

Sex-specific alterations in muscle health and related protein and amino acid kinetics in different stages of frailty

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Abstract

Presence of frailty in older adults is accompanied by declined muscle and bone health, and metabolic disturbances. Little is known about sex-specific changes in protein and amino acid kinetics along the frailty trajectory. We investigated whether older adults at different stages of frailty (non-frailty, pre-frailty, and frailty) exhibited a distinct functional and metabolic phenotype, influenced by biological sex, and the potential of net protein breakdown as a functional biomarker of frailty.

In 208 older adults, body composition and bone density (by DXA), skeletal muscle strength (by dynamometry), and functional capacity (by 6-minute walk test (6MWT)) were measured. In the fasted state, a pulse of a stable isotope tracer solution, containing multiple labeled amino acids, was administered. Plasma tracer enrichments were analyzed by LC-MS/MS to measure protein amino acid kinetics, and net protein breakdown as a global marker of health.

In the whole group, handgrip strength and 6MWT distance ($P < 0.05$) were lower in the pre-frail and frail than in the non-frail. Bone density and leg muscle strength were lowest in the frail. A decrease in leg muscle strength was present in males only ($P < 0.001$), but muscle and bone loss in females only ($P < 0.03$). Frailty severity was associated with lower whole-body and net protein breakdown in females ($r: -0.29, -0.27, P < 0.007$) and a trend toward increased myofibrillar protein breakdown in males ($r: 0.22, P = 0.06$). Decreased net protein breakdown was further associated with lower protein intake and age in both sexes ($r: -0.29, -0.27, P < 0.05$) and worse 6MWT performance in females ($r: 0.25, P = 0.016$).

Our findings indicate that the trajectory of frailty is characterized by early functional decline (independent of sex) and disturbances in protein metabolism, featuring lower whole-body and net protein breakdown (particularly in females) and increased myofibrillar protein breakdown (particularly in males), while lean mass and bone density are preserved in males but reduced in females. Sex-specific nutritional and therapeutic approaches therefore need to be considered to optimize muscle and bone health, personalized along the frailty trajectory. The strong association between reduced net protein breakdown and markers of poor functional outcomes suggests a potential role of net protein breakdown as a functional biomarker of frailty.

Introduction

Frailty is an aging-related clinical syndrome characterized by a decline in physiological reserve and multi-organ function, and increased vulnerability to stressors¹. Frailty in older adults leads to poor recovery from disability, higher perioperative morbidity, and postoperative mortality²⁻⁴. Besides its impact in clinical settings, frailty is consistently associated with poor mobility, quality of life, and higher risk of falls and mortality rates⁵⁻⁷. With the prevalence of frailty (age ≥ 60) in nursing homes (52%) and community-dwelling older adults (15%)^{8,9}, it is up to 25% in adults aged > 85 years¹⁰. Despite this substantial public health burden, there are currently no established pharmacological treatments, nutritional regimens, or

validated biomarkers for frailty¹¹. Thus, developing accessible and applicable methods to prevent and treat frailty, such as targeted nutritional intervention, is critical.

Age-associated changes, such as decline in muscle and bone mass, functional performance, and multi-organ damage, contribute to an increased risk of frailty¹², particularly in the case of physical inactivity and malnutrition^{12,13}. Interestingly, the prevalence of frailty is greater in females than in males^{13,14} at any age, possibly related to lower muscle mass, longer life expectancy, and a higher number of comorbidities. However, despite the higher frailty burden, women appear to be more resilient, as shown by lower mortality rates observed across age groups and severity of frailty^{14,15}. This phenomenon is often referred to as *the male-female health-survival paradox*¹⁶. Age-related decline in bone mineral density has been more pronounced in women than in men¹⁷, whereas that in muscle has provided conflicting results^{18,19}.

To understand the underlying biological mechanisms of frailty, several untargeted metabolomics studies have investigated circulating amino acid biomarkers. Lower plasma essential amino acid levels have been found in older adults with frailty²⁰, related to lower tryptophan, isoleucine, leucine, proline, and methionine²¹, while other studies found no differences²², or lower BCAAs and tryptophan and higher glutamate and glutamine levels^{23–26}.

Metabolomics studies generally provide data on static plasma amino acid concentrations but do not provide insights into the underlying dynamic kinetics. We recently observed increased tau-methylhistidine production and downregulation of amino acid metabolism and net protein breakdown with aging in adults > 50 years using stable isotope methodology²⁷. Lower net protein breakdown (or postabsorptive protein balance) was further associated with worse clinical outcomes, including muscle weakness, poor exercise capacity, and cognitive function^{27,28}. Furthermore, we observed sex-specific disturbances in amino acid metabolism in older adults with chronic diseases and conditions^{29–31}. Females who were critically ill showed a suppressed decrease in net protein breakdown compared to their male counterparts²⁹, and increased myofibrillar breakdown was present in male COPD patients only, while the female patients showed a suppressed whole-body production³¹. However, little is known about sex-specific alterations in protein and amino acid metabolism across the frailty trajectory and whether these may explain differences in functional outcomes.

To address this gap, this study investigated whether older adults at different stages of frailty (non-frailty, pre-frailty, and frailty) exhibit a specific functional and metabolic phenotype. Using a novel pulse isotope method with a compartmental analysis model, we quantified intracellular production and intra/extracellular pool sizes of amino acids known to play a role in muscle health, and evaluated whether the observed kinetic perturbations were associated with markers of poor functional performance. As secondary objectives, we investigated whether the (functional and metabolic) phenotype differed by biological sex and explored the potential of net protein breakdown as a biomarker of functional decline.

We hypothesized that specific metabolic disturbances are already present in the pre-frail stage but more pronounced with frailty severity, particularly in females, which contribute to distinct muscle/bone health trajectories between males and females. Identification of potential biomarkers of functional decline will guide more personalized (sex based) approaches to future nutritional and therapeutic interventions to prevent (further) functional decline in the different stages of frailty.

Results

General characteristics

We studied 208 older adults with an average age of 72.5, 76.7, and 78.8 years in the non-frail, pre-frail, and frail groups, respectively (Table 1). Sex distribution was comparable except for the frail group, where more females were present (% females in non-/pre-/frail: 44%, 47%, 78%). All groups were overweight (average BMI between 26–29 kg/m²). The frail group had a slightly higher Charlson Comorbidity Index score (average 1.1), which is considered mild. Average fasting plasma glucose was around 5.5 mmol/L in all groups, indicating no evidence of insulin resistance. The highest prevalence of the Fried Frailty physical domains in the pre-frail group was slow gait speed (58.7%), weakness (35.9%), and low physical activity (19.6%), while that in the frail group was slow gait speed (94.6%), weakness (86.3%), and low physical activity (73.5%) (Table 1). The prevalence of domains in males and females was comparable except for exhaustion (males: 1.6% vs females: 16.6%) in the pre-frail, and exhaustion (males: 80% vs females: 51.2%) and weight loss + BMI (males: 33.3% vs females: 50.9%) in the frail group. The highest comorbidities in the non-frail group were myocardial infarction (12.5%), cerebrovascular diseases with mild or no residue (8.3%), and diabetes without complications (8.3%). In the pre-frail group, these were chronic pulmonary disease (11.9%), myocardial infarction (10.8%), and diabetes without complications (9.8%), and in the frail group, chronic pulmonary disease (32.4%), congestive heart failure (13.2%), and diabetes without complications (11.8%). In addition, all groups showed other medical features such as hypertension, dyslipidemia, osteoarthritis, psychological issues, and obstructive sleep apnea. The average number of medications in the non-frail, pre-frail, and frail groups was 4.2, 4.2, and 6.9, respectively. Most commonly used medications in all groups included non-opioid analgesics and antipyretics, bone resorption inhibitors, and lipid-lowering, antiulcer, antianxiety/depressant, antihypertensive, and antihistamine drugs.

Table 1

Characteristics of study participants, prevalence of individual frailty scores, disease characteristics, medical features, and medications after stratification by stage of frailty in the whole group of older adults and in males and females separately

	Non-frail (WG: n = 48) (M: n = 27 / F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49 / F: n = 43)	Frail (WG: n = 68) (M: n = 15 / F: n = 53)
Characteristics of study participants			
Age (years)	WG: 72.7 (7.5) M: 74.8 (7.5), F: 70 (6.6)	WG: 76.7 (8.7) M: 78.4 (9.4), F: 74.7 (7.6)	WG: 78.8 (9.2) M: 80.8 (10.1), F: 78.3 (9)
BMI	WG: 28.6 (4.1) M: 27.8 (3.6), F: 29.6 (4.5)	WG: 29.4 (5.4) M: 29.2 (5), F: 29.7 (5.9)	WG: 26.1 (6.4) M: 28.6 (6.2), F: 25.4 (6.4)
Charlson Comorbidity Index	WG: 0.4 (0.7) M: 0.6 (0.8), F: 0.2 (0.4)	WG: 0.6 (0.8) M: 0.8 (0.9), F: 0.3 (0.5)	WG: 1.1 (1.5) M: 0.7 (0.8), F: 1.2 (1.6)
Plasma glucose concentration (mmol/L)	WG: 5.4 (0.4) M: 5.4 (0.4), F: 4.9 (0.3)	WG: 5.6 (1.5) M: 5.6 (1.8), F: 5.5 (1)	WG: 5.5 (1) M: 5.8 (1.4), F: 5.3 (0.8)
Raw frailty score	WG: 0 (0) M: 0 (0), F: 0 (0)	WG: 1.4 (0.5) M: 1.3 (0.5), F: 1.4 (0.5)	WG: 3.3 (0.5) M: 3.3 (0.6), F: 3.3 (0.5)
Prevalence of individual scores in the (pre) frail groups			
Weakness (%)		WG: 35.9 M: 30.6, F: 41.9	WG: 85.3 M: 80, F: 86.8
Slow gait speed (%)		WG: 58.7 M: 61.2, F: 55.8	WG: 94.3 M: 84.6, F: 97.6
Low physical activity (%)		WG: 19.6 M: 20.4, F: 18.6	WG: 73.5 M: 60, F: 77.3
Exhaustion (%)		WG: 13.4 M: 1.6, F: 16.6	WG: 58.9 M: 80, F: 51.2
Weight loss + low BMI (%)		WG: 9.8 M: 8.2, F: 11.6	WG: 47.1 M: 33.3, F: 50.9
Disease characteristics			

