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Molecular and physiological aspects of sarcopenia in the older person: Mechanisms, diagnostics and therapy

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Molecular and physiological aspects of sarcopenia in the older person: Mechanisms, diagnostics and therapy

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Editorial: Molecular and physiological aspects of sarcopenia in the older person: mechanisms, diagnostics and therapy

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sarcopenia, geriatric research, inflammaging, muscle mass assessment, aging and muscle health, muscle atrophy mechanisms

Editorial on the Research Topic
Molecular and physiological aspects of sarcopenia in the older person: mechanisms, diagnostics and therapy

In the first article of our exploration of the most recent research findings in the Molecular and Physiological Aspects of Sarcopenia in the Older Person RT, we highlight a significant connection between sarcopenia and serum Follicle-Stimulating Hormone (Ke et al.). As we delve into the domain of sarcopenia-related research, our attention is drawn to a study that examines the use of Xanthine Oxidoreductase (XOR) inhibitors and their potential impact on sarcopenia among patients undergoing hemodialysis (HD). Sarcopenia is a prevalent concern in this population, and this second study delves into the role of XOR inhibitors in addressing this condition (Kurajoh et al.). Indeed, there is a concerted effort to identify novel biochemical markers associated with sarcopenic conditions (1), as early detection, facilitated by precise diagnoses, can enhance the efficacy of comprehensive treatment approaches (2).

In the current quest to uncover the complexities of sarcopenia, we turn our attention to a groundbreaking study that delves into the underlying mechanisms and immunological profiles related to this condition (Abdelrahman et al.). Continuing our exploration, we shift our focus to a captivating study conducted in the agricultural and pastoral regions of China. This research unraveling the relationship between plasma tumor necrosis factor-α, and their association with sarcopenia among community-dwelling older adults (Wumaer et al.). The inspection of the intricate relationship between aging, physical activity (PA), and inflammation continues with a study focusing on inflammaging in physically active older women. This research delves into how inflammaging relates to body composition and physical factors, shedding light on this complex interplay (Santos et al.). In detail, these lastthree studies serve as an invitation for readers to scrutinizing the molecular mechanisms that underpin the connection between inflammation and muscle health (3), as well as exploring potential interventions aimed at ameliorating the impact of inflammation on sarcopenia (4).

Regular PA and exercise are cornerstones of healthy aging, especially for those in long-term care facilities. In our pursuit of advancing the health and wellbeing of older individuals, we now turn our attention to a promising approach: exergame-based exercise. This innovative method has the potential to address muscle loss, muscle strength, cognition, and functional performance among older adults residing in rural long-term care facilities (Tuan et al.). Screening and early detection of sarcopenia is essential for timely intervention and improved outcomes. In this study, we delve into the prospective relationship between physical performance tests and the risk of developing sarcopenia in individuals aged 55 and over in Malaysia. The research focuses on determining suitable cut-off values for screening activities and highlights the importance of early detection (Megasari et al.).

In fact, the early diagnosis of sarcopenia among community-dwelling older individuals is of paramount importance (2). Through timely identification, healthcare providers can implement multimodal interventions that encompass dietary re-education, targeted supplementation, and structured exercise regimens (4, 5). This "gold standard" approach not only addresses the progression of sarcopenia, and reduce the risk of adverse outcomes such as hospitalization, institutionalization, and premature mortality (6–8).

Regarding to unlock the secrets of longevity and vitality in aging, we arrive at a crucial study that explores the remarkable effects of caloric restriction (CR) on age-related muscle atrophy. Sarcopenia has been a pressing concern in geriatric research, and understanding the mechanisms behind CR's influence is of paramount importance (Lv et al.). In the continuing exploration of sarcopenia and its clinical implications, we shift our focus to a novel and cost-effective tool for assessing muscle mass and its prognostic value in hospitalized patients. This meta-analysis delves into the relationship between the creatinine/cystatin C ratio and sarcopenia, providing valuable insights into the utility of this biomarker (Zheng et al.). These two studies collectively contribute to our knowledge of the mechanisms involved in sarcopenia and highlight the significance of proper nutrition in maintaining muscle health in the older population (9). They underscore the importance of early detection and interventions to improve the nutritional status and overall wellbeing of older individuals affected by sarcopenia.

The link between muscle health and coronary heart disease is a critical area of investigation, as it can have profound implications for the wellbeing of patients. This study that delves into circulatory biomarkers associated with low muscle mass in individuals with coronary heart disease. These findings provide insights into the mechanisms of muscle health in this specific population (James et al.). In our continuing, we now turn our attention to a study conducted in Brazil, which investigates the prevalence and interplay of obesity, sarcopenia, and metabolic syndrome. These conditions are of critical concern in the aging population, but understanding their prevalence and associations is essential for effective intervention (Pinheiro et al.). These insights are crucial to enhance cardiovascular health and wellbeing in aging individuals. Furthermore, this understanding is essential for addressing the sarcopenic obesity (10), where individuals experience the double burden of muscle loss and excess fat, putting them at higher risk for cardiovascular issues.

In summary, this RT provided a comprehensive and illuminating exploration of diverse facets of aging-related research. It has successfully elucidated critical links between aging, muscle health, and a myriad of related factors, ranging from molecular mechanisms to clinical interventions. The Research Topic of studies within this issue has shed light on the complex interplay of inflammation, exercise, nutrition, and biomarkers, offering valuable insights for the field of sarcopenia-related research.

As a contemporary reflection, this RT is not only a cornerstone in advancing our understanding of the aging process but also plays a central role in addressing the Sustainable Development Goals (SDGs) by United Nations (11). Through emphasizing the significance of early detection and intervention, it contributes significantly to SDG 3 (Good Health and Wellbeing) by promoting the health and wellbeing of older adults. In addition to the objectives of this RT, it's essential to highlight that the knowledge presented here is in harmony with the global commitment to attaining Sustainable Development Goal 4 (SDG 4)—Quality Education (12). The wealth of knowledge presented herein not only expands the horizons of geriatric and gerontology research but also paves the way for a brighter, healthier future for our aging population, aligning with SDG 10 (Reduced Inequalities) by focusing on the specific needs of older individuals and ensuring inclusivity. Additionally, this research supports SDG 17 (Partnerships for the Goals) by fostering collaboration among researchers, healthcare providers, and policymakers to collectively address the challenges of aging and promote healthier aging globally.

Author contributions

GF: Conceptualization, Project administration, Writing—original draft, Writing—review & editing. MN: Conceptualization, Project administration, Writing—original draft. TD: Conceptualization, Project administration, Supervision, Writing—original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. El-Sebaie M, Elwakil W. Biomarkers of sarcopenia: an unmet need. *Egyptian Rheumatol Rehabil.* (2023) 50:45. doi: 10.1186/s43166-023-00213-w

2. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the extended group for EWGSOP2. *Age Ageing.* (2019) 48:16–31. doi: 10.1093/ageing/afy169

3. Rossi FE, Lira FS, Silva BSA, Freire APCF, Ramos EMC, Gobbo LA. Influence of skeletal muscle mass and fat mass on the metabolic and inflammatory profile in sarcopenic and non-sarcopenic overfat elderly. *Aging Clin Exp Res.* (2019) 31:629–35. doi: 10.1007/s40520-018-1029-3

4. Lozano-Montoya I, Correa-Pérez A, Abraha I, Soiza RL, Cherubini A, O'Mahony D, et al. Nonpharmacological interventions to treat physical frailty and sarcopenia in older patients: a systematic overview – the SENATOR project ONTOP series. *Clin Interv Aging.* (2017) 12:721–40. doi: 10.2147/CIA.S132496

5. Laosa O, Alonso C, Castro M, Rodriguez-Manas L. Pharmaceutical interventions for frailty and sarcopenia. *Curr Pharm Des.* (2014) 20:3068–82. doi: 10.2174/13816128113196660705

6. Sampaio RAC, Sewo Sampaio PY, Uchida MC, Arai H. Management of dynapenia, sarcopenia, and frailty: the role of physical exercise. *J Aging Res.* (2020) 2020:8186769. doi: 10.1155/2020/8186769

7. Batsis JA, Mackenzie TA, Barre LK, Lopez-Jimenez F, Bartels SJ. Sarcopenia, sarcopenic obesity and mortality in older adults: results from the National Health and Nutrition Examination Survey III. *Eur J Clin Nutr.* (2014) 68:1001–7. doi: 10.1038/ejcn.2014.117

8. Cramer JT, Cruz-Jentoft AJ, Landi F, Hickson M, Zamboni M, Pereira SL, et al. Impacts of high-protein oral nutritional supplements among malnourished men and women with sarcopenia: a multicenter, randomized, double-blinded, controlled trial. *J Am Med Dir Assoc.* (2016) 17:1044–55. doi: 10.1016/j.jamda.2016.08.009

9. Robinson SM, Reginster JY, Rizzoli R, Shaw SC, Kanis JA, Bautmans I, et al. Does nutrition play a role in the prevention and management of sarcopenia? *Clin Nutr.* (2018) 37:1121–32. doi: 10.1016/j.clnu.2017.08.016

10. Stenholm S, Harris T, Rantenen T, Visser M, Kritchevsky SB, Ferrucci L. Sarcopenic obesity-definition, etiology and consequences. *Curr Opin Clin Nutr Metab Care.* (2008) 11:693–700. doi: 10.1097/MCO.0b013e328312c37d

11. Pauw JB de, Gericke N, Olsson D, Berglund T. The effectiveness of education for sustainable development. *Sustainability (Switzerland).* (2015) 7:15693–717. doi: 10.3390/su71115693

12. Saini M, Sengupta E, Singh M, Singh H, Singh J. Sustainable development goal for quality education (SDG 4): a study on SDG 4 to extract the pattern of association among the indicators of SDG 4 employing a genetic algorithm. *Educ Inf Technol.* (2022) 28:2031–2069. doi: 10.1007/s10639-022-11265-4

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Association Between Serum Follicle-Stimulating Hormone and Sarcopenia and Physical Disability Among Older Chinese Men: Evidence From a Cross-Sectional Study

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Background: Sarcopenia is a geriatric syndrome characterized by progressive loss of muscle mass, function and quality and associated with a range of adverse health outcomes including disability. Despite a negative correlation between muscle mass and follicle-stimulating hormone (FSH) levels in postmenopausal women, it is unclear if FSH is associated with sarcopenia and its poor outcomes, especially in older men.

