Health (R01 AG075136) and by the University of Florida Claude D. Pepper Older Americans Independence Center (P30 AG028740).

Declaration of competing interest

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge the National Institutes of Health for supporting this study through grant R01 AG075136. We also thank the University of Florida Claude D. Pepper Older Americans Independence Center for their support in advancing aging research (P30 AG028740). In addition, we extend our sincere appreciation to the staff and colleagues at the Core Facility for Biomarker Research and Mitochondrial Biology for their valuable support and collaboration throughout the research process.

Finally, we are grateful to all study participants, without whom this research would not have been possible.

Appendix A

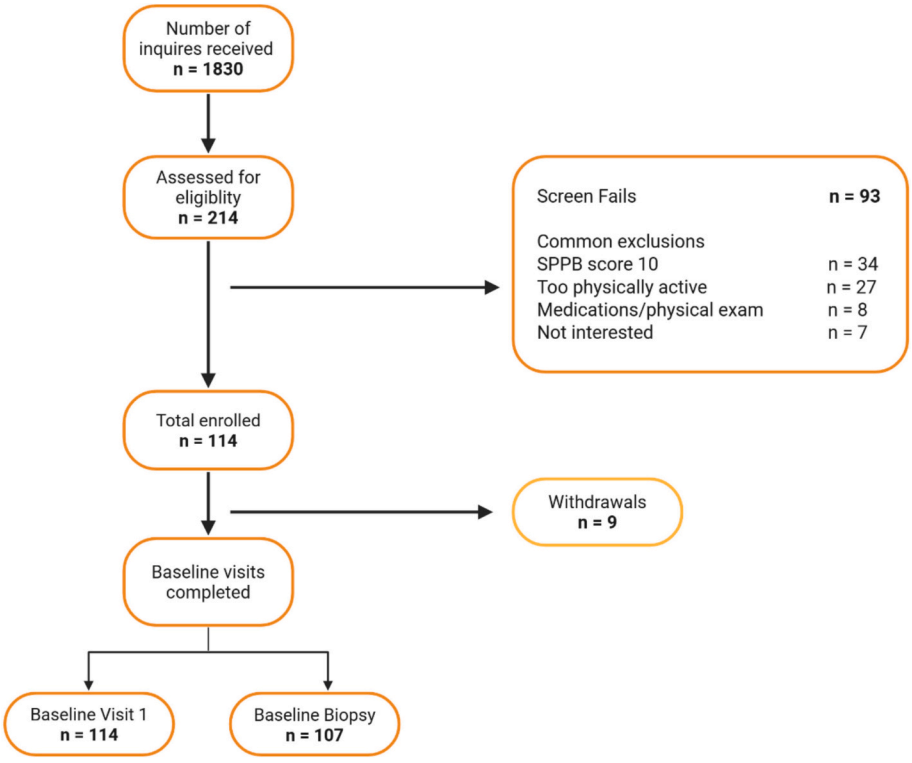


Fig. 1. Enrollment summary.
CONSORT-style diagram showing participant flow through the Muscle Iron Flow study. Out of 1830 initial inquiries, 214 individuals were assessed for eligibility. Common reasons for screen failure included SPPB score of 10, high physical activity levels, medication conflicts, or lack of interest. A total of 114 participants were enrolled, and 107 completed the baseline muscle biopsy. Nine participants withdrew after enrollment.
Created in https://BioRender.com.