Myocardial infarction (%)	WG: 12.5 M: 18.5, F: 4.7	WG: 10.8 M: 18.4, F: 2.3	WG: 2.9 M: 13.3, F: 0
Congestive heart failure (%)	WG: 2.1 M: 0, F: 4.7	WG: 5 M: 8, F: 2.3	WG: 13.2 M: 6.6, F: 15.1
Cerebrovascular disease (%): CVA or TIA with mild or no residue	WG: 8.3 M: 14.8, F: 0	WG: 5.4 M: 6.1, F: 4.6	WG: 5.9 M: 6.6, F: 5.6
Chronic pulmonary disease (%)	WG: 0 M: 0, F: 0	WG: 11.9 M: 14.3, F: 9.3	WG: 32.4 M: 6.6, F: 39.6
Diabetes without complications (%)	WG: 8.3 M: 7, F: 9.5	WG: 9.8 M: 10.2, F: 9.3	WG: 11.8 M: 26, F: 4.5
Moderate or severe renal disease (%)	WG: 2.1 M: 3.7, F: 0	WG: 1.1 M: 2, F: 0	WG: 5.9 M: 0, F: 7.5
Other medical features			
Hypertension (%)	WG: 47.9 M: 59.2, F: 33.3	WG: 55.4 M: 63.2, F: 46.5	WG: 45.6 M: 46.6, F: 45.3
Dyslipidemia (%)	WG: 45.8 M: 48.1, F: 42.8	WG: 50 M: 53.1, F: 46.5	WG: 32.4 M: 33.3, F: 32.1
OSA (%)	WG: 12.5 M: 14.8, F: 9.5	WG: 16.3 M: 16.3, F: 16.3	WG: 13.2 M: 26.6, F: 9.4
Osteoporosis (%)	WG: 2.1 M: 3.7, F: 0	WG: 9.5 M: 2, F: 16.3	WG: 29.4 M: 0, F: 37.8
Osteoarthritis (%)	WG: 39.6 M: 33.3, F: 47.6	WG: 39.1 M: 40.8, F: 37.2	WG: 38.2 M: 46.6, F: 35.8
Psychological issues (%)	WG: 14.6 M: 14.8, F: 14.3	WG: 17.4 M: 12.2, F: 23.2	WG: 20.6 M: 13.3, F: 22.6
Hypothyroidism (%)	WG: 8.3 M: 3, F: 14.3	WG: 19.6 M: 10.2, F: 30.2	WG: 17.6 M: 6.6, F: 20.8

Anemia (%)	WG: 8.8 M: 14.8, F: 9.5	WG: 13 M: 14.3, F: 11.6	WG: 7.3 M: 6.6, F: 7.5
Medications			
Number of medications (n)	WG: 4.2 (3.1) M: 3.8 (2.4), F: 4.7 (3.1)	WG: 4.2 (3.1) M: 4.2 (3.1), F: 4.2 (3.1)	WG: 6.9 (6.9) M: 7.2 (8.9), F: 6.8 (6.3)
Nonopioid analgesic and antipyretic (%)	WG: 28.6 M: 26, F: 31.6	WG: 16.8 M: 12.7, F: 21.4	WG: 29.3 M: 38.4, F: 26.6
Lipid-lowering agent (%)	WG: 35.7 M: 30.4, F: 42.1	WG: 48.3 M: 44.7, F: 52.3	WG: 29.3 M: 53.8, F: 22.2
Bone resorption inhibitor (%)	WG: 7.1 M: 0, F: 15.8	WG: 4.5 M: 0, F: 9.5	WG: 12 M: 0, F: 15.5
Antiulcer agent (%)	WG: 16.7 M: 17.3, F: 15.7	WG: 17.9 M: 14.8, F: 4.7	WG: 15.5 M: 23.1, F: 13.3
Antianxiety or antidepressant agent (%)	WG: 11.9 M: 8, F: 15.8	WG: 19.1 M: 8.5, F: 30.9	WG: 13.8 M: 15.4, F: 13.3
Antihypertensive drugs (%)	WG: 52.4 M: 56.5, F: 42.8	WG: 51.7 M: 57.4, F: 45.2	WG: 46.5 M: 61.5, F: 42.2
Antihistamine (%)	WG: 14.3 M: 21.7, F: 5.2	WG: 12.4 M: 14.9, F: 9.5	WG: 15.5 M: 0, F: 0
Values are estimated means (SD). Statistics are descriptive statistics.			
Abbreviations: BMI, body mass index; OSA, obstructive sleep apnea; WG, whole group; M, males; F, females			

Lifestyle factors, anthropometrics, body composition, muscle function and physical capacity

Habitual physical activity was lower in the pre-frail and frail compared with the non-frail ($P < 0.017$), with further reduction in the frail compared with the pre-frail ($P < 0.001$) (Table 2). No difference was observed in habitual dietary (including protein) intake among the three groups. Body weight and maximal inspiratory pressure were lower in the frail compared with the non-frail and pre-frail (all $P < 0.04$). Lower BMI, fat mass, lean soft tissue, and lean extremity mass were observed in the frail compared with the pre-frail (all $P < 0.02$), while fat mass and lean extremity mass were higher in the pre-frail than non-frail ($P < 0.01$). Bone mineral density (BMD) in the hip was lower in the frail compared with the non- and pre-

frail ($P < 0.003$). BMD in the hip neck showed lower values in the frail compared with the pre-frail ($P = 0.043$). Handgrip strength and 6MWT distance were lower in the pre-frail and frail compared with the non-frail ($P < 0.05$), and in the frail than in the pre-frail ($P < 0.001$). Additionally, we found lower peak leg torque extension in the frail than in the pre-frail ($P < 0.032$). The observed differences in handgrip strength and 6MWT distance between the groups remained after correction for lean soft tissue.

Table 2

Lifestyle, habitual intakes, anthropometrics, body composition, and muscle function after stratification by stage of frailty in the whole group of older adults and in males and females separately

	Sex	Non-frail (WG: n = 48) (M: n = 27) F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49) F: n = 43)	Frail (WG: n = 68) (M: n = 15) F: n = 53)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
<i>Lifestyle and habitual dietary intakes</i>							
Habitual physical activity (score)	WG	209.7 [191.3, 229.8]	177.9 [166.9, 189.5]	117.7 [108.7, 127.5]	0.016	< .001	< .001
	M	208.4 [185.8, 233.6]	174.2 [160.2, 189.4]	123.4 [106, 143.8]	0.052	< .001	< .001
	F	213 [185.4, 244.8]	182.5 [166.7, 199.8]	117.5 [107.7, 128.3]	0.207	< .001	< .001
Total calorie intake (kcal/day)	WG	2000 [1779, 2247]	1756 [1634, 1887]	1737 [1587, 1901]	0.198	0.214	0.997
	M	2126 [1831, 2468]	1863 [1688, 2057]	1842 [1537, 2207]	0.413	0.539	0.999
	F	1874 [1552, 2263]	1654 [1484, 1843]	1666 [1508, 1839]	0.604	0.659	1
Total carbohydrate intake (g/day)	WG	215.4 [187.9, 246.8]	198.4 [181.6, 216.9]	198.6 [177.3, 222.5]	0.694	0.778	1
	M	228.1 [192.7, 270.1]	209.8 [185.8, 236.8]	205.6 [163.8, 258.1]	0.817	0.85	0.998
	F	202.8	189.1	190.4	0.939	0.957	1

	Sex	Non-frail (WG: n = 48) (M: n = 27) F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49) F: n = 43)	Frail (WG: n = 68) (M: n = 15) F: n = 53)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
<i>Lifestyle and habitual dietary intakes</i>							
		[161.1, 255.3]	[165.4, 216.2]	[167.8, 216.1]			
Total fat intake (g/day)	WG	83.04 [71.38, 96.6]	72.88 [66.16, 80.29]	75.73 [66.91, 85.72]	0.412	0.763	0.952
	M	81.78 [67.42, 99.21]	76.8 [66.85, 88.23]	91.84 [70.8, 119.12]	0.939	0.87	0.585
Protein intake (g/day)	F	88.56 [69.18, 113.4]	69.39 [60.48, 79.62]	70.08 [61.54, 79.8]	0.293	0.349	0.999
	WG	78.33 [69.12, 88.76]	70.17 [64.68, 76.12]	69.23 [62.38, 76.84]	0.395	0.407	0.996
Total protein intake (g/kg body weight*day)	M	77.2 [66.48, 89.64]	74.44 [66.86, 82.88]	68.81 [56.26, 84.15]	0.972	0.744	0.862
	F	81.53 [65.89, 100.89]	65.71 [58.06, 74.37]	69.55 [61.87, 78.2]	0.271	0.542	0.887
Total protein intake (g/kg body weight*day)	WG	1.05 [0.91, 1.21]	0.96 [0.87, 1.05]	1.12 [1, 1.27]	0.622	0.874	0.124
	M	0.99 [0.84, 1.18]	0.96 [0.85, 1.08]	0.89 [0.7, 1.12]	0.985	0.818	0.896
Total protein intake (g/kg body weight*day)	F	1.12	0.94	1.22	0.547	0.899	0.029

	Sex	Non-frail (WG: n = 48) (M: n = 27) F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49) F: n = 43)	Frail (WG: n = 68) (M: n = 15) F: n = 53)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
Lifestyle and habitual dietary intakes							
		[0.88, 1.42]	[0.82, 1.08]	[1.07, 1.39]			
Anthropometrics and body composition							
Body Weight (kg)	WG	79.84 [75.08, 84.89]	81.94 [78.48, 85.55]	71.52 [67.75, 75.5]	0.87	0.035	< .001
	M	84.6 [78.43, 91.26]	87.88 [83.15, 92.88]	83.17 [75.19, 91.99]	0.808	0.991	0.706
	F	75.86 [68.7, 83.75]	76.26 [71.38, 81.48]	64.26 [60.27, 68.51]	1	0.031	0.001
BMI (kg/m2)	WG	28.5 [26.88, 30.21]	29.42 [28.24, 30.65]	26.19 [24.88, 27.58]	0.759	0.119	0.002
	M	27.88 [26.1, 29.78]	29.16 [27.79, 30.6]	28.47 [26.07, 31.08]	0.622	0.975	0.95
	F	29.38 [26.65, 32.4]	29.64 [27.77, 31.64]	25.38 [23.82, 27.03]	0.998	0.062	0.004
Fat mass (g)	WG	23760 [21320, 26480]	28910 [26790, 31200]	24180 [21970, 26600]	0.009	0.994	0.012
	M	21644 [18792, 24928]	26038 [23484, 28870]	25325 [20984, 30564]	0.103	0.497	0.991
	F	26277	31822	24753	0.143	0.919	0.006

	Sex	Non-frail (WG: n = 48) (M: n = 27) F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49) F: n = 43)	Frail (WG: n = 68) (M: n = 15) F: n = 53)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
<i>Lifestyle and habitual dietary intakes</i>							
		[22266, 31012]	[28487, 35546]	[22239, 27552]			
Lean soft tissue (g)	WG	47270 [45160, 49480]	49280 [47720, 50890]	44400 [42640, 46220]	0.368	0.148	< .001
	M	56332 [52818, 60081]	57132 [54504, 59886]	51747 [47495, 56380]	0.98	0.318	0.119
	F	39382 [36775, 42174]	42464 [40565, 44453]	38181 [36527, 39910]	0.186	0.852	0.004
	WG	825 [719, 946.6]	838.3 [759.9, 924.7]	702.3 [621.1, 794.1]	0.997	0.275	0.078
	M	877.2 [746.2, 1031.2]	893.7 [793, 1007.1]	912 [735.2, 1131.2]	0.997	0.989	0.998
	F	777.8 [617.2, 980.3]	782.7 [668, 917.1]	614.6 [529.1, 713.9]	1	0.327	0.101
Visceral adipose tissue (g)	WG	0.89 [0.828, 0.957]	0.892 [0.848, 0.938]	0.749 [0.704, 0.797]	1	0.002	< .001
	M	0.915 [0.858, 0.975]	0.957 [0.913, 1.002]	0.844 [0.778, 0.916]	0.596	0.336	0.023
	F	0.872	0.83	0.69	0.902	0.021	0.01
	WG	0.89 [0.828, 0.957]	0.892 [0.848, 0.938]	0.749 [0.704, 0.797]	1	0.002	< .001
	M	0.915 [0.858, 0.975]	0.957 [0.913, 1.002]	0.844 [0.778, 0.916]	0.596	0.336	0.023
	F	0.872	0.83	0.69	0.902	0.021	0.01
BMD hip total (g/cm2)	WG	0.89 [0.828, 0.957]	0.892 [0.848, 0.938]	0.749 [0.704, 0.797]	1	0.002	< .001
	M	0.915 [0.858, 0.975]	0.957 [0.913, 1.002]	0.844 [0.778, 0.916]	0.596	0.336	0.023
	F	0.872	0.83	0.69	0.902	0.021	0.01
	WG	0.89 [0.828, 0.957]	0.892 [0.848, 0.938]	0.749 [0.704, 0.797]	1	0.002	< .001
	M	0.915 [0.858, 0.975]	0.957 [0.913, 1.002]	0.844 [0.778, 0.916]	0.596	0.336	0.023
	F	0.872	0.83	0.69	0.902	0.021	0.01