Methods: We used cross-sectional data from 360 men aged over 80 who participated in health check-ups to investigate correlations between serum FSH and sarcopenia, individual sarcopenia components, low physical performance (gait speed $\leq$0.8 m/s) and instrumental activities of daily living (IADL) disability. Sarcopenia and severe sarcopenia were diagnosed according to the revised definition of the European Working Group on Sarcopenia in Old People (EWGSOP2).

Results: The prevalence of sarcopenia was 17.8% in this population. In binary logistic regression analysis, compared with higher FSH group, lower FSH group showed a significant reduction in the risk of low calf circumference (a surrogate for muscle mass; OR 0.308, 95% CI 0.109–0.868, $P = 0.026$) after adjusting potential confounders including age, waist circumference, education, exercise, associated biochemical parameters, other sex hormones and high-sensitivity C-reactive protein. The correlation between FSH and low handgrip strength was marginally significant (OR 0.390, 95% CI 0.151–1.005, $P = 0.051$). No associations were observed between FSH and sarcopenia, severe sarcopenia, and disability in adjusted models.

Conclusion: In older men, circulating FSH was not associated with sarcopenia, sarcopenia severity, the majority of its components and adverse health outcome (IADL disability), with the exception of low calf circumference. Further work is needed to better elucidate the association of FSH and low muscle quantity by adopting more accurate measurement method of appendicular skeletal muscle mass such as DXA, CT or MRI.

Keywords: follicle-stimulating hormone, sarcopenia, physical performance, disability, older men

INTRODUCTION

Sarcopenia has been defined as a geriatric syndrome characterized by progressive loss of muscle mass, muscle strength and physical performance that is associated with a range of adverse health outcomes including frailty, disability, falls and mortality (1). In addition to common risk factors such as advanced age, inactivity and poor nutritional condition (1), reproductive hormones, which also change with aging (2), have been associated with sarcopenia (3). Among reproductive hormones, the effects of androgens and estrogens on skeletal muscle anabolism and homeostasis are well-established, particularly combined with resistance exercise training (4, 5), although caution should be taken regarding side-effects when sex steroids supplementation is applied in clinical practice (6).

Progress and updates have been made in the association of reproductive hormones and sarcopenia since extragonadal actions of follicle-stimulating hormone (FSH) were described in animals and humans. FSH has the potential to induce lipid storage, redistribution, and ectopic deposition (7–9), all factors that have been linked to chronic low-grade inflammatory state which has been recognized as a cause of age-related sarcopenia (10). For example, high-sensitivity C-reactive protein (hs-CRP), a biomarker of systemic inflammation, was found to be independently associated with sarcopenia component in a recent meta-analysis (11). Accordingly, the potential regulation of FSH on skeletal muscle mass has been proposed in the past decade, in which lean mass (mainly muscle) is negatively correlated with FSH levels in both young and old postmenopausal women (12–15). Most recently, observations from Park et al. that a reduction in appendicular lean mass across menopausal stages was associated with higher FSH levels supplied further evidence for the potential unfavorable effect of FSH on skeletal mass loss (16). However, these studies were all conducted in postmenopausal or perimenopausal women as rapid elevation of FSH level during perimenopausal transition and remain high after menopause. Research focusing on older males, a subpopulation with a high prevalence of sarcopenia and associated adverse outcomes such as disability, is still insufficient, although a gradual and constant increase in circulating FSH also exists in men as aging (2) and the upregulation of FSH in males receiving androgen deprivation therapy promotes the development of several metabolic diseases such as metabolic syndrome, atherosclerotic cardiovascular disease, and insulin resistance (17). At present, current research has not investigated the link between FSH and muscle strength, a key component for defining sarcopenia, in both males and females. Thus, there is a need to investigate the effects of FSH in sarcopenia and associated muscle function in older individuals, especially in older males.

In this cross-sectional study based on males aged over 80 years old, we first determined the level of FSH in individuals with or without sarcopenia. Next, we assessed the associations between serum FSH level and sarcopenia, physical performance and disability after controlling for potential confounders including age, obesity, exercise, education level, lipid profiles, nutrition status, systemic inflammation, and other hormones.

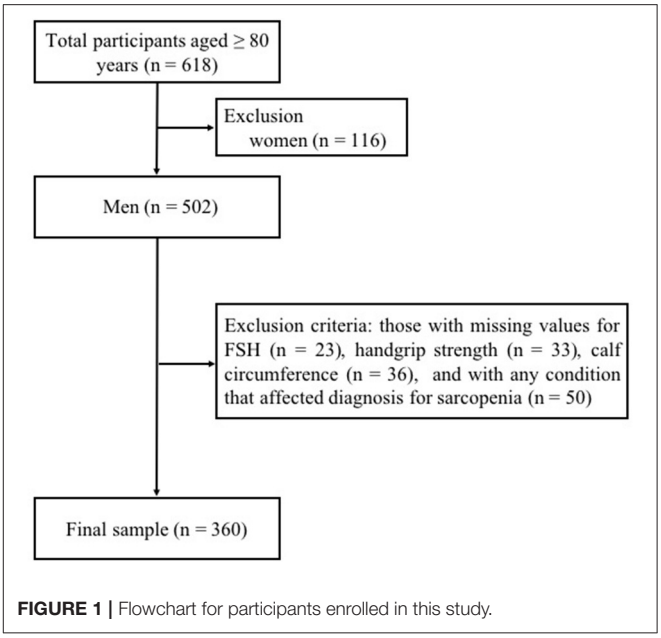

FIGURE 1 | Flowchart for participants enrolled in this study.

MATERIALS AND METHODS

Study Design

The cross-sectional baseline data used in the present study were from a single-center, prospective observational study which initiated to assess FSH, metabolic risks, and aging in elderly subjects underwent annual health examination in the Department of Geriatrics of the Shanghai Jiaotong University Affiliated Sixth People's Hospital (ChiCTR1800018015; www.chictr.org.cn). The baseline data were collected from January 2019 to December 2019.

Eligibility Criteria and Sample Selection

The recruitment criteria of the original prospective study were as follows: (i) 80 years of age or older; (ii) no problems of communication; (iii) no acute or end-stage illness; (iv) without taking any sex hormonal replacement therapy or with diseases involving in the hypothalamo-pituitary-gonadal/thyroid/adrenal axis. At baseline, 618 volunteers, of which 502 were men and 116 were women, were recruited *via* advertisement in the hospital. In order to investigate the cross-sectional association of FSH and sarcopenia in old men in the present analysis, subjects who had the following conditions at baseline were excluded from the study: (i) those with missing values for FSH ($n = 23$), handgrip strength ($n = 33$) or calf circumference ($n = 36$); (ii) females ($n = 116$); (iii) with any condition that affected detection for handgrip strength and calf circumferences, such as upper limb arthritis and edema of lower limbs ($n = 50$). Finally, 360 males aged 80–98 years were included in the study (**Figure 1**).

Outcome Measures

Data collection of all variables was organized by the principal investigator and was performed by a well-trained research team. Data on demographics, smoking, exercise, comorbidities,

anthropometric and circulating biochemical measurements were collected. Among the above indicators, only those which had shown statistical differences ($P < 0.05$) between sarcopenia subgroups in the comparison analysis were treated as covariates in the subsequent binary logistic regression analysis. As such, age, waist circumference, education, exercise, luteinizing hormone (LH), estradiol (E_2), total testosterone (TT), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), 25-hydroxyvitamin D [25(OH)D], albumin, free triiodothyronine (FT3) and hsCRP were selected as co-variables, all introduced in the adjusted regression models.

Assessment of Sarcopenia

For the diagnosis of sarcopenia, calf circumference, handgrip strength and 4-meter gait speed were assessed. Calf circumference was measured with the elderly participant in standing position, at the greatest circumference of the lower right leg, recorded in centimeters. The handgrip strength of the dominant hand was the maximum value evaluated three times using CAMRY hydraulic hand dynamometer (EH101; Camry, China). At least 1-min rest was required between each test. 4-meter gait speed test is performed twice to obtain the gait speed in which the participants walk 4 meters at their usual pace. The gait speed was calculated by dividing the distance (meter) by walking time (second). The European Working Group on Sarcopenia in Older People updated the consensus definition of sarcopenia in 2018 (EWGSOP2) (18) as the following criteria: (1) low muscle strength, (2) low muscle quantity or quality and (3) low physical performance. Probable sarcopenia is identified by criterion one, while the diagnosis is confirmed by additional documentation of criterion two. Severe sarcopenia is considered if all criteria are met. In the present study, handgrip strength <27 kg was considered low handgrip strength and calf circumference <31 cm was considered low muscle quantity (low calf circumference), according to EWGSOP2 recommended cutoffs. Low physical performance was defined as a 4-meter gait speed ≤0.8 m/s.