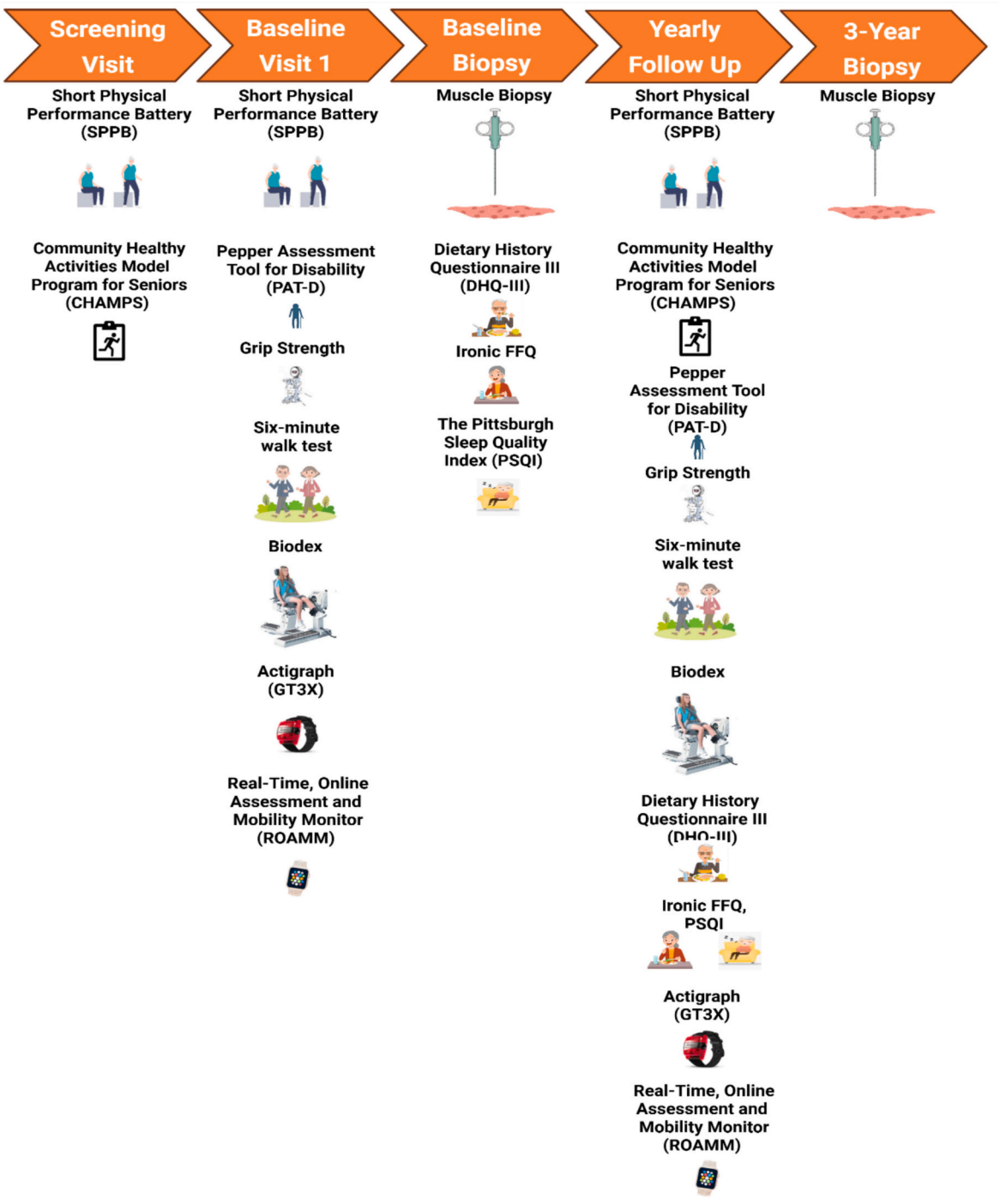


Fig. 2. Study timeline.
Schematic timeline of participant study visits and assessments across the Muscle Iron Flow study. Visits include a screening visit, two baseline assessments (Baseline Visit 1 and Baseline Biopsy), yearly follow-up visits, and a final 3-year biopsy. Assessments include functional measures (e.g., Short Physical Performance Battery [SPPB], six-minute walk test, grip strength), dietary and sleep questionnaires (e.g., CHAMPS, DHQ-III, PSQI), and device-based measures (e.g., Actigraph GT3X, ROAMM). Muscle biopsies are performed at the Baseline Biopsy and 3-year follow-up.
Created in https://BioRender.com.