	Sex	Non-frail (WG: n = 48) (M: n = 27) F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49) F: n = 43)	Frail (WG: n = 68) (M: n = 15) F: n = 53)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
Lifestyle and habitual dietary intakes							
		[0.764, 0.996]	[0.76, 0.907]	[0.637, 0.748]			
BMD hip neck (g/cm ²)	WG	0.735 [0.693, 0.781]	0.754 [0.723, 0.786]	0.694 [0.658, 0.731]	0.873	0.414	0.043
	M	0.771 [0.71, 0.838]	0.801 [0.754, 0.851]	0.7 [0.627, 0.782]	0.85	0.429	0.09
	F	0.699 [0.64, 0.763]	0.705 [0.665, 0.747]	0.67 [0.634, 0.708]	0.998	0.821	0.51
Muscle function							
MIP (cm H ₂ O)	WG	73.31 [65.91, 81.55]	71.27 [66.45, 76.44]	55.76 [50.83, 61.18]	0.962	0.001	< .001
	M	84.25 [73.9, 96.05]	78.63 [71.84, 86.07]	60.1 [50.36, 71.73]	0.783	0.008	0.015
	F	61.88 [52.02, 73.62]	64.22 [57.66, 71.52]	50.27 [45.05, 56.09]	0.977	0.186	0.007
Handgrip strength (N)	WG	228.6 [215.6, 242.5]	203.5 [195.2, 212.1]	168.6 [160, 177.6]	0.006	< .001	< .001
	M	280.7 [259.4, 303.7]	251.9 [237.8, 266.8]	186 [167.5, 206.6]	0.099	< .001	< .001
	F	183.1	162.5	144.1	0.095	< .001	0.017

	Sex	Non-frail (WG: n = 48) (M: n = 27) F: n = 21)	Pre-frail (WG: n = 92) (M: n = 49) F: n = 43)	Frail (WG: n = 68) (M: n = 15) F: n = 53)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
Lifestyle and habitual dietary intakes							
		[167.4, 200.2]	[153, 172.5]	[136, 152.7]			
Peak leg torque extension (Nm)	WG	81.32 [71.87, 92.01]	83.93 [77.57, 90.82]	71.16 [64.52, 78.48]	0.963	0.281	0.032
	M	111.5 [95.3, 130.4]	110.2 [100.5, 120.9]	68.4 [56.5, 82.9]	0.999	< .001	< .001
	F	57.6 [48.1, 68.9]	62.7 [55.5, 70.8]	59.7 [53.7, 66.3]	0.808	0.982	0.91
6MWT distance (m)	WG	492.4 [456.5, 531]	438.3 [416.3, 461.5]	352.9 [327.2, 380.7]	0.044	< .001	< .001
	M	490.2 [445.9, 538.9]	452.2 [422.4, 484.1]	389.1 [338.6, 447.2]	0.45	0.02	0.133
	F	501.3 [442.6, 567.7]	424.7 [392.3, 459.8]	333.2 [304.6, 364.5]	0.096	< .001	< .001
Values are estimated means [95% CI]. Statistics (JASP) are done with Generalized Linear Model (Family: Gamma, Link: Log) to examine the effect of frailty group with covariate age (and sex for the whole group analysis). Multiple comparisons for group differences were analyzed using Tukey post hoc. Bold is p < 0.05. Abbreviation: BMI, Body Mass Index; BMD, Bone Mineral Density; MIP, Maximal Inspiratory Pressure; 6MWT, 6-Minute Walk Test, WG, whole group; M, males; F, females							

As a secondary analysis, we performed GLM in males and females separately. In males, stage of frailty did not affect lifestyle, habitual dietary intake, and body composition, other than lower habitual physical

activity in the frail ($P < 0.001$), and hip BMD in the frail compared with the pre-frail ($P = 0.023$) (Table 2). Lower values for MIP, handgrip, and peak leg torque were observed in the frail compared with the non- and pre-frail groups (all $P < 0.02$) in males, and 6MWT distance was lower in frail than in non-frail male subjects ($P = 0.02$) (Table 2, **Fig. 1**). In females, lower body weight, habitual physical activity, and hip BMD were found in the frail compared with the non- and pre-frail (all $P < 0.04$), and lower BMI, fat mass, lean soft tissue, and lean extremity in the frail compared with the pre-frail (all $P < 0.04$). Interestingly, lean extremity in the pre-frail was higher than in the non-frail ($P < 0.001$). We observed lower MIP in the frail compared with the pre-frail, and lower handgrip strength and 6MWT distance in the frail compared with the non- and pre-frail (all $P < 0.02$). We did not find a decrease in peak leg torque extension in female (pre) frail subjects (Fig. 1).

Metabolic markers

Plasma concentrations, whole-body and intracellular production of amino acids

We observed lower plasma concentrations (**Supplemental Table 3**) of valine ($P < 0.001$), leucine ($P = 0.011$), and sum of BCAA ($P = 0.003$) in the frail older adults compared with the non-frail, and of glutamate, phenylalanine, sum of EAA and LNAA in the frail older adults compared with the non- and pre-frail groups (all $P < 0.03$). The plasma concentration of tau-methylhistidine was higher in the frail than in the pre-frail ($P = 0.039$), whereas the sum of amino acids was lower in the frail than in the pre-frail ($P = 0.018$). Sex-specific analysis showed that pre-frailty was associated with lower plasma aspartate concentration ($P = 0.034$), and frailty was associated with a higher tryptophan/LNAA ratio in males ($P = 0.028$) (**Supplemental Table 4**). In females, lower plasma concentration of glutamate, asparagine, valine, histidine, and LNAA was observed in the frail compared with the non-frail. Lower values for asparagine, serine, phenylalanine, histidine, lysine, essential amino acids, and sum amino acids were observed in the frail compared with the pre-frail (all $P < 0.04$) (**Supplemental Table 4**).

Whole body production (WBP) (Table 3) represents the turnover that can be observed in plasma and does not take into account the intracellular production. All metabolic parameters were corrected for age, sex, and lean soft tissue. We observed lower WBP of tyrosine in the frail compared with the non- and pre-frail ($P < 0.05$). WBP of hydroxyproline, leucine, and valine was lower in pre-frail older adults than in non-frail older adults, with no further change in the frail stage ($P = 0.019$). In addition, WBP of phenylalanine ($P < 0.007$) and net protein breakdown ($P < 0.05$) were lower in the frail compared with the non-frail and pre-frail. We found elevated WBP of tau-methylhistidine (marker of myofibrillar protein breakdown) in the frail compared with the pre-frail ($P = 0.027$). In males, lower WBP of glutamine and tyrosine was observed in the frail compared with the non-frail participants ($P < 0.05$) (Table 3). Valine WBP was lower in the pre-frail and frail groups compared with the non-frail group in males ($P < 0.02$), while net protein breakdown was lower in the frail group than in the pre-frail group. In females, we found lower WBP of isoleucine, leucine, and valine in the pre-frail compared with the non-frail ($P < 0.03$). WBP of phenylalanine and tyrosine, and net protein breakdown were lower in the female frail group than in the non-frail group ($P < 0.02$).

Table 3

Whole body production (WBP) of amino acids after stratification by stage of frailty in the whole group of older adults and in males and females separately

Glutamine	Sex	Non-frail (n=27m/21f)	Pre-frail (n=49m/43f)	Frail (n=15m/53f)	P value Non- frail vs Pre- frail	P value Non- frail vs Frail	P value Pre- frail vs Frail
	WG	412.1 [389.2, 436.3]	383.2 [367.8, 399.2]	382.2 [361.1, 404.4]	0.127	0.219	1
	M	469 [439.5, 500]	437.6 [417.1, 459]	399.9 [363.7, 439.6]	0.262	0.02	0.24
	F	373 [338.9, 411]	337.8 [316.5, 360.6]	354.2 [330.5, 379.5]	0.269	0.793	0.714
Glutamate	WG	125.3 [104, 150.9]	132.4 [115.7, 151.5]	160.2 [133, 192.9]	0.952	0.227	0.318
	M	115 [88, 149]	132 [108.5, 160]	138 [93.6, 203]	0.774	0.841	0.996
	F	140 [106, 183]	134 [111, 162]	175 [143.6, 214]	0.994	0.468	0.197
Hydroxy proline	WG	3.84 [3.31, 4.47]	2.94 [2.65, 3.25]	3.6 [3.14, 4.12]	0.019	0.902	0.076
	M	4.49 [3.75, 5.37]	3.6 [3.16, 4.1]	4.47 [3.5, 5.71]	0.174	1	0.384
	F	3.45 [2.65, 4.49]	2.37 [2.02, 2.78]	2.96 [2.51, 3.48]	0.088	0.732	0.193
Isoleucine	WG	44.22 [40.44, 48.35]	40.83 [38.35, 43.47]	40.06 [36.5, 43.97]	0.397	0.387	0.983
	M	46.6	49.6	42.2	0.634	0.573	0.103

		[42.5, 51.2]	[46.4, 53]	[36.6, 48.7]			
	F	43.8 [37.7, 50.8]	33.7 [30.5, 37.3]	36.1 [32.2, 40.5]	0.022	0.179	0.774
Leucine	WG	92.72 [87.64, 98.09]	84.04 [80.77, 87.45]	84.99 [80.4, 89.83]	0.019	0.113	0.985
	M	106.2 [99.1, 113.8]	100 [95, 105.2]	91.6 [82.8, 101.3]	0.426	0.051	0.31
	F	83.5 [76.2, 91.6]	71 [66.9, 75.5]	75.8 [71.1, 80.9]	0.017	0.286	0.403
Phenylalanine	WG	41.72 [39.79, 43.75]	40.82 [39.46, 42.23]	37.07 [35.26, 38.97]	0.841	0.005	0.006
	M	47.4 [44.34, 50.6]	48 [45.7, 50.4]	42.5 [38.36, 47]	0.985	0.213	0.081
	F	37.9 [35.36, 40.6]	35.1 [33.5, 36.8]	32.4 [30.69, 34.1]	0.209	0.003	0.087
Taurine	WG	16.93 [15.78, 18.17]	18.23 [17.33, 19.16]	18.83 [17.46, 20.32]	0.25	0.155	0.865
	M	18.46 [16.77, 20.32]	20.04 [18.67, 21.51]	21.28 [18.49, 24.48]	0.434	0.311	0.842
	F	15.84 [14.21, 17.66]	16.79 [15.6, 18.07]	17.21 [15.74, 18.81]	0.759	0.599	0.968
Tau-methyl histidine	WG	0.53 [0.47, 0.59]	0.52 [0.48, 0.57]	0.65 [0.57, 0.73]	0.999	0.081	0.027
	M	0.62 [0.52, 0.73]	0.64 [0.57, 0.72]	0.82 [0.63, 1.06]	0.976	0.262	0.334
	F	0.49 [0.41, 0.58]	0.44 [0.39, 0.49]	0.54 [0.48, 0.61]	0.729	0.713	0.072

Tyrosine	WG	38.58 [36.26, 41.05]	35.59 [34.06, 37.2]	32.63 [30.57, 34.83]	0.114	0.002	0.092
	M	43.08 [39.74, 46.71]	41.05 [38.68, 43.57]	35.55 [31.41, 40.25]	0.72	0.033	0.097
	F	35.94 [32.64, 39.58]	31.15 [29.18, 33.24]	29.67 [27.55, 31.95]	0.055	0.014	0.723
Valine	WG	91.52 [84.98, 98.56]	74.92 [71.09, 78.96]	81.58 [75.41, 88.25]	< .001	0.133	0.242
	M	104.1 [96.6, 112.2]	88.9 [84.2, 93.8]	85.3 [75.9, 95.8]	0.004	0.013	0.889
	F	82.1 [71.9, 93.8]	63.1 [57.7, 69.1]	73.6 [66.4, 81.7]	0.008	0.544	0.107
Net Protein Breakdown	WG	6.07 [5.38, 6.84]	5.7 [5.24, 6.21]	4.72 [4.16, 5.35]	0.794	0.023	0.042
	M	6.22 [5.33, 7.24]	6.88 [6.16, 7.7]	4.81 [3.77, 6.14]	0.628	0.211	0.013
	F	6.24 [5.21, 7.47]	4.74 [4.2, 5.35]	4.31 [3.76, 4.94]	0.057	0.013	0.69

Values are estimated means [95% CI].