Assessment of Disability

Physical disability was assessed by instrumental activities of daily living (IADL) (19, 20). In brief, the IADL index includes eight tasks—shopping, preparing meals, using the telephone, housekeeping, laundry, transportation, taking medications, and managing money. Each IADL item was scored 0 to 1 according to the ability of participants to perform each task and total score was eight. Scores ranged from 0 to 8 with a lower score denoting the need for assistance with IADLs. A score ≤7 was defined as IADL disability.

Laboratory Measurements

After an overnight fast, venous blood samples were collected from all individuals and the samples were used to measure fasting blood glucose (FPG), hemoglobin A1c (HbA1c), TG, total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol (HDL-C), hsCRP, albumin, 25(OH)D and hormones. Plasma fasting glucose was measured using the glucose oxidase method (detection range: 0.078–55.51 mmol/L). HbA1c was measured by high-performance liquid chromatography (Bio-Rad Laboratories) (detection range: 3.5–20%). Serum lipids were measured by standard enzymatic methods using a Hitachi 747 analyzer (Castle Hill) (detection range of TC: 0.05–12.90 mmol/L, TG: 0.01–22.58 mmol/L, LDL-C:0.05–10.20 mmol/L, HDL-C: 0.10–2.59 mmol/L). hsCRP was detected by particle-enhanced immunoturbidimetry (Dade Behring Inc.) (detection range: 0.175–500 mg/L). Serum albumin was measured by the bromocresol green method (Shanghai Kehua Bio-Engineering Co, Ltd) (detection range: 10.0–60.0 g/L). Hormones including serum 25(OH)D (detection range: 3.0–70.0 ng/ml), FT3 (detection range: 0.4–50.0 pmol/L), free thyroxine (FT4, detection range: 0.3–100.0 pmol/L) and thyroid-stimulating hormone (TSH, detection range: 0.06–99 mIU/L) were quantified by an electrochemiluminescence immunoassay method (Roche Diagnostics GmbH) on a Cobas e601 analyzer.

Reproductive hormones TT (detection range: 0.087–52.000 nmol/L), E_2 (detection range: 18.35–15781.00 pmol/L), FSH (detection range: 0.5–160.0 IU/L), and LH (detection range: 0.1–200.0 IU/L) were detected by chemiluminescence (Abbott GmbH & Co. KG) with inter- and intra-assay coefficients of variations of <5%. The normal range was 6.68–25.7 nM for TT, 41.4–159 pM for E_2, 1.5–12.4 IU/L for FSH, and 1.7–8.6 IU/L for LH.

Other Covariates

Waist circumference was measured at the middle point between the lower edge of the rib cage and iliac crests. Education was dichotomized with a cutoff for college graduation vs. no college graduation due to the high proportion of subjects with college graduation (80.6 %). Subjects with at least a college graduation were regarded as having high education. Living alone was defined based on living without a partner. We designated routine exercises with moderate to strenuous intensity, three times a week or more frequently. Non-smoker were defined as those who had never smoked or had smoked fewer than 100 cigarettes in the past. Comorbidities included type two diabetes (T2DM), coronary heart disease (CHD), hypertension, chronic obstructive pulmonary disease (COPD), arrhythmia, chronic kidney disease (CKD), osteoporosis, history of fracture, osteoarthritis, and neoplasms. And the number of comorbidities in every participant was calculated.

Statistical Analysis

Data were tested for normality using the Shapiro-Wilk test, and continuous variables are presented as mean ± standard deviation for normally distributed variables or median (25th percentile to 75th percentile) for skewed variables, whereas categorical variables are expressed as numbers with percentages. Differences between two groups (non-sarcopenia and sarcopenia) were compared by the Student's *t*-test for data with a normal distribution or the Mann-Whitney U test for data with a skewed distribution. For categorical variables, intergroup comparisons were analyzed using the chi-square test. The association between FSH (categorical variables) and sarcopenia, severe sarcopenia, two components for sarcopenia diagnosis, lower physical performance, and IADL disability was assessed using a binary logistic regression model, organized in crude and adjusted

models, and results are expressed as odds ratios (OR) with a 95% confidence interval (CI). All statistical analyses were performed using SPSS 26.0 (SPSS Inc., Chicago, IL). A two-sided $P < 0.05$ was considered statistically significant.

RESULTS

Overall, we enrolled 360 old men (with a mean age of 86.2 ± 4.4 years). The mean number of comorbidities was 3.8 ± 1.7 per volunteer. Of the total number of participants, 64 (17.8%) had sarcopenia according to the EWGSOP2 definition. **Table 1** summarized the general demographic and laboratory characteristics of these subjects according to their sarcopenia status. As expected, those with sarcopenia had a higher hsCRP but a lower 25(OH)D (both $P < 0.05$), FT3 and proportion of high education (both $P < 0.01$), than those without sarcopenia. In addition, sarcopenic individuals had a decreased proportion of routine exercises when compared to those of non-sarcopenic individuals ($P < 0.01$). Poorer nutritional status indicated as lower TG, LDL-C (both $P < 0.05$), albumin and waist circumference (both $P < 0.01$) was also detected in sarcopenic participants compared to non-sarcopenic ones. Moreover, men with sarcopenia were older ($P < 0.01$), coupled with higher circulating FSH and LH levels (both $P < 0.05$), whereas no differences in TT and E_2 were found between the two groups.

We next stratified participants into two groups: lower FSH and higher FSH groups, according to the median FSH value measured in our assays (20.42 IU/L). As shown in **Table 2**, compared with the lower FSH group, men with a higher FSH had decreased handgrip strength and calf circumference (both $P < 0.05$). There was no difference in 4-meter gait speed between two groups.

Table 3 shows the association of FSH with sarcopenia, its defining components and measures of physical function in aged men analyzed by multinomial logistic regressions. We calculated the ORs in models with and without adjustment for potential confounders. In the crude analysis, higher FSH group had increased ORs for sarcopenia (0.575, 95% CI 0.331–0.999, $P = 0.049$), low calf circumference (0.522, 95% CI 0.307–0.887, $P = 0.016$), and low handgrip strength (0.386, 95% CI 0.235–0.636, $P < 0.001$). Adjusted Model was adjusted for age, waist circumference, education, exercise, LH, E_2, TT, TG, LDL-C, 25(OH)D, albumin, FT3, and hsCRP. There were no significant associations observed between FSH and sarcopenia (OR 0.511, 95% CI 0.178–1.467, $P = 0.212$) or severe sarcopenia (OR 0.400, 95% CI 0.136–1.175, $P = 0.096$) after controlling above confounders, although the prevalence of sarcopenia was significant different between two groups ($P < 0.05$). For sarcopenia components, lower FSH men had decreased ORs for low calf circumference (OR 0.308, 95% CI 0.109–0.868, $P = 0.026$) compared with higher FSH men. The correlation between FSH and low handgrip strength was marginally significant (OR 0.390, 95% CI 0.151–1.005, $P = 0.051$). No significant association was observed between FSH and low physical performance between two groups ($P = 0.446$).

As sarcopenia was found to be an independent risk factor for several adverse health outcomes including disability (21), we next assessed the association between physical disability defined as an IADL score ≤ 7 and FSH levels (**Table 4**). No relationship of FSH and disability was observed in adjusted model although the significant association was observed in the crude model ($P < 0.001$) and the apparent difference in the proportion of IADL disability between two groups ($P < 0.01$).

TABLE 1 | Characteristics of study population according to sarcopenia status.

Variable	non-sarcopenia ($n = 296$)	Sarcopenia ($n = 64$)	*P*
Age (years)	85.8 ± 4.0	88.1 ± 4.3	<0.001
Waist circumference (cm)	91.2 ± 9.3	86.2 ± 8.9	<0.001
Non-smoker (%, *n*)	74.3 (220)	81.3 (52)	0.242
High education (%, *n*)	84.8 (251)	67.2 (43)	0.001
Living alone (%, *n*)	8.8 (26)	7.8 (5)	0.802
Routine exercise (%, *n*)	55.1 (163)	26.6 (17)	<0.001
Number of Comorbities	3.7 ± 1.6	4.0 ± 1.8	0.120
FPG (mmol/L)	5.1 (4.7–5.7)	5.0 (4.7–5.4)	0.387
HbA1c (%)	6.2 ± 0.9	6.4 ± 1.2	0.145
TC (mmo/L)	4.1 ± 1.0	3.8 ± 0.9	0.071
TG (mmo/L)	1.1 ± 0.7	0.9 ± 0.5	0.038
HDL-C (mmo/L)	1.2 ± 0.3	1.3 ± 0.4	0.111
LDL-C (mmo/L)	2.2 ± 0.8	2.0 ± 0.7	0.036
25(OH)D (ng/mL)	16.6 (12.2–24.5)	16.0 (9.9–19.0)	0.038
Albumin (g/L)	40.2 ± 3.4	37.8 ± 4.7	<0.001
hsCRP (mg/L)	1.00 (0.40–2.44)	1.53 (0.82–4.52)	0.024
FT3 (pmol/L)	4.1 ± 0.6	3.7 ± 0.7	<0.001
FT4 (pmol/L)	16.4 ± 2.3	16.3 ± 1.7	0.702
TSH (mIU/L)	3.2 ± 2.5	3.3 ± 3.0	0.968
TT (nmol/L)	13.0 ± 6.3	14.3 ± 7.7	0.137
E_2 (pmol/L)	130 ± 46	142 ± 49	0.061
FSH (IU/L)	18.7 (12.1–38.3)	29.5 (14.8–37.0)	0.038
LH (IU/L)	9.4 (6.8–19.0)	12.5 (9.6–23.9)	0.033

Data are presented as means ± standard deviation (SD) for data with a normal distribution, medians (interquartile range) for data with a skewed distribution, or as a number with a proportion for categorical variables. The differences between non-sarcopenia and sarcopenia were compared by the Student's t-test for data with a normal distribution or the Mann-Whitney U test for data with a skewed distribution. For categorical variables, differences were analyzed using the chi-square test.