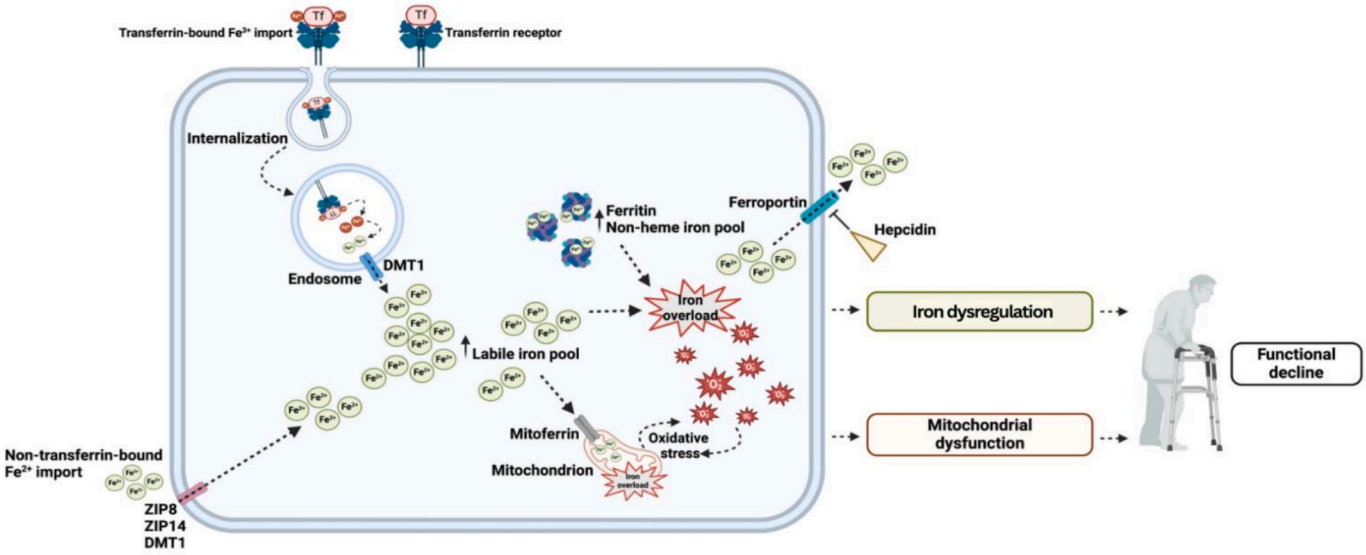


Fig. 3. Iron dysregulation and mitochondrial dysfunction drive decline.
Illustration of iron import, regulation, and its impact on mitochondrial function. Disruptions in iron homeostasis lead to iron overload, increasing oxidative stress and mitochondrial dysfunction. These processes contribute to cellular damage and functional decline in aging individuals.
Created in https://BioRender.com.

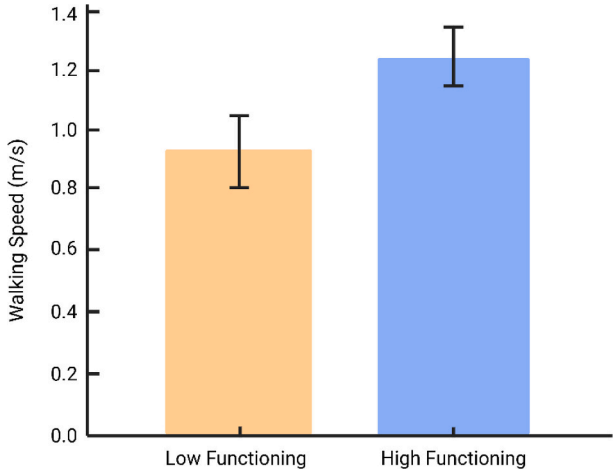


Fig. 4. Walking speed by functional status.
Mean usual walking speed (m/s) for the high-functioning and low-functioning groups at baseline. High-functioning participants walked significantly faster than low-functioning participants (1.22 ± 0.20 m/s vs. 0.96 ± 0.23 m/s; $p < 0.0001$). Bars represent group means, and error bars indicate standard deviations.
Created in https://BioRender.com.

Data availability

Data will be made available on request.

References

Abrams, P.A., 2004. Evolutionary biology: mortality and lifespan. Nature 431 (7012), 1048 (Oct 28).

Aggio, D., Papachristou, E., Papacosta, O., Lennon, L.T., Ash, S., Whincup, P.H., et al., 2019. Twenty-year trajectories of physical activity types from midlife to old age. Med. Sci. Sports Exerc. 51 (3), 481–489 (Mar).

Al Maqbali, M., Hughes, C., Gracey, J., Rankin, J., Dunwoody, L., Hacker, E., 2020. Validation of the Pittsburgh Sleep Quality Index (PSQI) with Arabic cancer patients. Sleep Biol. Rhythms 18 (3), 217–223 (July).

Ali, M.Y., Oliva, C.R., Flor, S., Griguer, C.E., 2022. Mitoferrin, cellular and mitochondrial iron homeostasis. Cells 11 (21), 3464 (Jan).

Allen, L.A., Felker, G.M., Mehra, M.R., Chiong, J.R., Dunlap, S.H., Ghali, J.K., et al., 2010. Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. J. Card. Fail. 16 (3), 230–238 (Mar).