Statistics (JASP) are done with Generalized Linear Model (Family: Gamma, Link: Log) to examine the effect of frailty group with covariates age and lean soft tissue (and sex for the whole group analysis).

Multiple comparisons for group differences were analyzed using Tukey post hoc. Bold is $p < 0.05$.

Abbreviations: WG, whole group; M, males; F, females

Compartmental analysis (Table 4) enables calculation of intracellular amino acid production. We observed lower intracellular production of glutamine and leucine in the pre-frail and frail compared with the non-frail (all $P < 0.05$) (Fig. 2A). Interestingly, intracellular production of glutamate ($P = 0.043$) and hydroxyproline (marker of collagen breakdown) ($P = 0.002$) was lower in the pre-frail compared with the non-frail, without further changes in the frail stage. Intracellular production of methionine, phenylalanine (marker of whole-body protein breakdown), and tyrosine, and net protein breakdown (measured by

hydroxylation rate of phenylalanine to tyrosine) were reduced in frail older adults as compared to the non- and pre-frail older adults (all $P < 0.05$). In males, Intracellular production of glutamine and valine was lower in subjects with pre-frailty and frailty compared with non-frail subjects (all $P < 0.05$) (Table 4, **Fig. 2B**). We further observed lower intracellular production of hydroxyproline in the pre-frail compared with the non-frail ($P = 0.013$). In females, lower intracellular production of phenylalanine and tyrosine, and net protein breakdown were observed in the frail subjects compared with the non-frail subjects (all $P < 0.02$), with a further decrease in intracellular production of phenylalanine in the frail compared with the pre-frail ($P = 0.026$) (Table 4, **Fig. 2C**). Intracellular valine production was lower in the pre-frail compared with the non-frail ($P = 0.045$) in females.

Table 4

Intracellular production of amino acids after stratification by stage of frailty in the whole group of older adults and in males and females separately

Glutamine		Non-frail (n=27m/21f)	Pre-frail (n=49m/43f)	Frail (n=15m/53f)	P value Non-frail vs Pre-frail	P value Non-frail vs Frail	P value Pre-frail vs Frail
	WG	859.7 [808.7, 913.9]	760.8 [728.4, 794.6]	767 [721.2, 815.7]	0.006	0.042	0.995
	M	1026 [947.9, 1110]	864.6 [816.9, 915.2]	861.2 [765, 969.4]	0.003	0.048	1
	F	734 [672.7, 801]	672.8 [633.5, 714.6]	683.6 [641.3, 728.7]	0.298	0.517	0.98
Glutamate	WG	1227 [1045, 1441]	947.5 [846, 1061]	1035 [883.4, 1213]	0.043	0.41	0.77
	M	1260 [995, 1595]	1021 [857.5, 1215]	1071 [753, 1523]	0.442	0.83	0.993
	F	1215 [972, 1519]	866 [746.8, 1005]	993 [849, 1162]	0.065	0.449	0.541
Hydroxy proline	WG	5.61 [4.8, 6.56]	3.93 [3.56, 4.35]	4.88 [4.27, 5.57]	0.002	0.492	0.051
	M	6.62 [5.53, 7.92]	4.66 [4.11, 5.3]	6.41 [5.07, 8.11]	0.013	0.995	0.101
	F	4.96 [3.76, 6.54]	3.27 [2.79, 3.83]	3.95 [3.36, 4.64]	0.066	0.478	0.312
Isoleucine	WG	104.6 [92.28, 118.6]	101 [92.49, 110.2]	99.55 [87.45, 113.3]	0.955	0.938	0.997
	M	120.6	117.6	100.3	0.986	0.318	0.342

		[105.8, 137.5]	[107.2, 129.1]	[82.4, 122.1]			
	F	92.3 [73.8, 115.5]	86.9 [74.8, 101]	91.9 [77.4, 109.1]	0.96	1	0.953
Leucine	WG	221.2 [207.7, 235.5]	200.8 [192, 210]	196.5 [184.8, 209]	0.046	0.036	0.927
	M	243.3 [226.8, 261]	238.9 [226.8, 251.6]	213.6 [192.8, 236.6]	0.966	0.114	0.139
	F	206.7 [181.7, 235.1]	174.5 [160.2, 190.2]	178.1 [162.8, 194.9]	0.112	0.224	0.985
Phenylalanine	WG	91.39 [86.44, 96.62]	91.06 [87.56, 94.7]	80.03 [75.53, 84.8]	0.999	0.007	0.001
	M	101.5 [94.93, 108.5]	105.6 [100.6, 110.8]	93.9 [84.95, 103.9]	0.72	0.51	0.101
	F	84.3 [76.95, 92.4]	79.8 [75, 84.9]	70 [65.2, 75.1]	0.686	0.011	0.026
Taurine	WG	38.57 [35.64, 41.74]	40.54 [38.31, 42.91]	39.49 [36.28, 42.98]	0.669	0.973	0.942
	M	43.48 [39.07, 48.38]	43.29 [39.97, 46.88]	46.56 [39.83, 54.42]	1	0.865	0.807
	F	34.48 [30.57, 38.88]	38.64 [35.59, 41.96]	35.36 [32.03, 39.04]	0.309	0.986	0.456
Tau-methyl histidine	WG	0.7 [0.63, 0.78]	0.68 [0.63, 0.73]	0.79 [0.71, 0.88]	0.93	0.426	0.111
	M	0.78 [0.68, 0.89]	0.82 [0.74, 0.9]	1.05 [0.84, 1.32]	0.928	0.115	0.184

	F	0.68 [0.58, 0.8]	0.58 [0.52, 0.64]	0.65 [0.57, 0.73]	0.287	0.941	0.472
Tyrosine	WG	72.8 [67.88, 78.07]	67.4 [64.14, 70.82]	60.37 [56.1, 64.98]	0.221	0.002	0.046
	M	82.82 [75.69, 90.63]	75.64 [70.82, 80.79]	70.25 [61.36, 80.44]	0.31	0.133	0.688
	F	66.15 [59.26, 73.85]	60.97 [56.59, 65.68]	53.48 [49.06, 58.29]	0.541	0.019	0.086
Valine	WG	162.5 [149.7, 176.4]	134.6 [127, 142.7]	149.4 [137, 163]	0.001	0.465	0.168
	M	187.7 [173.6, 202.8]	159.7 [151, 169]	157.2 [139.3, 177.5]	0.005	0.045	0.994
	F	143.8 [123.8, 167]	113.6 [102.7, 125.7]	134.4 [119.8, 150.9]	0.045	0.882	0.12
Net Protein Breakdown	WG	11.68 [10.33, 13.22]	10.66 [9.76, 11.63]	8.7 [7.64, 9.91]	0.553	0.008	0.032
	M	12.06 [10.27, 14.15]	12.49 [11.12, 14.02]	9.69 [7.55, 12.43]	0.979	0.368	0.149
	F	11.67 [9.64, 14.12]	9.22 [8.11, 10.49]	7.76 [6.7, 8.99]	0.157	0.011	0.257
Values are estimated means [95% CI]. Statistics (JASP) are done with Generalized Linear Model (Family: Gamma, Link: Log) to examine the effect of frailty group with covariates age and lean soft tissue (and sex for the whole group analysis). Multiple comparisons for group differences were analyzed using Tukey post hoc. Bold is p < 0.05. Abbreviations: WG, whole group; M, males; F, females							

Extracellular and intracellular pools of amino acids

The extracellular pool size of amino acids (**Supplemental Table 5**) represents the plasma concentration, except when the volume of the extracellular pool is changed. We observed a lower extracellular pool size of hydroxyproline ($P = 0.019$) and valine ($P = 0.003$) in the pre-frail older adults compared with the non-frail. Glutamate extracellular pool sizes were larger in the frail group compared with the non-frail group ($P = 0.004$), with glutamate extracellular pool size higher compared with the pre-frail as well ($P = 0.02$). The extracellular pool size of tau-methylhistidine was higher in the pre-frail ($P = 0.002$) and frail ($P < 0.001$) compared with the non-frail, whereas that of glutamine was higher in the frail compared with the pre-frail ($P = 0.007$).

The intracellular pool size of glutamate was larger in the frail compared with the pre-frail ($P = 0.028$), while that of methionine ($P = 0.002$) and phenylalanine ($P < 0.05$) was smaller in frail older adults as compared with the non- and pre-frail older adults (**Supplemental Table 5**). Histidine intracellular pool size was lower in the frail compared with the non-frail ($P = 0.042$), while that of valine showed a lower value in the pre-frail compared with the non-frail ($P = 0.01$).

Associations between absolute frailty score and protein metabolism markers

Whole group analysis, including both males and females, showed a negative association between the numerical frailty score and intracellular whole-body protein breakdown (PB) ($r: -0.131$, trend $P = 0.082$) and intracellular net protein breakdown (PB) ($r: -0.168$, $P = 0.022$) (**Supplemental Fig. 1**), but a positive association between the numerical frailty score and intracellular myofibrillar protein breakdown ($r: 0.161$, $P = 0.034$). In male subjects, we observed a trend toward significance in the association between a higher frailty score and a greater intracellular myofibrillar protein breakdown ($r: 0.215$, $P = 0.061$) (Fig. 3). In female subjects, a higher frailty score was associated with lower intracellular whole-body ($r: -0.27$, $P = 0.006$) and net protein breakdown ($r: -0.29$, $P = 0.004$). No association was found between myofibrillar protein breakdown and the frailty score in females and between whole-body/net protein breakdown and the frailty score in males.

Whole group analysis showed a positive correlation of intracellular protein breakdown with daily protein intake ($r: 0.316$, $P < 0.001$) and daily protein intake per body weight ($r: 0.288$, $P < 0.001$), and a trend toward positive correlation between intracellular net protein breakdown and 6MWT distance ($r: 0.143$, $P = 0.06$) (**Supplemental Fig. 2**). Our sub-analysis on sex-specific data showed that intracellular net protein breakdown was positively associated with daily protein intake per body weight ($r: 0.251$, $P = 0.04$) (Fig. 4), and no association between intracellular net protein breakdown and 6MWT distance was observed in males. In females, intracellular net protein breakdown was positively associated with 6MWT distance ($r: 0.25$, $P = 0.016$), and daily protein intake per body weight ($r: 0.288$, $P = 0.011$). Age showed a negative association with net protein breakdown in both males ($r: -0.491$, $P < 0.001$) and females ($r: -0.321$, $P = 0.001$).