FPG, fasting blood glucose; HbA1c, hemoglobin A1c; TC, serum total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; hsCRP, high-sensitivity C-reactive protein; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; TT, total testosterone; E_2, estradiol; FSH, follicle-stimulating hormone; LH, luteinizing hormone.

TABLE 2 | Sarcopenia associated components in older males stratified by the follicle-stimulating hormone (FSH) median value.

Variable	Lower-FSH ($n = 181$)	Higher-FSH ($n = 179$)	*P*
Handgrip strength (kg)	22.9 ± 7.0	21.3 ± 6.5	0.029
Calf circumference (cm)	32.8 ± 3.0	31.6 ± 4.0	0.020
Gait speed (m/s)	0.78 ± 0.31	0.73 ± 0.27	0.111

Data are presented as means ± standard deviation (SD).

TABLE 3 | Multivariate-adjusted logistic regression analyses of associations between follicle-stimulating hormone (FSH) levels and sarcopenia, severe sarcopenia, and individual sarcopenia components.

	Prevalence (%, *n*)	Crude model	*P*	Adjusted model[a]	*P*
Sarcopenia					
Higher-FSH	21.8 (39)*	1.00		1.00	
Lower-FSH	13.8 (25)	0.575 (0.331–0.999)	0.049	0.511 (0.178, 1.467)	0.212
Severe sarcopenia					
Higher-FSH	19.6 (35)	1.00		1.00	
Lower-FSH	12.7 (23)	0.599 (0.338–1.062)	0.079	0.400 (0.136–1.175)	0.096
Low calf circumference					
Higher-FSH	25.1 (45)*	1.00		1.00	
Lower-FSH	14.9 (27)	0.522 (0.307–0.887)	0.016	0.308 (0.109, 0.868)	0.026
Low handgrip strength					
Higher-FSH	84.4 (151)**	1.00		1.00	
Lower-FSH	65.7 (119)	0.386 (0.235–0.636)	<0.001	0.390 (0.151,1.005)	0.051
Low physical performance					
Higher-FSH	59.8 (108)	1.00		1.00	
Lower-FSH	58.0 (105)	0.930 (0.611–1.415)	0.734	0.717 (0.305,1.685)	0.446

*Data were a proportion with a number for categorical variables and odds ratios (95% confidence interval). Low calf circumference: calf circumference <31 cm; Low handgrip strength: handgrip strength <27 kg; Low physical performance: 4-meter gait speed ≤0.8 m/s. All models constructed by logistic regression analysis: a: adjusted for age, waist circumference, education, exercise, LH, E_2, TT, TG, LDL-C, 25(OH)D, albumin, FT3, and hsCRP. *P < 0.05, **P < 0.01, Lower-FSH group vs. Higher-FSH group.*

TABLE 4 | Multivariate-adjusted logistic regression analyses of associations between follicle-stimulating hormone (FSH) levels and IADL disability.

	Prevalence (%, *n*)	Crude model	P	Adjusted model[a]	*P*
IADL disability					
Higher-FSH	67.6 (121)**	1.00		1.00	
Lower-FSH	49.2 (89)	0.464 (0.302-0.711)	<0.001	0.664 (0.300, 1.467)	0.311

*Data were a proportion with a number for IADL disability and odds ratio (95% confidence interval). IADL disability: score ≤7. All models constructed by logistic regression analysis: a: adjusted for age, waist circumference, education, exercise, LH, E_2, TT, TG, LDL-C, 25(OH)D, albumin, FT3, and hsCRP. **P < 0.01, Lower-FSH group vs. Higher-FSH group.*

DISCUSSION

Here, we provide a comprehensive examination of the cross-sectional associations between FSH levels and sarcopenia and associated poor outcomes in men over 80 years of age after adjusting for potential confounders of clinical significance. To our knowledge, although there are a limited number of studies showing associations between FSH and muscle mass in females (12–15), there is currently no human study that has explored the relationship between FSH and sarcopenia in men. Hence, this is the first study that has assessed the link between serum FSH and sarcopenia and sarcopenia-associated functional outcomes including physical disability in males. After adjusting for potential variables involved in sarcopenia, we found that higher FSH concentrations may correlate with reduced skeletal muscle mass indicated as low calf circumference, but not sarcopenia, sarcopenia severity, low muscle strength and IADL disability.

Our study is consistent with previous observational studies conducted in young and old postmenopausal females, in which high FSH levels were associated with lean (mainly muscle) mass (12–15, 22). In men, there is only one study (AGES-Reykjavik study) that has examined this association, showing no correlation (14). There are three possible explanations for differences between the AGES-Reykjavik study and ours. One possibility is that our older Chinese men were exposed to higher circulating concentrations of FSH than that of older men in Iceland (mean FSH: 28 IU/L vs. 19 IU/L, both detected by ELISA), which could surpass the threshold level of FSH to affect muscle mass. Furthermore, we adjusted for potential covariates including age, waist circumference, education, exercise, LH, E_2, TT, TG, LDL-C, 25(OH)D, albumin, FT3, and hsCRP, while the AGES-Reykjavik study only adjusted for age, subgroup, E_2, and TT. Hence, the results of our study were more rigorous than those of the AGES-Reykjavik study. Third, the number of analytic samples in the present study ($n = 360$) is larger than those of the AGES-Reykjavik study ($n = 245$), which could lead to higher statistical power.

We found a possible association between skeletal muscle mass and FSH, but not between muscle strength and FSH after adjusting for all the covariates although muscle quantity is the basis for muscle strength. This suggests that the effect of FSH on muscle strength is small. In fact, other reproductive hormones such as androgens also presented a similar characteristic of inducing a greater promotion in muscle mass than muscle strength (23, 24). Thus, hormonal regulation of FSH in association with resistance exercise training may be a promising new strategy to manage sarcopenia since exercise remains a valid countermeasure against muscle atrophy and androgen treatment is not recommended due to intolerable adverse events in older men (6, 25). Notably, the genotype of FSH receptors may affect the role of circulating FSH on target tissues. A recent study reported that men with the GG genotype of the FSH receptor rs6166 SNP have lower levels of blood glucose than those with the AA genotype and their FSH concentrations were inversely correlated with insulin and insulin resistance. Meanwhile, the FSH receptor rs6166 A/G genotype did not affect glucose metabolism in healthy men (26). Thus, genotype-specific effects of FSH receptors on muscle mass, muscle strength and function should be explored in the future study.

Sarcopenia is associated with an increased likelihood of several adverse health outcomes including disability (21). We did not observe any association between FSH and gait speed or disability in the present study. A prospective study of Japanese community-dwelling older adults found that there were no increased risks of incident disability for participants with only one sarcopenia component (21). Another prospective study with 9.1 years

follow-up revealed that gait speed was a powerful predictor of disability, in men, compared with sarcopenia components (27). Thus, it is reasonable for the loss of association between FSH and gait speed and disability in our study. In addition, functional disability in older men has multiple causes, of which muscle weakness is only one. Other risk factors such as dietary pattern and anemia, may also play important roles in the presence of disability among older adults, which were not analyzed in the present study (28, 29).

We acknowledge some limitations to the present study. First, although a possible association between FSH and skeletal muscle mass was observed, as a cross-sectional study, the causality of this relationship cannot be established. Second, in the current study, calf circumference was not as accurate as bioelectrical impedance analysis or dual-energy X-ray absorptiometry for evaluating muscle mass especially in individuals with a high percentage of body fat, as it can equally predict lean and fat mass. However, calf circumference, as a validated surrogate measure of skeletal muscle mass, was suggested by EWGSOP2 as it is easy to perform in clinical and population settings. Finally, the participants were from a population receiving regular medical examination as opposed to a community or a general population, so our results might not be generalizable.

CONCLUSION

In our cross-sectional study of elderly Chinese men aged over 80, FSH was not associated with sarcopenia, sarcopenia severity, the majority of its components and sarcopenia-associated adverse outcome (IADL disability). Interestingly, FSH seems to be a independent risk factor for low skeletal muscle mass indicated as low calf circumference although calf circumference is not a gold standard method to evaluate muscle mass. Further study is needed to confirm the association of FSH and low muscle quantity by adopting more accurate measurement method of appendicular skeletal muscle mass such as DXA, CT or MRI.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Shanghai Jiaotong University Affiliated Sixth People's Hospital (Reference number: 2018–109). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

YK and YZ: designed the study. YK, XZ, and YZ: collected and analyzed the data. YK: wrote the manuscript. QG and YZ: participated in the critical review of the manuscript. All authors read and approved the final manuscript.