Ani, C., Ovbiagele, B., 2009. Elevated red blood cell distribution width predicts mortality in persons with known stroke. J. Neurol. Sci. 277 (1–2), 103–108 (Feb 15).

Anton, S.D., Woods, A.J., Ashizawa, T., Barb, D., Buford, T.W., Carter, C.S., et al., 2015. Successful aging: advancing the science of physical independence in older adults. Ageing Res. Rev. 24 (00), 304–327 (Nov).

Arantes-Oliveira, N., Berman, J.R., Kenyon, C., 2003. Healthy animals with extreme longevity. Science 302 (5645), 611 (Oct 24).

Recruitment for research studies & clinical trials, BUMP. . Available from: https://recruitment.bumpdigitalmarketing.com/ (Internet, cited 2025 May 29).

Buysse, D.J., Reynolds, C.F., Monk, T.H., Berman, S.R., Kupfer, D.J., 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 28 (2), 193–213 (May).

Callahan, K.E., Lovato, L., Miller, M.E., Marsh, A.P., Fielding, R.A., Gill, T.M., et al., 2018. Self-reported physical function as a predictor of hospitalization in the lifestyle interventions and independence for elders study. J. Am. Geriatr. Soc. 66 (10), 1927–1933 (Oct).

Cawthon, P.M., Travison, T.G., Manini, T.M., Patel, S., Pencina, K.M., Fielding, R.A., et al., 2020. Establishing the link between lean mass and grip strength cut points with mobility disability and other health outcomes: proceedings of the sarcopenia definition and outcomes consortium conference. J. Gerontol. A Biol. Sci. Med. Sci. 75 (7), 1317–1323 (June 18).

Chang, M., Cohen-Mansfield, J., Ferrucci, L., Leveille, S., Volpato, S., de Rekeneire, N., et al., 2004. Incidence of loss of ability to walk 400 meters in a functionally limited older population. J. Am. Geriatr. Soc. 52 (12), 2094–2098 (Dec).