A negative correlation between frailty score and hip BMD was observed in the whole-group analysis ($r: -0.256$, $P < 0.001$) and in females ($r: -0.309$, $P = 0.001$), whereas no association was found between hip BMD and frailty score in males (Fig. 5). We found a positive association between lean soft tissue and hip

BMD ($r: 0.293, P < 0.001$), and between physical activity and hip BMD ($r: 0.293, P < 0.001$) (**Fig. 5D, 5E**) in whole group analysis. A positive correlation between physical activity level and hip BMD was observed in females ($r: 0.296, P = 0.002$) (**Fig. 5F**) but not in males (graph not shown).

Discussion

Using the Fried frailty phenotype, we studied 208 older adults at different stages of frailty to unravel disruptions in protein and amino acid metabolism that may underlie poor muscle and bone health, and to examine the role of biological sex. Our study showed that the trajectory of frailty in older adults on the group level was characterized by early functional decline (independent of sex) and disturbances in protein metabolism, featuring lower whole-body and net protein breakdown (particularly in females) and increased myofibrillar protein breakdown (particularly in males), while lean mass was preserved in males but reduced in females. Frailty in males was therefore characterized by muscle weakness, possibly due to a more muscle-specific (myofibrillar) catabolic phenotype despite muscle mass preservation, while poor muscle health (weakness and loss) in females was associated with a reduction in (net) whole-body protein metabolism. These findings indicate that sex differences exist in the metabolic trajectories underlying early functional decline in older adults, suggesting the need for early sex specific therapies to reduce the risk of frailty progression.

General characteristics of studied population and bone health

There have been several definitions and criteria of frailty proposed in the literature^{32–35}. Although different clinical instruments have been developed to measure the degree of frailty, two approaches, “frailty phenotype”³² and “frailty index”³⁴, have predominated. Consistent with previous literature identifying advanced age and female sex as primary risk factors for frailty¹³, subjects in our (pre) frail cohorts showed a slightly higher age than the non-frail, and a notable female predominance (78%) in the frail group. While BMI was lower in the frail, significance disappeared after adjusting for age and biological sex. Physical inactivity and muscle weakness were observed in the pre-frail and frail stages in line with the observed prevalences of the Fried frailty phenotype domains of low physical activity (19.6% in pre-frail and 73.5% in frail) and low handgrip strength (weakness) (35.9% in pre-frail and 85.3% in frail) (Table 1). Phenotypic patterns shifted from predominantly muscle/functional-related impairments, such as the combination of weakness and slow gait in the pre-frail stage, toward more systemic manifestations, including exhaustion and unintended weight loss or low BMI, in the frail stage. These observations suggest a trajectory in which functional decline occurs before the onset of systemic symptoms and significant body composition changes. Future longitudinal investigations are needed to confirm this finding.

Previous studies have reported an association between frailty and increased prevalence of osteoporosis and reduced bone density in older populations^{36,37}. We found that bone mineral density at the hip and femoral neck was lower in frail older adults compared to non-frail and pre-frail subjects, independent of

age and sex, and the decline was more pronounced in females (Table 2). Furthermore, the absolute frailty score was negatively correlated with hip bone mineral density (Fig. 5A). In sex-stratified sub-analyses, this association remained significant only among female participants (Fig. 5B, C), indicating that the bone loss associated with frailty severity may be female-specific. The observed decline in bone mineral density was also linked to reduced low muscle mass in both sexes and physical activity (Fig. 5D, 5E), consistent with previous studies^{38,39}. This positive relationship between physical activity levels/muscle health and bone health emphasizes the critical role of physical engagement and suggests that resistance-based exercise interventions may mitigate frailty-related bone deterioration⁴⁰. The relationship between low physical activity and low hip BMD observed only in females indicates that female older adults are more vulnerable to disuse-induced bone loss and highlights the importance of exercise in females to mitigate frailty and disuse-associated bone loss. Prospective exercise rehabilitation trials focusing on bone health outcomes are warranted to validate these therapeutic strategies across the frailty trajectory.

Body composition and muscle health changes in relation to frailty severity

We found greater adiposity in the pre-frail than in the non-frail participants, yet no differences in fat mass were observed in the frail groups. Previous literature investigating the association between body mass index or adiposity and frailty showed conflicting results, as some found a higher frailty incidence and risk of frailty in the overweight/obese^{41–43} and those with higher fat mass⁴⁴, whereas others found a higher risk in those with lower BMI⁴⁵, also called *the obesity paradox*. In our study, the highest prevalence of the individual frailty domains within the pre-frail group was, besides muscle weakness and low physical activity, slow gait speed (58.7%). No differences in disease characteristics and other medical features were observed except for a higher prevalence of chronic pulmonary disease, commonly associated with being overweight^{46,47}. While other studies also found increased visceral adipose tissue in individuals with pre-frailty⁴⁸, this was not present in our pre-frail subjects.

The prevalence of sarcopenia among the non-, pre-, and frail groups ranges from 40% to 72% based on the specific diagnostic criteria applied⁴⁹. Our results indicated a reduction in lean soft tissue and appendicular mass in frail individuals when compared with the pre-frail, likely influenced by the higher incidence of overweight and obesity within the pre-frail cohort. Interestingly, no marked differences in lean mass were detected between the frail and non-frail groups, which may be attributable to the baseline characteristics of our study participants, who were generally overweight, with BMIs between 26 and 29 kg/m². Although multiple clinical studies report a consistent relationship between low lean mass and frailty^{50–52}, this link remains inconsistent across different cohorts⁵¹, indicating that while closely linked, sarcopenia is not a determining marker of frailty^{51,53}.

Our sex-specific analysis showed that alterations in body composition (i.e., lower body weight, BMI, fat mass, and lean mass) were observed only in the female frail cohort (Table 2). Research examining sex-

specific changes in body composition during frailty remains inconsistent. Some investigations found that increased waist circumference was associated with frailty only in males⁵⁴, or that higher adiposity correlated with frailty status exclusively in men⁵⁵. Conversely, other studies have reported that frail women exhibit higher BMIs and waist circumferences, whereas frailty in men is characterized by BMI reduction and muscle loss⁵⁶. Further longitudinal research is required to elucidate these sex-specific phenotypic trajectories in body composition with frailty, as well as the role of co-existing comorbidities as an independent contributing factor to poor muscle health in the frailty trajectory.

Both male and female frail older adults showed poor muscle function (i.e., lower inspiratory pressure and handgrip strength) and 6MWT distance, except that decline in leg muscle strength with advanced frailty was present in males but not females. Though lower leg muscle strength is not one of the criteria for the Fried frailty phenotype, lower leg strength has been associated with frailty by both the chair stand test and knee extension^{57,58}. Previous studies found sex-specific muscle function and intramuscular changes^{59,60}. The association between muscle weakness and smaller diameter of type II myofibers was observed in male older adults, while muscle weakness in female older adults was associated with intramuscular inflammation⁶⁰. In addition, greater age-associated decline in muscle function was observed in male COPD patients compared with female counterparts⁴⁶. Males also have a greater distribution of type II fibers than women^{61,62}, which is known to decrease more with the aging process than type I fibers⁵⁹. This might contribute to a greater decrease in lower-limb muscle strength with frailty progression observed in males but not females. Our data suggest that pre-frailty and frailty were associated with functional decline without changes in body weight, BMI, and/or body composition, especially in males. Our data also indicate that women are more resistant to frailty-associated decline in leg muscle strength but not muscle loss compared to men. Muscle mass and strength are related but not always linearly associated⁶³. As maintaining muscle function is more important than muscle mass due to its effect on physical performance and disability⁶⁴, a resistance exercise-based regimen might be recommended for older adults at risk of frailty.

Increased myofibrillar protein breakdown in (male) frail subjects

Plasma concentration and whole-body production of tau-methylhistidine were increased in the frail in line with the association observed between absolute frailty score and higher myofibrillar protein breakdown (**Supplemental Fig. 1**). Since over 90% of tau-methylhistidine is found in skeletal muscle, it serves as a reliable marker for myofibrillar protein breakdown, being released during muscle degradation but not reutilized or further metabolized^{65,66}. Similar patterns of downregulated protein turnover with increased myofibrillar breakdown have been seen in older adults over 70 years²⁷, while elevated whole-body production and plasma concentration of tau-methylhistidine also occur in older or frail adults^{27,67,68} and cancer patients⁶⁶. Moreover, we observed that frailty, particularly in males, was associated with increased myofibrillar protein breakdown (trend, $P = 0.06$) (Fig. 3B). This association between absolute frailty score and intracellular production of tau-methylhistidine (myofibrillar protein breakdown) was not

present in females (Fig. 3E). These observations align with our previous findings in COPD patients, where male subjects showed increased myofibrillar protein breakdown, which was absent in female subjects³¹. This sex-specific increase in myofibrillar protein breakdown may contribute to a greater frailty-associated decline in muscle function observed in males in our study. As mentioned above, men have a greater distribution of type II fibers than women^{61,62}, which are lost more with aging than the type I fibers, which may contribute to sex-specific myofibrillar protein catabolism. In addition, low testosterone has been associated with increased risk of frailty mainly due to its association with worse physical function (e.g., handgrip strength and gait speed)⁶⁹. Testosterone declines with aging and frailty, and regulates skeletal muscle protein metabolism partly through muscle-specific ubiquitin ligases, including MuRF1 and atrogin-1^{70,71}, indicating that possibly low testosterone in male frail subjects might contribute to the sex-specific myofibrillar protein breakdown observed in males.

Downregulated whole-body protein metabolism, particularly in frail females

Although plasma amino acid concentrations remained unchanged in the pre-frail stage, individuals with frailty showed lower levels of several amino acids, including glutamate, valine, leucine, and phenylalanine, as well as total branched-chain and essential amino acids. These findings are consistent with previous reports of lower plasma essential amino acid levels in older adults with frailty^{20,21}. However, untargeted metabolomic studies investigating circulating biomarkers have provided inconsistent results. While some research identified elevated phenylalanine, glutamate, and glutamine in frail cohorts^{23,24,72}, other studies observed no discernible differences²². Such discrepancies might be due to differences in covariate adjustments, as those studies often did not account for age and body composition (e.g., BMI or lean mass), or population characteristics among studies. In line with a lower plasma amino acid profile, we observed an overall downregulated protein metabolism with frailty, characterized by lower intracellular production of phenylalanine and tyrosine, and net protein breakdown, particularly in females (Table 4, **Fig. 3D, 3F**). Being female and frail were associated with a decline in whole-body protein breakdown and net protein breakdown, while no reduction in whole-body and net protein breakdown was found in males (Fig. 4). These findings indicate that frailty in older adults is characterized by a sex specific reduction in whole-body protein metabolism in females.

Daily dietary intake was comparable between the frailty groups, except for the lower protein intake relative to body weight in the female pre-frail group. Furthermore, habitual physical activity was reduced in frail older adults in both males and females (Table 2), indicating that physical inactivity as well as poor dietary intake do not fully explain the suppressed protein turnover observed in frail females.