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REFERENCES

1. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet.* (2019) 393:2636–46. doi: 10.1016/S0140-6736(19)31138-9
2. Xia F, Wang N, Han B, Li Q, Chen Y, Zhu C, et al. Hypothalamic-pituitary-gonadal axis in aging men and women: increasing total testosterone in aging men. *Neuroendocrinology.* (2017) 104:291–301. doi: 10.1159/000446656
3. Sipilä S, Narici M, Kjaer M, Pöllänen E, Atkinson RA, Hansen M, et al. Sex hormones and skeletal muscle weakness. *Biogerontology.* (2013) 14:231–45. doi: 10.1007/s10522-013-9425-8
4. Diel P. The role of the estrogen receptor in skeletal muscle mass homeostasis and regeneration. *Acta Physiol.* (2014) 212:14–6. doi: 10.1111/apha.12341
5. Dubois V, Laurent M, Boonen S, Vanderschueren D, Claessens F. Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions. *Cell Mol Life Sci.* (2012) 69:1651–67. doi: 10.1007/s00018-011-0883-3
6. Laurent MR, Dedeyne L, Dupont J, Mellaerts B, Dejaeger M, Gielen E. Age-related bone loss and sarcopenia in men. *Maturitas.* (2019) 122:51–6. doi: 10.1016/j.maturitas.2019.01.006
7. Liu XM, Chan HC, Ding GL, Cai J, Song Y, Wang TT, et al. FSH regulates fat accumulation and redistribution in aging through the Gαi/Ca (2+)/CREB pathway. *Aging Cell.* (2015) 14:409–20. doi: 10.1111/acel.12331
8. Wang N, Li Q, Han B, Chen Y, Zhu C, Chen Y, et al. Follicle-stimulating hormone is associated with non-alcoholic fatty liver disease in Chinese women over 55 years old. *J Gastroenterol Hepatol.* (2016) 31:1196–202. doi: 10.1111/jgh.13271
9. Cui H, Zhao G, Liu R, Zheng M, Chen J, Wen J, et al. stimulates lipid biosynthesis in chicken adipose tissue by upregulating the expression of its receptor FSHR. *J Lipid Res.* (2012) 53:909–17. doi: 10.1194/jlr.M025403
10. Wiedmer P, Jung T, Castro JP, Pomatto LCD, Sun PY, Davies KJA, et al. Sarcopenia - Molecular mechanisms and open questions. *Ageing Res Rev.* (2021) 65:101200. doi: 10.1016/j.arr.2020.101200
11. Shokri-Mashhadi N, Moradi S, Heidari Z, Saadat S. Association of circulating C-reactive protein and high-sensitivity C-reactive protein with components of sarcopenia: a systematic review and meta-analysis of observational studies. *Exp Gerontol.* (2021) 150:111330. doi: 10.1016/j.exger.2021.111330
12. Gourlay ML, Specker BL, Li C, Hammett-Stabler CA, Renner JB, Rubin JE. Follicle-stimulating hormone is independently associated with lean mass but not BMD in younger postmenopausal women. *Bone.* (2012) 50:311–6. doi: 10.1016/j.bone.2011.11.001
13. García-Martín A, Reyes-García R, García-Castro JM, Muñoz-Garach A, Escobar-Jiménez F, Muñoz-Torres M. Gonadotropins are related to lean mass in healthy postmenopausal women. *Endocr Res.* (2013) 38:119–24. doi: 10.3109/07435800.2012.733987
14. Veldhuis-Vlug AG, Woods GN, Sigurdsson S, Ewing SK, Le PT, Hue TF, et al. Serum FSH is associated with BMD, bone marrow adiposity, and body composition in the AGES-Reykjavik study of older adults. *J Clin Endocrinol Metab.* (2021) 106:e1156–69. doi: 10.1210/clinem/dgaa922

15. Sowers M, Zheng H, Tomey K, Karvonen-Gutierrez C, Jannausch M, Li X, et al. Changes in body composition in women over six years at midlife: ovarian and chronological aging. *J Clin Endocrinol Metab.* (2007) 92:895–901. doi: 10.1210/jc.2006-1393
16. Park YM, Jankowski CM, Ozemek C, Hildreth KL, Kohrt WM, Moreau KL. Appendicular lean mass is lower in late compared with early perimenopausal women: potential role of FSH. *J Appl Physiol (1985).* (2020) 128:1373–80. doi: 10.1152/japplphysiol.00315.2019
17. Crawford ED, Schally AV, Pinthus JH, Block NL, Rick FG, Garnick MB, et al. The potential role of follicle-stimulating hormone in the cardiovascular, metabolic, skeletal, and cognitive effects associated with androgen deprivation therapy. *Urol Oncol.* (2017) 35:183–91. doi: 10.1016/j.urolonc.2017.01.025
18. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* (2019) 48:16–31. doi: 10.1093/ageing/afz046
19. Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. *Gerontologist.* (1969) 9:179–86. doi: 10.1093/geront/9.3_Part_1.179
20. Tong A, Man DW. The validation of the Hong Kong Chinese version of the lawton instrumental activities of daily living scale for institutionalized elderly persons. *OTJR: Occup Particip Health.* (2002) 22:132–42. doi: 10.1177/153944920202200402
21. Kitamura A, Seino S, Abe T, Nofuji Y, Yokoyama Y, Amano H, et al. Sarcopenia: prevalence, associated factors, and the risk of mortality and disability in Japanese older adults. *J Cachexia Sarcopenia Muscle.* (2021) 12:30–8. doi: 10.1002/jcsm.12651
22. Jaff NG, Norris SA, Snyman T, Toman M, Crowther NJ. Body composition in the Study of Women Entering and in Endocrine Transition (SWEET): a perspective of African women who have a high prevalence of obesity and HIV infection. *Metabolism.* (2015) 64:1031–41. doi: 10.1016/j.metabol.2015.05.009
23. Falqueto H, Júnior JLR, Silvério MNO, Farias JCH, Schoenfeld BJ, Manfredi LH. Can conditions of skeletal muscle loss be improved by combining exercise with anabolic-androgenic steroids? A systematic review and meta-analysis of testosterone-based interventions. *Rev Endocr Metab Disord.* (2021) 22:161–78. doi: 10.1007/s11154-021-09634-4
24. Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, et al. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J Clin Endocrinol Metab.* (2004) 89:2085–98. doi: 10.1210/jc.2003-032006
25. Jardí F, Laurent MR, Dubois V, Kim N, Khalil R, Decallonne B, et al. Androgen and estrogen actions on male physical activity: a story beyond muscle. *J Endocrinol.* (2018) 238:R31–52. doi: 10.1530/JOE-18-0125
26. Cannarella R, Musso N, Condorelli RA, Musmeci M, Stefani S, Aversa A, et al. The 2039 A/G FSH receptor gene polymorphism influences glucose metabolism in healthy men. *Endocrine.* (2020) 70:629–34. doi: 10.1007/s12020-020-02420-3
27. Cesari M, Rolland Y, Abellan Van Kan G, Bandinelli S, Vellas B, Ferrucci L. Sarcopenia-related parameters and incident disability in older persons: results from the "invecchiare in Chianti" study. *J Gerontol A Biol Sci Med Sci.* (2015) 70:457–63. doi: 10.1093/gerona/glu181
28. Matsuyama S, Zhang S, Tomata Y, Abe S, Tanji F, Sugawara Y, et al. Association between improved adherence to the Japanese diet and incident functional disability in older people: The ohsaki cohort 2006 study. *Clin Nutr.* (2020) 39:2238–45. doi: 10.1016/j.clnu.2019.10.008
29. Owusu C, Cohen HJ, Feng T, Tew W, Mohile SG, Klepin HD, et al. Anemia and functional disability in older adults with cancer. *J Natl Compr Canc Netw.* (2015) 13:1233–9. doi: 10.6004/jnccn.2015.0152

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Xanthine Oxidoreductase Inhibitor Use Associated With Reduced Risk of Sarcopenia and Severe Sarcopenia in Patients Undergoing Hemodialysis

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Background: Xanthine oxidoreductase (XOR) inhibition reduces reactive oxygen species (ROS) production and enhances adenosine triphosphate (ATP) synthesis. We investigated the protective effects of XOR inhibitor treatment on sarcopenia, frequently observed in patients undergoing hemodialysis (HD), in which increased ROS and ATP shortage are known to be involved.

Methods: This retrospective cross-sectional study included 296 HD patient (203 males, 93 females). Muscle mass, physical performance, and muscle strength were assessed using dual-energy X-ray absorptiometry, five-time chair stand testing, and handgrip strength, respectively. The Asian Working Group for Sarcopenia 2019 criteria were used to define low muscle mass, low physical performance, and low muscle strength, as well as sarcopenia and severe sarcopenia.

Results: Sarcopenia and severe sarcopenia prevalence rates were 42.2 and 20.9%, respectively. XOR inhibitor users ($n = 119$) showed a significantly ($p < 0.05$) lower prevalence of sarcopenia and severe sarcopenia, as well as reduced muscle mass, physical performance, and muscle strength than non-users ($n = 177$). Multivariate logistic regression analyses also revealed XOR inhibitor use to be significantly associated with low muscle mass [odds ratio (OR), 0.384; 95% confidence interval (CI), 0.183–0.806; $p = 0.011$] and low physical performance (OR, 0.286; 95% CI, 0.142–0.578; $p < 0.001$), while significance with low muscle strength was borderline. Furthermore, XOR inhibitor use was significantly associated with sarcopenia (OR, 0.462; 95% CI, 0.226–0.947; $p = 0.035$) and severe sarcopenia (OR, 0.236; 95% CI, 0.091–0.614; $p = 0.003$).

Conclusions: XOR inhibitor use was significantly associated with reduced risk of sarcopenia/severe sarcopenia in HD patients, suggesting that XOR inhibitor treatment has protective effects on sarcopenia in HD patients.

Keywords: XOR inhibitor, hemodialysis, sarcopenia, prevalence, AWGS 2019

INTRODUCTION

Sarcopenia is characterized as a decline in skeletal muscle mass and function (1), and frequently observed in patients undergoing hemodialysis (HD) (2, 3), which is termed uremic sarcopenia. Furthermore, HD patients have increased mortality (4–6), which has been shown to occur a higher rate in those with as compared to without sarcopenia (3, 7), thus emphasizing the need for prevention and treatment of this conditions in patients undergoing that therapy.