Chen, Y., Yu, W., Benali, A., Lu, D., Kok, S.Y., Wang, R., 2024. Towards human-like walking with biomechanical and neuromuscular control features: personalized attachment point optimization method of cable-driven exoskeleton. Front. Aging Neurosci. 16. Available from: https://www.frontiersin.org/journals/aging-neuroscience/articles/10.3389/fnagi.2024.1327397/full (Internet, Feb 2, cited 2025 Nov 18).
Cheng, X., Huang, J., Li, H., Zhao, D., Liu, Z., Zhu, L., et al., 2024. Quercetin: a promising therapy for diabetic encephalopathy through inhibition of hippocampal ferroptosis. Phytomedicine 126, 154887 (Apr 1).
Chistiakov, D.A., Sobenin, I.A., Revin, V.V., Orekhov, A.N., Bobryshev, Y.V., 2014. Mitochondrial aging and age-related dysfunction of mitochondria. Biomed. Res. Int. 2014, 238463.
Clark, D.J., Manini, T.M., Ferris, D.P., Hass, C.J., Brumback, B.A., Cruz-Almeida, Y., et al., 2019. Multimodal imaging of brain activity to investigate walking and mobility decline in older adults (mind in motion study): hypothesis, theory, and methods. Front. Aging Neurosci. 11, 358.
Cleveland Clinic. Actigraphy (Actigraph). Available from: https://my.clevelandclinic.org/health/drugs/25196-actigraphy (Internet, cited 2025 May 29).
Cummings, S.R., Newman, A.B., Coen, P.M., Hepple, R.T., Collins, R., Kennedy Ms, K., et al., 2023. The Study of Muscle, Mobility and Aging (SOMMA): a unique cohort study about the cellular biology of aging and age-related loss of mobility. J. Gerontol. A Biol. Sci. Med. Sci. 78 (11), 2083–2093 (Oct 28).
Deng, Y., Liu, Z., Lu, M., 2024. Extracellular vesicles deviced from hypoxia-3D-GMSCs rescue the mitochondrial dysfunction of aging-GMSCs. Biochem. Biophys. Res. Commun. (717), 150021 (July 12).
Dixon, S.J., Lemberg, K.M., Lamprecht, M.R., Skouta, R., Zaitsev, E.M., Gleason, C.E., et al., 2012. Ferroptosis: an iron-dependent form of non-apoptotic cell death. Cell 149 (5), 1060–1072 (May 25).
Drew, L., 2018. Fighting the inevitability of ageing. Nature 555 (7695), S15–S17 (Mar 8).
Drouin, J.M., Valovich-mcLeod, T.C., Shultz, S.J., Gansneder, B.M., Perrin, D.H., 2004. Reliability and validity of the Biodex system 3 pro isokinetic dynamometer velocity, torque and position measurements. Eur. J. Appl. Physiol. 91 (1), 22–29 (Jan).
Diet History Questionnaire III (DHQ III), EGRP/DCCPS/NCI/NIH. . Available from: https://epi.grants.cancer.gov/dhq3/index.html (Internet, cited 2025 May 29).
Felker, G.M., Allen, L.A., Pocock, S.J., Shaw, L.K., McMurray, J.J.V., Pfeffer, M.A., et al., 2007. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. J. Am. Coll. Cardiol. 50 (1), 40–47 (July 3).
Figueiredo, P.A., Powers, S.K., Ferreira, R.M., Appell, H.J., Duarte, J.A., 2009. Aging impairs skeletal muscle mitochondrial bioenergetic function. J. Gerontol. A Biol. Sci. Med. Sci. 64 (1), 21–33 (Jan).
Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12 (3), 189–198 (Nov).
Fuke, S., Kametani, M., Kato, T., 2008. Quantitative analysis of the 4977-bp common deletion of mitochondrial DNA in postmortem frontal cortex from patients with bipolar disorder and schizophrenia. Neurosci. Lett. 439 (2), 173–177 (July 11).
Furda, A., Santos, J.H., Meyer, J.N., Van Houten, B., 2014. Quantitative PCR-based measurement of nuclear and mitochondrial DNA damage and repair in mammalian cells. Methods Mol. Biol. 1105, 419–437.
Głąbska, D., Guzek, D., Ślązak, J., Włodarek, D., 2017. Assessing the validity and reproducibility of an Iron dietary intake questionnaire conducted in a group of young Polish women. Nutrients 9 (3), 199 (Feb 27).
Guralnik, J.M., Simonsick, E.M., Ferrucci, L., Glynn, R.J., Berkman, L.F., Blazer, D.G., et al., 1994. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. J. Gerontol. 49 (2), M85–M94 (Mar).
Guralnik, J.M., Ferrucci, L., Simonsick, E.M., Salive, M.E., Wallace, R.B., 1995. Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. N. Engl. J. Med. 332 (9), 556–561 (Mar 2).