Age- and frailty-related impairment in proteostasis, particularly reduced autophagy pathways and altered proteasome function, has been implicated in the accumulation of damaged organelles and misfolded proteins, contributing to cellular dysfunction^{73,74}. Whether the lower whole-body protein breakdown observed in frail females is a compensatory/adaptive mechanism to conserve existing protein/amino

acid stores and energy (ATP) in the body, or reflective of the impaired capacity to maintain protein turnover, warrants further study.

Net protein breakdown: a biomarker of functional outcomes?

The reduced net protein breakdown observed in the frail group has also been observed by us in other (clinical) conditions, including aging²⁷, COPD^{28,31}, and critical illness⁷⁵. We previously observed a strong association between lower net protein breakdown and higher blood pressure and systemic inflammation, as well as lower protein intake, survival, handgrip strength, and 6MWT distance walked in both healthy and COPD patients²⁸. In our previous study of 575 older adults, lower net protein breakdown was related to worse muscle function²⁷. Our current study also showed that lower net protein breakdown was associated with worse 6MWT distance, especially in females, independent of age and lean mass (Fig. 4), and the association with lower levels of protein intake was observed in both males and females. Our findings therefore suggest that greater daily dietary protein intake and physical activity/exercise rehabilitation may help maintain net protein breakdown, functional capacity and muscle health, especially in frail females.

Given the consistent positive association between net protein breakdown and muscle health and 6MWT distance, and that 6MWT is a well-established and validated predictor of clinical outcomes in older adults^{27,76,77}, our findings suggest that net protein breakdown may be considered a promising biomarker of functional outcomes. Future longitudinal studies are warranted to evaluate whether the decreased net protein breakdown independently predicts adverse functional performance and whether interventions that preserve net protein breakdown indeed are able to improve/restore muscle health in older adults. More recently, net protein breakdown measured after an overnight fast was shown to provide an accurate estimate of the total net protein lost over 24 hours⁷⁸, as indicated by our and others' previous feeding studies⁷⁹⁻⁸¹. This suggests that net protein breakdown might also be seen as a marker of protein requirement for maintenance, but more research is needed to confirm this.

Altered exercise capacity in the prefrail and BCAA and glutamate metabolism in female pre-frailty

We observed lower values for handgrip strength and 6MWT distance in the pre-frail on the whole group level. However, sex-specific analysis showed trends or no significant difference in the pre-frail in males and females, likely due to the small number of subjects in each group, resulting in higher variability. Lower 6MWT distance has also been observed in other studies in pre-frail subjects^{82,83}, indicating that functional decline is already present and detectable at an early stage of frailty. This emphasizes pre-frailty as a potentially important stage for early identification and intervention.

Furthermore, lower WBP of hydroxyproline, leucine, and valine, and intracellular production of glutamate, leucine, and valine were observed in the pre-frail group. While WBP and intracellular production of valine were reduced in both male and female pre-frail, the decrease in intracellular glutamate production (trend, $P = 0.065$) and glutamate concentration in pre-frail and frail groups, respectively, were present in females only. Production of glutamate is facilitated by branched-chain amino acid (BCAA) catabolism via transamination by BCAT. Despite no observed changes in kinetics in isoleucine or leucine among males, female pre-frail subjects showed overall downregulated BCAA metabolism, as shown by lower whole-body production. These findings suggest that the impaired intracellular glutamate production (trend, $P = 0.065$) in female pre-frail subjects may result from disrupted BCAA metabolism. The mechanism underlying the decline in valine kinetics, independent of other BCAAs in pre-frail males, requires further investigation into specific enzymatic or transporter activities.

Potential clinical significance of findings and future guidelines

The observed muscle-specific catabolic phenotype in frail males and suppressed whole-body protein metabolism suggest that sex-specific differences in nutritional treatment need to be considered when treating older adults at risk of and/or with frailty. For female older adults, adequate high quality protein intake combined with weight-bearing exercise may help increase protein turnover and muscle/bone gain⁴⁰. Furthermore, nutritional strategies supporting bone health, such as adequate calcium and vitamin D intake, are recommended^{84,85}. In contrast, for older male adults, strategies targeting lowering inflammation, such as ω -3 fatty acids, curcumin, and Vitamin D, might be recommended to suppress the accelerated myofibrillar protein breakdown observed in higher frailty severity. Furthermore, increased chronic inflammation, especially elevated IL-6, has been shown to hinder anabolic potential and increase muscle protein degradation^{86,87}, probably due to upregulated catabolic signals^{88,89}. Interestingly, frailty-associated decline in leg muscle strength was seen in males only, although no lean mass change was observed. Therefore, resistance exercise regimen focusing on lower-limb strengthening, such as sit-to-stands and/or chair squats, might improve leg muscle strength and prevent functional declines in (pre) frail males⁹⁰.

Limitations

Several limitations should be considered when interpreting our findings. While we had three study groups, the pre-frail subset was heterogeneous with respect to the Fried frailty domains, which increased variability⁹¹ and reduced the potential to identify a specific phenotype. Although frailty is known to be predominantly present in females, the 77% of females in the frail group resulted in a low absolute number of frail males, which reduced our statistical power to find sex-specific differences (and thus phenotypes), particularly in frail males. Although we mentioned that elevated inflammation might contribute to disturbances in protein metabolism markers, we did not measure inflammation markers such as IL-6 and CRP in this study. Future studies should investigate the relationship between the basal inflammation markers and protein metabolism alterations. Finally, our study was a cross-sectional study.

Future longitudinal follow-up of older adults with different stages of frailty is therefore needed to confirm our findings, provide more causal relationships, and understand underlying mechanisms.

Conclusion

Our findings suggest that frailty in older adults is associated with early functional decline and disturbance in protein metabolism, including lower whole-body and net protein breakdown and increased myofibrillar protein breakdown. Importantly, frailty in males was characterized by a muscle-specific catabolic phenotype and decline in lower limb strength, while that in females was associated with a reduction in whole-body and net protein breakdown and BCAA metabolism, and muscle and bone loss, indicating that the trajectory of amino acid metabolism and muscle/bone health in frailty is sex-specific. Furthermore, net protein breakdown was associated with functional status, suggesting the possibility of net protein as a promising biomarker. Our study highlights the importance of sex-specific and personalized approaches for future nutritional and therapeutic interventions for frailty when treating (pre) frail older adults.

Methods

Study design and population

We conducted a cross-sectional study using baseline measurements of 13 trials between 2022 and 2025 (NCT05240885, NCT05395390, NCT06628349, NCT01734473, NCT01787682, NCT01871350, NCT01624792, NCT02082418, NCT06801964, NCT03327181, NCT03796455, NCT04461236, NCT04459156). Participants were included using “age > 60y” and “the availability of all 5 domains for frailty phenotype assessment”³² as primary selectors and “the availability of body composition and amino acid kinetic data” as secondary selectors. This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines⁹². Participants were stratified into “non-frail”, “pre-frail”, and “frail” groups based on their frailty phenotype scores³², obtaining 48 non-frail, 92 pre-frail, and 68 frail older adults (**Figure S1**). Participants with untreated metabolic and renal diseases, active malignancy, and the use of oral steroids in the past month were not eligible to participate in the studies. Participants were recruited via mass emails and advertisements in senior resource magazines, newspapers, and radio in the College Station/Bryan, Texas, area. We also recontacted previous participants of the Center for Translational Research in Aging and Longevity (CTRAL), College Station, Texas. We obtained written informed consent from all participants after the study was explained. All studies were approved by the Institutional Review Board of Texas A&M University. The study consisted of 2 visits. During the first visit, participants arrived in the morning after an overnight fast, and body composition assessment and blood samples for metabolic markers were collected. During the second visit, typically conducted within a week of the first visit, muscle strength measurements were completed.

Assessment of frailty

Frailty status was determined by five criteria adapted from the Fried Frailty Phenotype³². Frailty was defined as having at least three of the following five domains; (a) weakness, determined by maximum handgrip strength; (b) slowness, assessed via a 4-meter gait speed test; (c) low physical activity levels, measured using the Physical Activity Scale for the Elderly (PASE); (d) exhaustion, evaluated through specific Center for Epidemiological Studies–Depression (CES-D) scale questions; and (e) low BMI and/or significant annual weight loss. Participants with one or two of the five domains were defined as pre-frail, and participants with none of the five domains were defined as non-frail. Cut-off points for weakness, slowness, low physical activity, and low BMI were defined by the lowest quintile derived from our previous large-scale cross-sectional study of 575 older adults in which sex-specific adjustments were applied to the cut-offs for weakness, slowness, and physical activity²⁷. To assess self-reported exhaustion, participants answered questions: “Have you felt that everything has been an effort in the last month?” “Is it hard to get going?” and “Have you felt unusually weak in the past month?” Finally, the low BMI and/or weight loss domain was satisfied if a participant exhibited a BMI < 23 kg/m² or reported unintentional weight loss exceeding 5% within the past six months.

Lifestyle, anthropometrics, body composition, and muscle function

Habitual physical activity and dietary intake were assessed by a 3-day dietary recall and Physical Activity Scale for the Elderly (PASE) questionnaire, respectively. Charlson Comorbidity Index (CCI) was used to assess the prevalence of comorbidities in our participants. Body mass index (BMI) was calculated using body weight and height measured by a digital beam scale and stadiometer, respectively. We measured body composition by dual-energy X-ray absorptiometry (DXA) (Hologic ADR 4500/Version 12.7.3.1 [Bedford, MA]). Maximal handgrip strength and peak leg torque extension were assessed via a handgrip dynamometer (Vernier dynamometry; Vernier Software and Technology [Beaverton, OR]) and lower limb muscle strength by maximal single-leg extension force using isokinetic dynamometry (Kin-Com; Isokinetic International, Chattanooga [TN, USA]), respectively. Exercise capacity was assessed using total distance covered during the 6-minute walk test (6MWT)⁹³.

Assessment of amino acid kinetics using stable isotope IV pulse

Participants were asked to maintain their habitual diet and refrain from strenuous exercise at least 48 hours before the study day. Participants arrived in the morning after an overnight fast. A research nurse placed a peripheral line into a dorsal hand vein or a lower arm vein. A hand was placed in a controlled hot box with an internal temperature of 60°C to simulate arteriovenous blood sampling⁹⁴. Following the baseline blood sampling for background enrichment, a pulse containing multiple amino acid isotopes was administered (**Supplemental Table 1**), and subsequent blood sampling was done at times (t = 10, 20, 30, 60, and 120 min)^{27,67}. We immediately stored samples on ice to prevent enzymatic reactions and centrifuged to yield plasma. Part of the plasma was pipetted into tubes that contained 0.1 vol of 33% (w/w) trichloroacetic acid and vortexed.

Biochemical analysis and calculations of metabolic parameters

We stored samples at -80°C until analysis of tracer enrichments (tracer:tracee ratio [TTR]) and amino acid concentrations by LC-MS/MS using double-isotope dilution and isotope-dilution methods, respectively⁹⁵. All TTRs were corrected for the background TTR measured in baseline plasma samples before the pulse administration. Following sample analysis by LC-MS/MS, the TTR decay at each time point was fitted with a 2-exponential model with the formula ($TTR = a \cdot \exp(-k_1 \cdot t) + b \cdot \exp(-k_2 \cdot t)$) using a nonlinear regression model in GraphPad Prism (v11). We used the fitted parameters obtained with the robust fitting option: a, b, k_1 , and k_2 , to calculate the predicted whole body production (WBP) and parameters of compartmental analysis. All formulas for compartmental calculations can be found in **Supplemental Table 2**.