Xanthine oxidoreductase (XOR) is an enzyme that generates reactive oxygen species (ROS), and also catalyzes oxidation from hypoxanthine to xanthine and then xanthine to uric acid in the purine degradation pathway (8). Thus, inhibition of XOR reduces ROS production, while it also enhances adenosine triphosphate (ATP) synthesis along with an increase in the purine salvage pathway by use of hypoxanthine (9–12). Since increased ROS and ATP shortage in skeletal muscle are known to be involved in sarcopenia, especially uremic sarcopenia cases (13–15), we speculated that XOR inhibitor treatment has effects to protect against sarcopenia through preservation of skeletal muscle mass and function by decreased ROS production and ATP enhancement in HD patient skeletal muscle tissues.

However, to the best of our knowledge, no report showing the effects of XOR inhibitor treatment on sarcopenia has been presented. To evaluate possible protective effects on sarcopenia, the present study was conducted to examine the association of XOR inhibitor use with sarcopenia in HD patients based on the Asian Working Group for Sarcopenia (AWGS) 2019 criteria (16).

MATERIALS AND METHODS

Study Design

This was a retrospective cross-sectional observational study conducted at Inoue Hospital (Osaka, Japan), where HD treatment was generally performed three times per week for about 4 h each time. As part of routine clinical care at that institution, muscle mass, physical performance, and muscle strength in HD patients are generally measured at the start of the first HD session that occurs during the week of their birthday. The association of XOR inhibitor use with sarcopenia and its components was investigated.

Study Participants

The study participants were enrolled based on the following criteria. Inclusion criteria: (1) patient undergoing HD, and (2) measurements of muscle mass, physical performance, and muscle strength performed between January 2018 and December 2018, and (3) age ≥20 years. Exclusion criteria: (1) HD therapy period <6 months, (2) presence of debilitating disease, such as liver cirrhosis, malignancy, infection, or acute illness, and (3) treated with corticosteroids.

The need/requirement for informed consent was waived by The Inoue Hospital Ethics Committee (approval no. 236) owing to the retrospective nature of the investigation. Following approval of the study protocol, all data subjected to analysis were collected from relevant patient medical records. This study was conducted in full accordance with the Declaration of Helsinki.

XOR Inhibitor User

An XOR inhibitor user was defined as a patient treated with allopurinol, febuxostat, or topiroxostat, each of which have been approved for therapeutic use as an XOR inhibitor in Japan.

Determination of Muscle Mass

Muscle mass was assessed using whole body dual-energy X-ray absorptiometry (Horizon A; Hologic, Massachusetts, USA) (17). Extremity fat-free mass minus bone mineral contents was considered to be appendicular skeletal muscle, and height-adjusted muscle mass was calculated as appendicular skeletal muscle divided by height in meters squared (kg/m^2) (3). Low muscle mass was defined as height-adjusted muscle mass <7.0 kg/m^2 for males and <5.4 kg/m^2 for females (16).

Measurement of Physical Performance

Physical performance was assessed using a five-time chair stand test. Patients were asked to stand and sit as fast as possible five times from a sitting position on a chair with a seat 40 cm high and straight back without arm rests, with their arms crossed on the chest and hands on their shoulders, as previously reported in the CHAIR [change hemodialysis patients' activity and impaired functions by chair stand exercise] study (18). They initiated performance of the test when an experienced research staff member spoke the word "start" and the time (seconds) until the end of the fifth repetition was recorded (19, 20). Low physical performance was defined as a test time ≥12 s (16).

Measurement of Muscle Strength

Muscle strength was assessed by determining handgrip strength using a Smedley type hand dynamometer (T.K.K.5101; Takei, Niigata, Japan), performed by an experienced research staff member (21, 22). Patients were instructed to hold the grip in an upright position with maximum force and with their arm extended. Measurements were repeated two times with each hand and the highest value (kg) was recorded. Low muscle strength was defined as handgrip strength <28 kg for males and <18 kg for females (16).

Diagnosis of Sarcopenia and Severe Sarcopenia

The AWGS 2019 criteria were used for diagnosis of sarcopenia and severe sarcopenia (16). Briefly, sarcopenia was defined as low muscle mass, plus low physical performance or low muscle strength, while severe sarcopenia was defined as low muscle mass, plus low physical performance, and low muscle strength.

Other Variables

Information regarding medication that may affect sarcopenia, including insulin, growth or sex hormones, and angiotensin converting enzyme (ACE) inhibitor/angiotensin II receptor blocker (ARB), as well as vitamin D use was obtained. The diagnosis of diabetes mellitus was based on a history of diabetes mellitus or the American Diabetes Association criteria (23). Past history of cerebrovascular disease was defined as

prior occurrence of ischemic stroke, transient ischemic attack, intracerebral hemorrhage, and/or subarachnoid hemorrhage. Blood samples were collected from each patient before the start of the first HD session of the week, with standard laboratory parameters including serum albumin, C-reactive protein (CRP), and uric acid levels analyzed using a routine laboratory method at the hospital (24).

Statistical Analysis

To compare variables between groups, Mann–Whitney's *U*-test (continuous variables) and a chi-squared test (categorical variables) were used. Serum CRP level was logarithmically transformed (log) to follow a normal distribution, before submitting to multivariate logistic regression analysis. Multivariate logistic regression analyses were performed to determine whether XOR inhibitor administration was independently associated with the presence of sarcopenia or severe sarcopenia in addition to that of low muscle mass, low physical performance, or low muscle strength, following adjustments based on various factors, including age, gender, degree of obesity, duration of hemodialysis, cerebrovascular disease, diabetes mellitus, vitamin D, nutritional status, inflammation, and uric acid, each of which are known to be associated with sarcopenia in HD patients (3, 7, 13, 25–28). The consistency of the associations between XOR inhibitor administration and presence of sarcopenia or severe sarcopenia was also examined in relationship to age (≤/>68 years), gender, body mass index (BMI) (≤/>22.6 kg/m^2), duration of hemodialysis (≤/>6.5 years), cerebrovascular disease (presence/absence), diabetes mellitus (presence/absence), use of oral and/or intravenous vitamin D (presence/absence), serum albumin (≤/>3.6 g/dL), CRP (≤/>0.1 mg/dL), uric acid level (≤/>6.8 mg/dL), diabetic nephropathy (presence/absence), and chronic glomerulonephritis (presence/absence). All statistical analyses were performed using the Statistical Package for the Social Sciences software (PASW Statistics, version 22.0). All reported *p*-values are two-tailed and were considered to be statistically significant at <0.05.

RESULTS

Study Population

Of 305 HD patients 20 years or older who underwent measurements of muscle mass, physical performance, and muscle strength during the study period, those who had been receiving HD therapy for <6 months were excluded ($n = 3$). Furthermore, those with liver cirrhosis, malignancy, infection, or acute illness were also excluded from analysis ($n = 6$). None of the patients were receiving corticosteroid treatment. As a result, 296 HD patients (203 males, 93 females) were retrospectively analyzed. The cause of renal failure was diabetic nephropathy in 87 (29.4%), chronic glomerulonephritis in 80 (27.0%), nephrosclerosis in 25 (8.4%), polycystic kidney disease in 19 (6.4%), graft loss in 16 (5.4%), congenital anomaly in the kidneys or urinary tract in 4 (1.4%), unclassifiable nephritis in 4 (1.4%), kidney or urinary tract tumor in 3 (1.0%), malignant hypertension in 3 (1.0%), pregnancy-induced hypertension in 3 (1.0%), autoimmune nephritis in 1 (0.3%), chronic pyelonephritis in 1 (0.3%), kidney disease-related gout in 1 (0.3%), urinary tract obstruction in 1 (0.3%), other disease in 4 (1.4%), and unknown etiology in 44 (14.9%).

Clinical Characteristics of Subjects, and Comparisons Between XOR Inhibitor Users and Non-users

Subject characteristics ($n = 296$) are shown in **Table 1**. The prevalence of sarcopenia and severe sarcopenia in the entire cohort were 42.2% ($n = 125$) and 20.9% ($n = 62$), respectively. Among XOR inhibitor users ($n = 119$; allopurinol: 58, febuxostat, 61), height-adjusted muscle mass and handgrip strength were significantly ($p < 0.01$) higher, and the five-time chair stand test time was significantly lower as compared to the non-users ($n = 177$; **Table 1**). In addition, the prevalence of low muscle mass (47.1 vs. 65.0%), low physical performance (25.2 vs. 44.1%), and low muscle strength (47.1 vs. 64.4%) was significantly ($p < 0.01$) lower in XOR inhibitor users than in non-users. Finally, the prevalence rates for sarcopenia (33.6 vs. 48.0%) and severe sarcopenia (12.6 vs. 26.6%) were significantly ($p < 0.05$) reduced in the XOR inhibitor users.