Guralnik, J.M., Ferrucci, L., Pieper, C.F., Leveille, S.G., Markides, K.S., Ostir, G.V., et al., 2000. Lower extremity function and subsequent disability: consistency across studies, predictive models, and value of gait speed alone compared with the short physical performance battery. J. Gerontol. A Biol. Sci. Med. Sci. 55 (4), M221–M231 (Apr).
Hekler, E.B., Buman, M.P., Haskell, W.L., Conway, T.L., Cain, K.L., Sallis, J.F., et al., 2012. Reliability and validity of CHAMPS self-reported sedentary-to-vigorous intensity physical activity in older adults. J. Phys. Act. Health 9 (2), 225–236 (Feb).
Hofer, T., Marzetti, E., Xu, J., Seo, A.Y., Gulec, S., Knutson, M.D., et al., 2008. Increased iron content and RNA oxidative damage in skeletal muscle with aging and disuse atrophy. Exp. Gerontol. 43 (6), 563–570 (June).
Janssen, L., Allard, N.A.E., Saris, C.G.J., Keijer, J., Hopman, M.T.E., Timmers, S., 2020. Muscle toxicity of drugs: when drugs turn physiology into pathophysiology. Physiol. Rev. 100 (2), 633–672 (Apr 1).
Joseph, A.M., Adhihetty, P.J., Buford, T.W., Wohlgemuth, S.E., Lees, H.A., Nguyen, L.M.D., et al., 2012. The impact of aging on mitochondrial function and biogenesis pathways in skeletal muscle of sedentary high- and low-functioning elderly individuals. Aging Cell 11 (5), 801–809 (Oct).
Joseph, S., Li, M., Zhang, S., Horne, L., Stacpoole, P.W., Wohlgemuth, S.E., et al., 2022. Sodium dichloroacetate stimulates cardiac mitochondrial metabolism and improves cardiac conduction in the ovine fetus during labor. Am. J. Phys. Regul. Integr. Comp. Phys. 322 (1), R83–R98 (Jan 1).
Jung, S.H., DeRuisseau, L.R., Kavazis, A.N., DeRuisseau, K.C., 2008. Plantaris muscle of aged rats demonstrates iron accumulation and altered expression of iron regulation proteins. Exp. Physiol. 93 (3), 407–414 (Mar).
Kheirkhahan, M., Tudor-Locke, C., Axtell, R., Buman, M.P., Fielding, R.A., Glynn, N.W., et al., 2016. Actigraphy features for predicting mobility disability in older adults. Physiol. Meas. 37 (10), 1813–1833 (Oct).
Kotze, M.J., van Velden, D.P., van Rensburg, S.J., Erasmus, R., 2009. Pathogenic mechanisms underlying iron deficiency and Iron overload: new insights for clinical application. EJIFCC 20 (2), 108–123 (Aug).
Lee, J.H., Chung, H.J., Kim, K., Jo, Y.H., Rhee, J.E., Kim, Y.J., et al., 2013. Red cell distribution width as a prognostic marker in patients with community-acquired pneumonia. Am. J. Emerg. Med. 31 (1), 72–79 (Jan).
Li, W.Q., Wu, J.Y., Xiang, D.X., Luo, S.L., Hu, X.B., Tang, T.T., et al., 2019. Micelles loaded with puerarin and modified with triphenylphosphonium cation possess mitochondrial targeting and demonstrate enhanced protective effect against isoprenaline-induced H9c2 cells apoptosis. Int. J. Nanomedicine 14, 8345–8360.
Li, Y., Lei, J., Qin, X., Li, G., Zhou, Q., Yang, Z., 2023. A mitochondria-targeted dual-response sensor for monitoring viscosity and peroxynitrite in living cells with distinct fluorescence signals. Bioorg. Chem. 138, 106603 (Sept 1).
López-Otín, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2023. Hallmarks of aging: an expanding universe. Cell 186 (2), 243–278 (Jan 19).
Lorente, L., Martín, M.M., Abreu-González, P., Solé-Violán, J., Ferreres, J., Labarta, L., et al., 2014. Red blood cell distribution width during the first week is associated with severity and mortality in septic patients. PLoS One 9 (8), e105436 (Aug 25).
Manini, T.M., Anton, S.D., Beavers, D.P., Cauley, J.A., Espeland, M.A., Fielding, R.A., et al., 2017. Enabling reduction of low-grade inflammation in SEniors pilot study: concept, rationale, and design. J. Am. Geriatr. Soc. 65 (9), 1961–1968 (Sept).
Manini, T.M., Buford, T.W., Kairalla, J.A., McDermott, M.M., Vaz Fragoso, C.A., Fielding, R.A., et al., 2018. Meta-analysis identifies mitochondrial DNA sequence variants associated with walking speed. GeroScience 40 (5–6), 497–511 (Dec).
Mankowski, R.T., Anton, S.D., Axtell, R., Chen, S.H., Fielding, R.A., Glynn, N.W., et al., 2017. Device-measured physical activity as a predictor of disability in mobility-limited older adults. J. Am. Geriatr. Soc. 65 (10), 2251–2256 (Oct).