Compartmental analysis of the tracer enrichment data was performed to calculate the metabolic parameters, including intracellular production and the extracellular (Q1) and intracellular pool sizes (Q2), using a 2-pool model. The conversion of phenylalanine to tyrosine equals net protein breakdown. It is measured using the WBP or intracellular production of the product amino acid multiplied by the estimated ratio between the product's TTR and substrate's TTR⁹⁶. This compartmental analysis calculation and pulse tracer method modeling were based on the books^{97,98} and validated in our publications⁹⁶.

Statistical analysis

Data are expressed as estimated means [95% CI], except for subject and disease characteristics and medical features in Table 1, which are expressed as means (SD). We performed statistical analysis using a generalized linear model (GLM) to examine the effect of frailty groups on main outcomes (i.e., compartmental amino acid metabolism, body composition, and muscle function), adjusting for age and sex as confounders; metabolic data were additionally corrected for lean soft tissue in GLM. We further performed linear regression analyses to investigate i) the association between protein metabolism markers (dependent variable) and frailty score (0–5); ii) the association between intracellular markers (dependent variable) and muscle health markers and protein intake, adjusted for age, sex, and lean tissue, and iii) the association between hip bone mineral density (dependent variable) and frailty score (0–5), lean mass and physical activity level. For our secondary analysis, the GLM and linear regression models were performed separately by sex, adjusting only for age (and lean soft tissue for metabolic markers). All statistical analyses were performed using JASP v0.98.1⁹⁹ (Amsterdam, NL). We used GraphPad Prism (v11) to visualize data. The significance level was set at $p < 0.05$.

Declarations

Competing Interests

The authors have no conflict of interest to declare.

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Author Contribution

All authors had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. NEPD, LER, and MPKJE designed the study concept. MCK and LER were involved in the conduct of the study. MCK performed the statistical analysis and data and sample analysis. MCK and MPKJE wrote the initial version of the manuscript. MCK, NEPD, and MPKJE reviewed the first draft and edited the final text. All authors participated in the writing, reading, and editing, and approved the final version of the manuscript submitted for publication.

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Data availability

Data described in the manuscript, codebook, and analytic code will be made available upon request, pending approval of the principal investigator (mpkj.engelen@ctr.al.org).

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Figures

Figure 1. Group differences in muscle health and exercise capacity in the whole group, males, and females

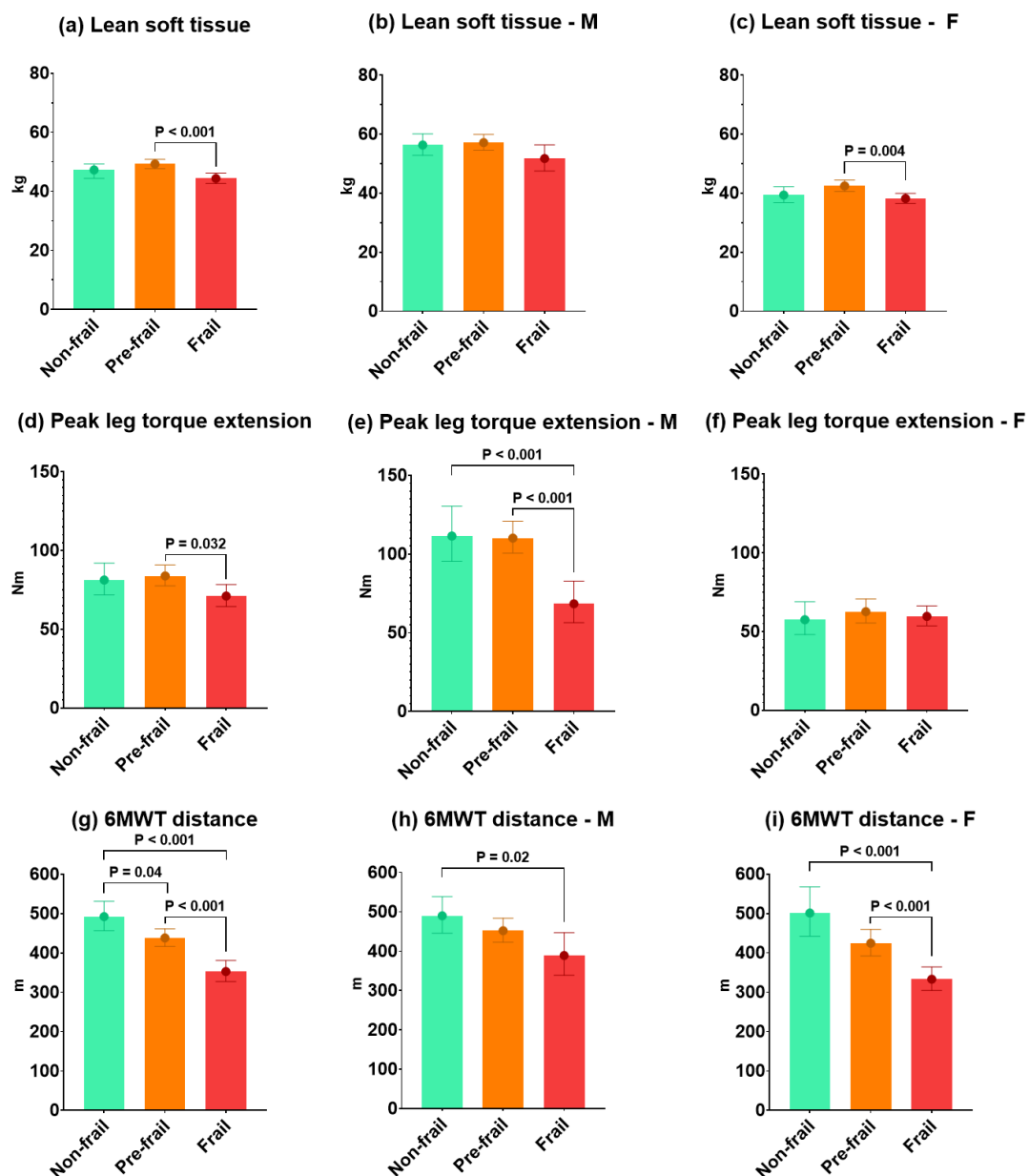


Figure 1

Group differences in muscle health and exercise capacity in the whole group, males, and females; peak leg torque extension (a), 6MWT distance (d), and lean soft tissue (g) in whole group, and peak leg torque extension (b), 6MWT distance (e), and lean soft tissue (h) in males, and peak leg torque extension (c), 6MWT distance (f), and lean soft tissue (i) in females. Abbreviations: 6MWT, 6-minute walk test

Figure 2. Intracellular production of amino acids in the whole group, males, and females in different stages of frailty

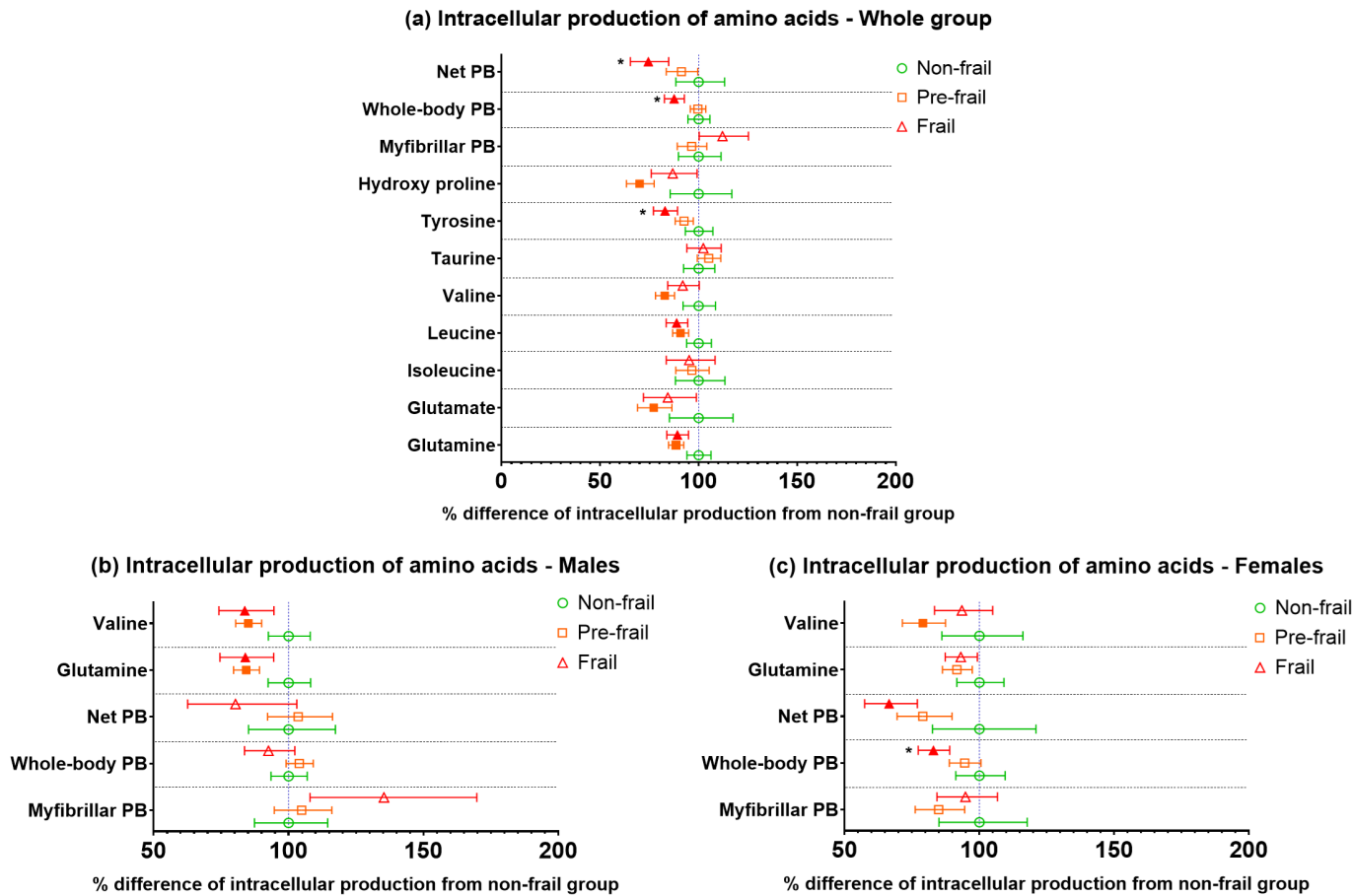


Figure 2

% difference in intracellular production of amino acids from the non-frail group in the whole group (a), males (b), and females (c). Net PB: phenylalanine hydroxylation to tyrosine; whole-body PB: intracellular production of phenylalanine; myofibrillar PB: intracellular production of tau-methylhistidine. Abbreviations: PB, protein breakdown

Figure 3. Association between absolute frailty score and protein metabolism markers in males and females