Associations of XOR Inhibitor Use With Low Muscle Mass, Low Physical Performance, and Low Muscle Strength

To examine whether use of an XOR inhibitor is independently associated with low muscle mass, low physical performance, and/or low muscle strength, multivariate logistic regression analyses were performed (**Table 2**). XOR inhibitor use was shown to be significantly associated with low muscle mass [odds ratio (OR), 0.384; 95% confidence interval (CI), 0.183–0.806; $p = 0.011$] and low physical performance (OR, 0.286; 95% CI, 0.142–0.578; $p < 0.001$), while a borderline significance with low muscle strength was also noted (OR, 0.500; 95% CI, 0.248–1.006; $p = 0.052$). Additionally, serum uric acid level demonstrated a significant association with low physical performance (OR, 0.710; 95% CI, 0.560–0.899; $p = 0.005$). Furthermore, gender, BMI, and log CRP were significantly associated with low muscle mass, while age, cerebrovascular disease, use of vitamin D, and log CRP were significantly associated with low physical performance, and age, duration of hemodialysis, cerebrovascular disease, and diabetes mellitus were significantly associated with low muscle strength. On the other hand, serum albumin level was not significantly associated with low muscle mass, low physical performance, or low muscle strength (**Table 2**).

Associations of XOR Inhibitor Use With Sarcopenia and Severe Sarcopenia

To further examine whether XOR inhibitor use is independently associated with sarcopenia or severe sarcopenia, multivariate logistic regression analyses were again performed (**Table 3**). These results showed that use of an XOR inhibitor was significantly associated with sarcopenia (OR, 0.462; 95% CI, 0.226–0.947; $p = 0.035$) and severe sarcopenia (OR, 0.236; 95% CI, 0.091–0.614; $p = 0.003$). Notably, there was no remarkable

TABLE 1 | Clinical characteristics of subjects ($n = 296$), and comparisons between XOR inhibitor users and non-users.

	Total	XOR inhibitor users ($n = 119$)	XOR inhibitor non-users ($n = 177$)	P
Age, years	68.0 (57.0–74.0)	66.0 (54.0–71.0)	68.0 (61.0–77.0)	0.008
Male, *n*	203 (68.6)	89 (74.8)	114 (66.4)	0.036
BMI, kg/m^2	22.6 (20.3–25.3)	22.9 (20.7–26.0)	22.5 (19.9–24.8)	0.004
Duration of hemodialysis, years	6.5 (3.0–16.8)	6.0 (2.0–17.0)	7.0 (3.0–16.0)	0.367
Diabetes mellitus, *n*	171 (57.8)	56 (47.1)	115 (65.0)	0.295
Cerebrovascular disease, *n*	42 (14.2)	19 (16.0)	23 (13.0)	0.472
Vitamin D user, *n*	242 (81.8)	97 (81.5)	145 (81.9)	0.279
Insulin user, *n*	33 (11.1)	11 (9.2)	22 (12.4)	0.393
ACE inhibitor/ARB user, *n*	121 (40.9)	52 (43.7)	69 (39.0)	0.419
Albumin, g/dL	3.6 (3.4–3.8)	3.6 (3.4–3.8)	3.6 (3.4–3.8)	0.175
CRP, mg/dL	0.1 (0.1–0.3)	0.1 (0.1–0.4)	0.1 (0.1–0.2)	0.899
Uric acid, mg/dL	6.8 (5.8–7.5)	6.0 (4.9–7.2)	7.1 (6.3–7.7)	<0.001
Height-adjusted muscle mass, kg/m^2	6.2 (5.6–7.0)	6.7 (5.8–7.5)	6.1 (5.6–6.8)	<0.001
Five-time chair stand test, seconds	10.0 (8.1–13.4)	9.1 (7.7–12.0)	10.9 (8.6–14.9)	0.001
Handgrip strength, kg	24.1 (18.2–29.6)	26.5 (20.2–31.3)	22.5 (16.8–28.0)	0.001
Low muscle mass, *n*	171 (57.8)	56 (47.1)	115 (65.0)	<0.001
Low physical performance, *n*	108 (36.5)	30 (25.2)	78 (44.1)	0.005
Low muscle strength, *n*	170 (57.4)	56 (47.1)	114 (64.4)	0.006
Sarcopenia, *n*	125 (42.2)	40 (33.6)	85 (48.0)	0.003
Severe sarcopenia, *n*	62 (20.9)	15 (12.6)	47 (26.6)	0.013

Values are expressed as median (interquartile range) or number (%). P-values are shown for comparisons between XOR inhibitor users and non-users. BMI, body mass index; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker; CRP, C-reactive protein; XOR, xanthine oxidoreductase.

TABLE 2 | Multivariate logistic regression analysis of possible factors associated with low muscle mass, low physical performance, and low muscle strength.

	Low muscle mass		Low physical performance		Low muscle strength	
Variables	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age	1.015 (0.985-1.045)	0.340	1.084 (1.049–1.120)	<0.001	1.143 (1.101–1.187)	<0.001
Gender (male/female = 1/0)	12.709 (5.695–28.361)	<0.001	0.600 (0.326–1.103)	0.100	1.142 (0.576–2.264)	0.703
BMI	0.608 (0.534–0.693)	<0.001	1.069 (0.993–1.150)	0.076	1.038 (0.958–1.124)	0.360
Duration of hemodialysis	0.996 (0.961–1.033)	0.837	1.026 (0.994–1.059)	0.107	1.089 (1.047–1.134)	<0.001
Cerebrovascular disease (yes/no = 1/0)	2.429 (0.773–7.636)	0.129	3.034 (1.348–6.830)	0.007	4.275 (1.334–13.700)	0.014
Diabetes mellitus (yes/no = 1/0)	1.715 (0.825–3.564)	0.148	1.062 (0.575–1.958)	0.848	2.536 (1.266–5.083)	0.009
Vitamin D (yes/no = 1/0)	1.902 (0.848–4.267)	0.119	0.443 (0.213–0.922)	0.030	0.518 (0.233–1.151)	0.107
Albumin	0.460 (0.145–1.462)	0.188	0.790 (0.285–2.187)	0.649	0.396 (0.127–1.235)	0.111
Log CRP	6.429 (1.938–21.333)	0.002	3.001 (1.238–7.274)	0.015	1.250 (0.455–3.434)	0.665
Uric acid	0.925 (0.727–1.176)	0.524	0.710 (0.560–0.899)	0.005	0.816 (0.636–1.047)	0.110
XOR inhibitor (yes/no = 1/0)	0.384 (0.183–0.806)	0.011	0.286 (0.142–0.578)	<0.001	0.500 (0.248–1.006)	0.052

BMI, body mass index; CRP, C-reactive protein; XOR, xanthine oxidoreductase; OR, odds ratio; CI, confidence interval.

inconsistency observed among the results for the series of subgroups (**Figures 1A,B**). Age, gender, BMI, and history of cerebrovascular diseases were significantly associated with both sarcopenia and severe sarcopenia, while serum uric acid as well as CRP level were significantly associated with severe sarcopenia (OR, 0.715; 95% CI, 0.524–0.977; $p = 0.035$; **Table 3**). In contrast, serum albumin level, duration of hemodialysis, diabetes mellitus, and vitamin D use were not significantly associated with sarcopenia or severe sarcopenia (**Table 3**). When presence of XOR inhibitor was replaced by type and presence of XOR inhibitor, use of allopurinol and febuxostat (ref. absence of XOR inhibitor) was similarly associated with sarcopenia (OR 0.435, 95% CI 0.185–1.027, $p = 0.058$; OR 0.509, 95% CI 0.182–1.425, $p = 0.198$, respectively) and severe sarcopenia (OR 0.298, 95% CI 0.099–0.892, $p = 0.030$; OR 0.163, 95% CI 0.042–0.634, $p = 0.009$, respectively), suggesting that differences among types of XOR inhibitors have little effect on sarcopenia/severe sarcopenia. When use of insulin or ACE inhibitors/ARBs was added to the multivariate logistic regression model, use of XOR inhibitors remained significantly associated with sarcopenia (OR

TABLE 3 | Multivariate logistic regression analysis of possible factors associated with sarcopenia and severe sarcopenia.

	Sarcopenia		Severe sarcopenia	
Variables	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Age	1.090 (1.054–1.127)	<0.001	1.086 (1.041–1.133)	<0.001
Gender (male/female = 1/0)	8.746 (4.085–18.724)	<0.001	4.178 (1.768–9.874)	0.001
BMI	0.799 (0.726–0.880)	<0.001	0.773 (0.684–0.874)	<0.001
Duration of hemodialysis	1.029 (0.995–1.064)	0.101	1.013 (0.975–1.052)	0.510
Cerebrovascular disease (yes/no = 1/0)	3.649 (1.370–9.721)	0.010	2.703 (1.106–6.608)	0.029
Diabetes mellitus (yes/no = 1/0)	1.769 (0.911–3.437)	0.092	1.063 (0.493–2.289)	0.876
Vitamin D (yes/no = 1/0)	0.713 (0.332–1.533)	0.387	0.431 (0.178–1.039)	0.061
Albumin	0.421 (0.139–1.273)	0.126	0.665 (0.187–2.372)	0.530
Log CRP	1.421 (0.529–3.823)	0.486	4.254 (1.501–12.054)	0.006
Uric acid	0.836 (0.653–1.071)	0.157	0.715 (0.524–0.977)	0.035
XOR inhibitor (yes/no = 1/0)	0.462 (0.226–0.947)	0.035	0.236 (0.091–0.614)	0.003

BMI, body mass index; CRP, C-reactive protein; XOR, xanthine oxidoreductase; OR, odds ratio; CI, confidence interval.

0.467, 95% CI 0.228–0.957, $p = 0.038$; OR 0.463, 95% CI 0.226–0.948, $p = 0.035$, respectively) and severe sarcopenia (OR 0.238, 95% CI 0.091–0.620, $p = 0.003$; OR, 0.236, 95% CI 0.091–0.614, $p = 0.003$, respectively).