Mankowski, R.T., Wohlgemuth, S.E., Bresciani, G., Martin, A.D., Arnaoutakis, G., Martin, T., et al., 2023. Intraoperative hemi-diaphragm electrical stimulation demonstrates attenuated mitochondrial function without change in oxidative stress in cardiothoracic surgery patients. Antioxidants 12 (5), 1009 (Apr 27).
Manzar, M.D., BaHammam, A.S., Hameed, U.A., Spence, D.W., Pandi-Perumal, S.R., Moscovitch, A., et al., 2018. Dimensionality of the Pittsburgh Sleep Quality Index: a systematic review. Health Qual. Life Outcomes 16 (1), 89 (May 9).
Marsh, A.P., Applegate, W.B., Guralnik, J.M., Jack Rejeski, W., Church, T.S., Fielding, R.A., et al., 2016. Hospitalizations during a physical activity intervention in older adults at risk of mobility disability: analyses from the lifestyle interventions and independence for elders randomized clinical trial. J. Am. Geriatr. Soc. 64 (5), 933–943 (May).
Masaldan, S., Clatworthy, S.A.S., Gamell, C., Meggyesy, P.M., Rigopoulos, A.T., Haupt, S., et al., 2018. Iron accumulation in senescent cells is coupled with impaired ferritinophagy and inhibition of ferroptosis. Redox Biol. 14, 100–115 (Apr).
Mavaddatiyan, L., Naeini, S., Khodabandeh, S., Hosseini, F., Skelton RhysJP, Azizi, V., et al., 2025. Exploring the association between aging, ferroptosis, and common age-related diseases. Arch. Gerontol. Geriatr. 135, 105877 (Aug 1).
Meys, R., Janssen, S.M.J., Franssen, F.M.E., Vaes, A.W., Stoffels, A.a.F., van Hees, H.W.H., et al., 2023. Test-retest reliability, construct validity and determinants of 6-minute walk test performance in adult patients with asthma. Pulmonology 29 (6), 486–494.
Miwa, S., Kashyap, S., Chini, E., von Zglinicki, T., 2022. Mitochondrial dysfunction in cell senescence and aging. J. Clin. Invest. 132 (13), e158447.
National Institute on Aging, 2021. Older adults with declining physical function at greater risk of dying. Available from: https://www.nia.nih.gov/news/older-adults-declining-physical-function-greater-risk-dying (Internet, cited 2025 May 29).
Nguyen, L.T., Buse, J.D., Baskin, L., Sadrzadeh, S.M.H., Naugler, C., 2017. Influence of diurnal variation and fasting on serum iron concentrations in a community-based population. Clin. Biochem. 50 (18), 1237–1242 (Dec).
O'Neill, D., Forman, D.E., 2020. The importance of physical function as a clinical outcome: assessment and enhancement. Clin. Cardiol. 43 (2), 108–117 (Feb).
Pavasini, R., Guralnik, J., Brown, J.C., di Bari, M., Cesari, M., Landi, F., et al., 2016. Short Physical Performance Battery and all-cause mortality: systematic review and meta-analysis. BMC Med. 14 (1), 215 (Dec 22).
Perlstein, T.S., Weuve, J., Pfeffer, M.A., Beckman, J.A., 2009. Red blood cell distribution width and mortality risk in a community-based prospective cohort. Arch. Intern. Med. 169 (6), 588–594 (Mar 23).
Picca, A., Mankowski, R.T., Kamenov, G., Anton, S.D., Manini, T.M., Buford, T.W., et al., 2019a. Advanced age is associated with iron dyshomeostasis and mitochondrial DNA damage in human skeletal muscle. Cells 8 (12), 1525 (Nov 27).
Picca, A., Guerra, F., Calvani, R., Bucci, C., Lo Monaco, M.R., Bentivoglio, A.R., et al., 2019b. Mitochondrial dysfunction and aging: insights from the analysis of extracellular vesicles. Int. J. Mol. Sci. 20 (4), 805 (Feb 13).
Picca, A., Saini, S.K., Mankowski, R.T., Kamenov, G., Anton, S.D., Manini, T.M., et al., 2020. Altered expression of Mitoferrin and Frataxin, larger labile iron pool and greater mitochondrial DNA damage in the skeletal muscle of older adults. Cells 9 (12), 2579 (Dec 2).
Picca, A., Triolo, M., Wohlgemuth, S.E., Martenson, M.S., Mankowski, R.T., Anton, S.D., et al., 2023. Relationship between mitochondrial quality control markers, lower extremity tissue composition, and physical performance in physically inactive older adults. Cells 12 (1), 183 (Jan 2).
Porto, J.M., Nakaishi, A.P.M., Cangussu-Oliveira, L.M., Freire Júnior, R.C., Spilla, S.B., de Abreu, D.C.C., 2019. Relationship between grip strength and global muscle strength in community-dwelling older people. Arch. Gerontol. Geriatr. 82, 273–278.