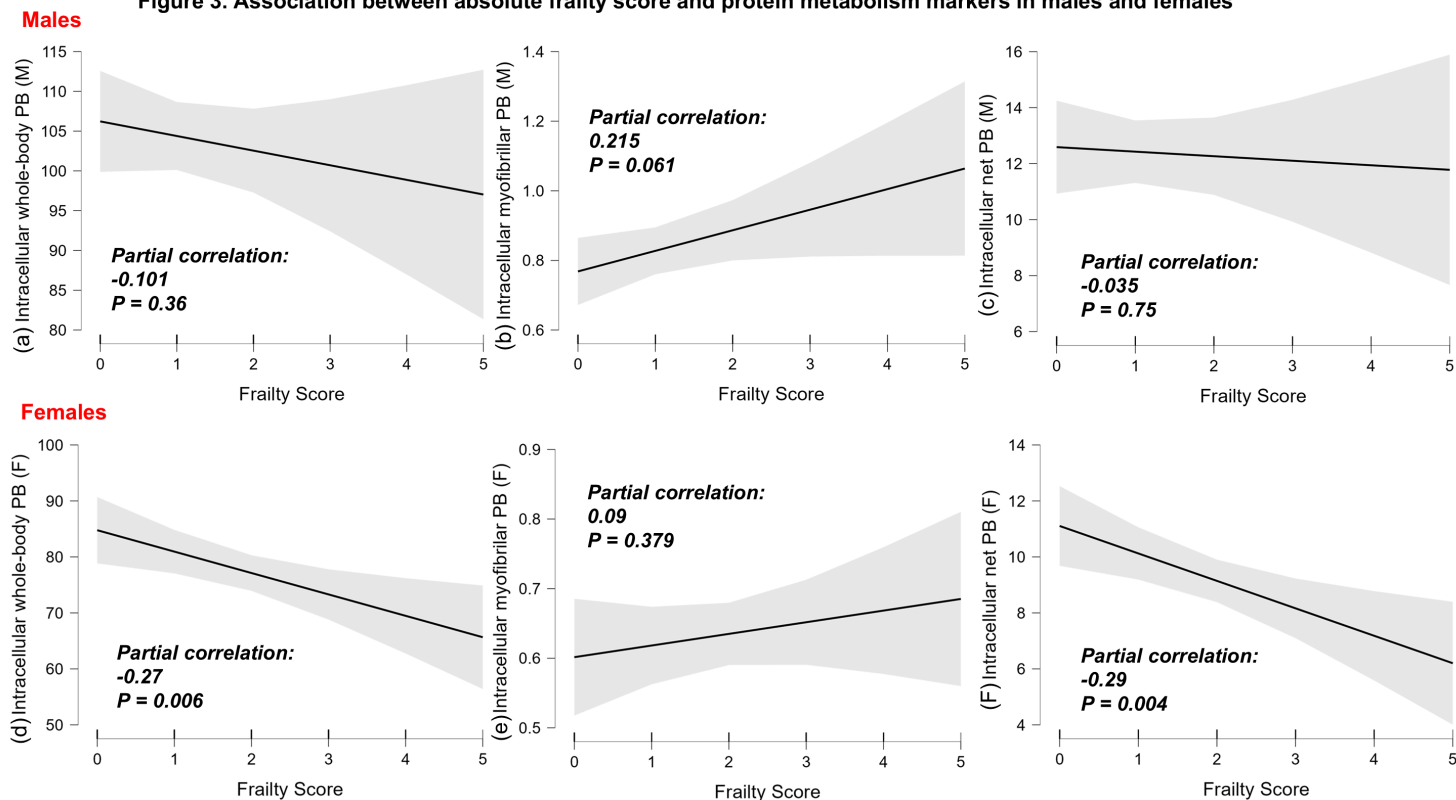


Figure 3

Association between absolute frailty score and protein metabolism markers in males and females. The y-axis shows adjusted intracellular whole-body protein breakdown (PB), myofibrillar protein breakdown (PB), and net protein breakdown (PB) for age and lean soft tissue. Net PB: phenylalanine hydroxylation to tyrosine; whole-body PB: intracellular production of phenylalanine; myofibrillar PB: intracellular production of tau-methylhistidine. Abbreviations: PB, protein breakdown

Figure 4. Association between net protein breakdown and 6MWT distance, protein intake, and age in males and females

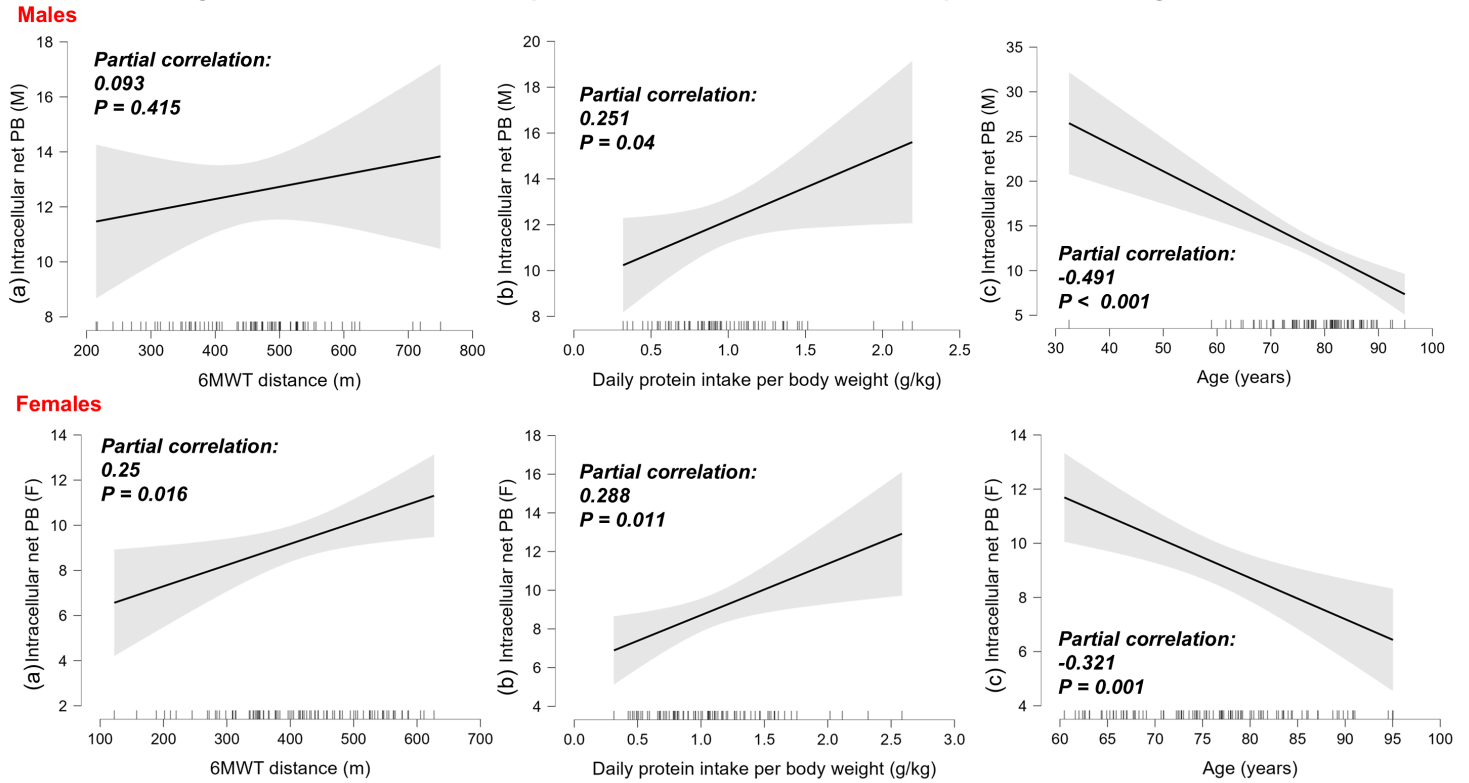


Figure 4

Association between intracellular net protein breakdown and 6MWT distance, protein intake, and age in males and females. The y-axis shows adjusted intracellular net protein breakdown for age and lean soft tissue. Net PB: phenylalanine hydroxylation to tyrosine. Abbreviations: 6MWT, 6-minute walk test

Fig 5. Association between bone mineral density and absolute frailty score in the whole group and males and females, and lean soft tissue and physical activity level in the whole group and females

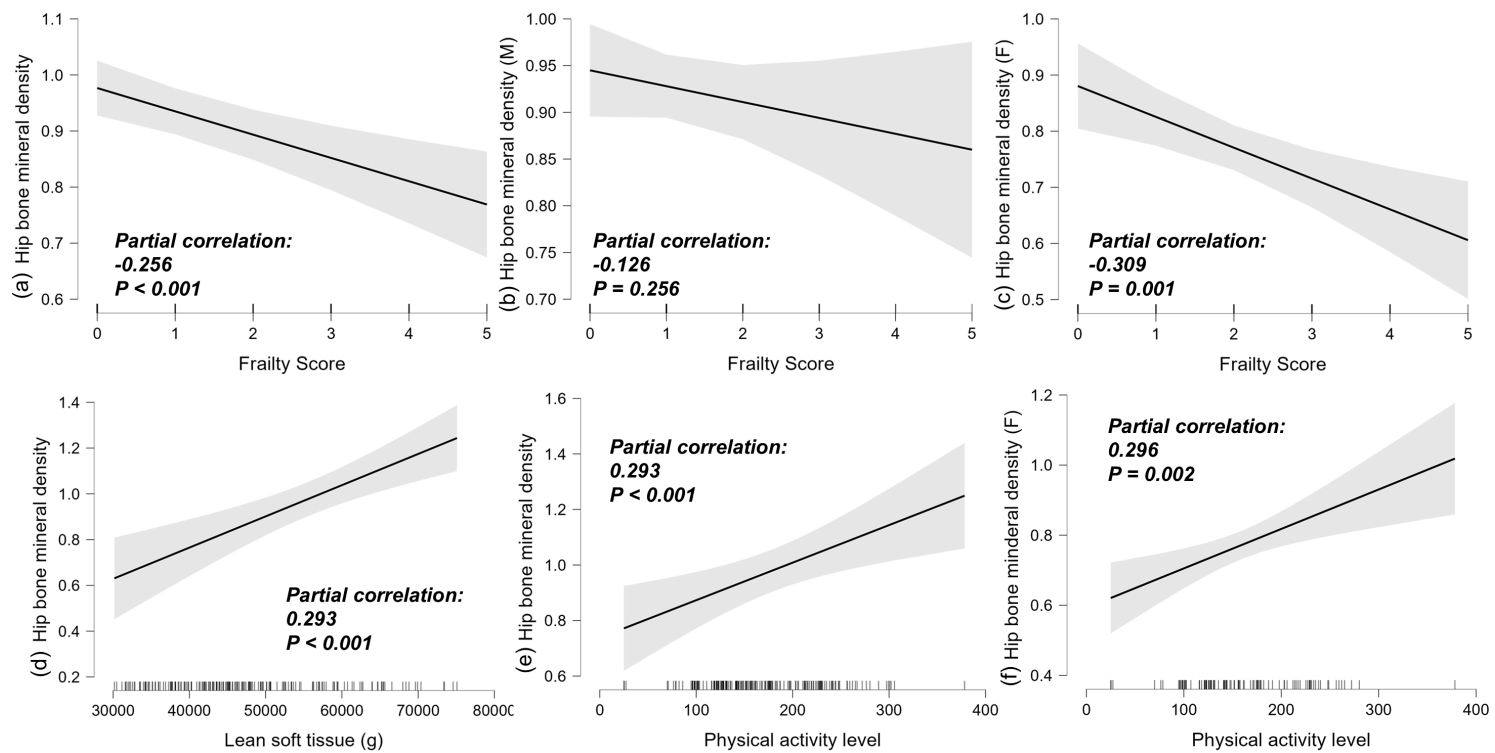


Figure 5

Association between bone mineral density and absolute frailty score in the whole group (a), and males (b), and females (c), and lean soft tissue (d), and physical activity level (e) in the whole group and females (f). The y-axis shows adjusted hip bone mineral density values for age and sex (a,d,e), and age only (b,c,f).

Supplementary Files

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