Psaty, B.M., Lee, M., Savage, P.J., Rutan, G.H., German, P.S., Lyles, M., 1992. Assessing the use of medications in the elderly: methods and initial experience in the Cardiovascular Health Study. The Cardiovascular Health Study Collaborative Research Group. J. Clin. Epidemiol. 45 (6), 683–692 (June).

Rebouche, C.J., Wilcox, C.L., Widness, J.A., 2004. Microanalysis of non-heme iron in animal tissues. J. Biochem. Biophys. Methods 58 (3), 239–251 (Mar 31).

Rejeski, W.J., Ip, E.H., Marsh, A.P., Miller, M.E., Farmer, D.F., 2008. Measuring disability in older adults: the International Classification System of Functioning, Disability and Health (ICF) framework. Geriatr Gerontol Int 8 (1), 48–54 (Mar).

Rejeski, W.J., Marsh, A.P., Anton, S., Chen, S.H., Church, T., Gill, T.M., et al., 2013. The MAT-sf: clinical relevance and validity. J. Gerontol. A Biol. Sci. Med. Sci. 68 (12), 1567–1574 (Dec).

Said, A.S., Spinella, P.C., Hartman, M.E., Steffen, K.M., Jackups, R., Holubkov, R., et al., 2017. RBC distribution width: biomarker for red cell dysfunction and critical illness outcome? Pediatr. Crit. Care Med. 18 (2), 134–142 (Feb).

Seo, A.Y., Xu, J., Servais, S., Hofer, T., Marzetti, E., Wohlgemuth, S.E., et al., 2008. Mitochondrial iron accumulation with age and functional consequences. Aging Cell 7 (5), 706–716 (Oct).

Shlisky, J., Bloom, D.E., Beaudreault, A.R., Tucker, K.L., Keller, H.H., Freund-Levi, Y., et al., 2017. Nutritional considerations for healthy aging and reduction in age-related chronic disease. Adv. Nutr. 8 (1), 17–26 (Jan).

Shokolenko, I., Venediktova, N., Bochkareva, A., Wilson, G.L., Alexeyev, M.F., 2009. Oxidative stress induces degradation of mitochondrial DNA. Nucleic Acids Res. 37 (8), 2539–2548 (May).

Stewart, A., Mills, K.M., King, A.C., Haskell, W.L., Gillis, D., Ritter, P.L., 2001. CHAMPS physical activity questionnaire for older adults: outcomes for interventions. Med. Sci. Sports Exerc. 33 (7), 1126–1141. PMID 11445760.

Subar, A.F., Thompson, F.E., Kipnis, V., Midthune, D., Hurwitz, P., McNutt, S., et al., 2001. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. Am. J. Epidemiol. 154 (12), 1089–1099 (Dec 15).

Sun, X.H., Ma, T., Yao, S., Chen, Z.K., Xu, W.D., Jiang, X.Y., et al., 2020. Associations of sleep quality and sleep duration with frailty and pre-frailty in an elderly population Rugao longevity and ageing study. BMC Geriatr. 20 (1), 9 (Jan 6).

Tatullo, M., Rengo, S., Sammartino, G., Simeone, M., Valletta, A., Gasparro, R., 2024. Inflammaging, epigenetic damage, mitochondrial dysfunctions, and macrophage alterations are the main missing pieces in the complex mosaic of stem cell physiology. Discov. Med. 36 (188), 1772–1779 (Sept).

van der Rijt, S., Molenaars, M., McIntyre, R.L., Janssens, G.E., Houtkooper, R.H., 2020. Integrating the hallmarks of aging throughout the tree of life: a focus on mitochondrial dysfunction. Front. Cell Dev. Biol. 8, 594416.

Wang, T., Wu, Y., Li, W., Li, S., Sun, Y., Li, S., et al., 2019. Weak grip strength and cognition predict functional limitation in older Europeans. J. Am. Geriatr. Soc. 67 (1), 93–99 (Jan).

Wojciak, R.W., 2014. Effect of short-term food restriction on iron metabolism, relative well-being and depression symptoms in healthy women. Eat Weight Disord. 19 (3), 321–327.

Wu, B., Xiao, Q., Zhu, L., Tang, H., Peng, W., 2024. Icariin targets p53 to protect against ceramide-induced neuronal senescence: implication in Alzheimer's disease. Free Radic. Biol. Med. (224), 204–219 (Nov 1).

Xu, J., Knutson, M.D., Carter, C.S., Leeuwenburgh, C., 2008. Iron accumulation with age, oxidative stress and functional decline. PLoS One 3 (8), e2865 (Aug 6).

Xu, J., Hwang, J.C.Y., Lees, H.A., Wohlgemuth, S.E., Knutson, M.D., Judge, A.R., et al., 2012. Long-term perturbation of muscle iron homeostasis following hindlimb suspension in old rats is associated with high levels of oxidative stress and impaired recovery from atrophy. Exp. Gerontol. 47 (1), 100–108 (Jan).

Zeidan, R.S., Martenson, M., Tamargo, J.A., McLaren, C., Ezzati, A., Lin, Y., et al., 2024. Iron homeostasis in older adults: balancing nutritional requirements and health risks. J. Nutr. Health Aging 28 (5), 100212 (May).

Zhang, M.J., Pisco, A.O., Darmanis, S., Zou, J., 2021. Mouse aging cell atlas analysis reveals global and cell type-specific aging signatures. eLife 10, e62293.