DISCUSSION

In this first known study to investigate the association of XOR inhibitor treatment with sarcopenia in HD patients, the following primary findings were obtained. First, the rates of prevalence of sarcopenia and severe sarcopenia, determined based on the AWGS 2019 criteria, were 42.2 and 20.9%, respectively (**Table 1**). Furthermore, XOR inhibitor use was shown to be significantly associated with reduced risk of low muscle mass and low physical performance, while a borderline significance with low muscle strength was also found (**Table 2**). Finally, XOR inhibitor use was significantly associated with reduced risk of both sarcopenia and severe sarcopenia (**Table 3**). Together, the present results suggest that treatment with an XOR inhibitor exerts protective effects to reduce the risk of sarcopenia and severe sarcopenia, which are frequently observed in HD patients.

A diagnostic algorithm for sarcopenia has been proposed by several different western groups, including the Foundation for the National Institutes of Health Sarcopenia Project (29), European Working Group on Sarcopenia in Older People (30, 31), and International working group on sarcopenia (32). However, because of anthropometric as well as cultural and/or lifestyle-related differences in Asia as compared with Western populations, the AWGS proposed a diagnostic algorithm for sarcopenia based on Asian data in 2014 (33), which was updated in 2019 (16). Previous studies including ours have reported the prevalence of sarcopenia in HD patients based on the AWGS 2014 criteria as ranging from 33.7 to 40% (2, 3), though no studies reporting that prevalence in HD patients based on the AWGS 2019 criteria have been presented prior to the present report (**Table 1**).

Previous clinical studies that examined Tour de France cyclists and professional soccer players have found that administration of an XOR inhibitor protects against skeletal muscle damage caused by exhaustive exercise (34, 35). Furthermore, other studies have noted that XOR inhibitor administration protects against skeletal muscle atrophy caused by immobilization following an ankle sprain in male subjects (36) and improved functional outcomes after rehabilitation in older patients (37), suggesting that XOR inhibitor administration contributes to protect against sarcopenia. However, to the best of our knowledge, no investigation of the association of XOR inhibitor treatment with sarcopenia has been presented. The present results are the first to demonstrate associations of XOR inhibitor use with reduced levels of sarcopenia and severe sarcopenia (**Table 3**), as well as its protection against low levels of muscle mass, physical performance, and muscle strength (**Table 2**).

Although it remains unclear why XOR inhibitor use has been found to be associated with those factors, decreased ROS production could be a potential reason. XOR is involved in production of ROS and uric acid, and expressed in the vascular endothelium of skeletal muscle (38), as well as in the liver and intestines (39, 40). Previous studies have shown that administration of an XOR inhibitor protected against skeletal muscle atrophy caused by unloading in mice and rats by inhibiting activation of the ubiquitin-proteasome pathway via decreased ROS production in skeletal muscle (36, 41). In addition, XOR inhibitor administration was reported to increase maximal isometric force in aged mice by decreasing ROS production in skeletal muscle (42). Therefore, treatment with an XOR inhibitor may contribute to preservation of skeletal muscle mass and improvement of skeletal muscle function by reducing ROS production in skeletal muscle. On the other hand, increased uric acid level was found to be significantly associated with a reduced risk of low physical performance (**Table 2**) and severe sarcopenia (**Table 3**). Uric acid has been shown to gain antioxidant properties by scavenging ROS in a hydrophilic condition, such as by circulating in blood (43), and when present

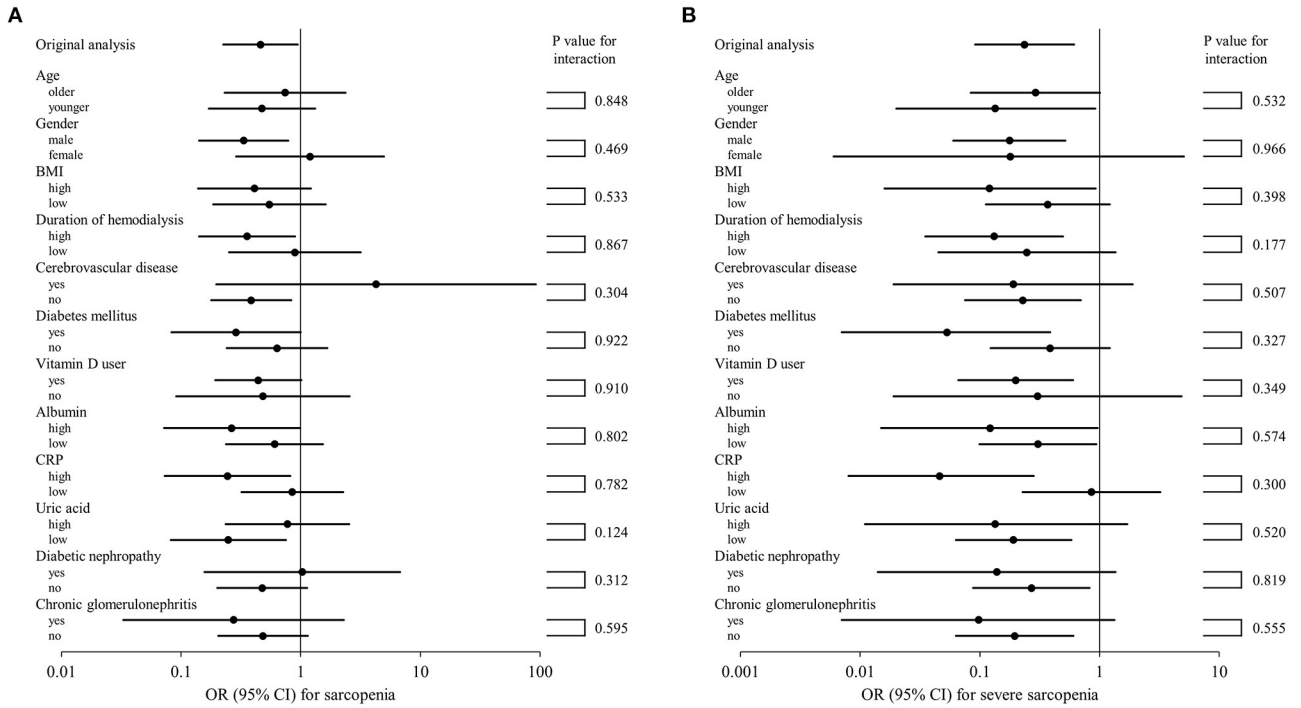

FIGURE 1 | Subgroup analysis of association of XOR inhibitor use with sarcopenia **(A)** and severe sarcopenia **(B)**. Forrest plots showing OR (95% CI) in squares and lines for each category are presented. The 95% CI values shown were calculated within each category and *p*-values for subgroup analyses represent tests for interaction. The higher and lower groups were defined as greater and less than or equal to the median, respectively. BMI, body mass index; CRP, C-reactive protein; XOR, xanthine oxidoreductase; OR, odds ratio; CI, confidence interval.

in the circulation is thought to have a protective effect against sarcopenia (44–46). Since renal excretion of uric acid is reduced in HD patients, administration of an XOR inhibitor may exert a protective effect against sarcopenia by increasing the level of uric acid level relative to XOR activity in circulating blood.

Another possibility that can potentially explain the protective effects of XOR inhibitor treatment is its effect to conserve the intracellular level of ATP, thus providing energy necessary for muscle contractions (47). In skeletal muscle, ATP production has shown to be decreased under a uremic condition (48) and ATP levels have been shown to be significantly lower in uremic patients as compared to control subjects (15, 49). Of importance, administration of an XOR inhibitor enhances the intracellular ATP level by increasing the purine salvage pathway through use of hypoxanthine and decreasing energy expenditure by reducing *de novo* purine synthesis (9–12). Taken together, these findings indicate that XOR inhibitor treatment might exert protective effects on sarcopenia in HD patients through preservation of skeletal muscle mass and improvement of skeletal muscle function by ATP enhancement in skeletal muscle.

Previous studies have found associations of age, gender, degree of obesity, duration of hemodialysis, cerebrovascular disease, diabetes mellitus, vitamin D use, inflammation, and uric acid with sarcopenia or its components in HD patients (3, 7, 13, 25–27). In the present investigation as well, age, gender, BMI, duration of hemodialysis, history of cerebrovascular disease, presence of diabetes mellitus, use of oral and/or intravenous vitamin D, CRP, and uric acid level were shown to be significantly associated with some or all of the factors low muscle mass, low physical performance, low muscle strength, sarcopenia, and severe sarcopenia. On the other hand, while nutritional status has also been reported to be associated with sarcopenia in HD patients (28), serum albumin level showed no significant association with low muscle mass, low physical performance, low muscle strength, sarcopenia, or severe sarcopenia in our study (**Tables 2**, **3**). Although serum albumin level is considered to be an indicator of nutritional status, that level may not adequately reflect nutritional status in HD patients, since it did not show a significant association with sarcopenia or related components. In addition, nutritional intake has been reported to be associated with sarcopenia in older subjects (50), though unfortunately information regarding nutritional intake in the present subjects was not obtained. In future investigations of the relationship between XOR inhibitors and sarcopenia, it will be necessary to include accurate information regarding nutritional intake and status in the analysis.

This study has several limitations. First is the cross-sectional design, thus even though relationships were explored in predictive terms, the results cannot be interpreted to show causal relationships. Second, while physical activity has been shown to influence XOR activity (51–53), no survey of physical activity was conducted in the present study. Furthermore, we

were unable to fully investigate the association of dosage, duration, or indication of XOR inhibitor administration with sarcopenia. Third, because of the methods used, ROS, oxidative stress, and ATP levels in skeletal muscle and blood were not determined. Fourth, since muscle mass, physical performance, and muscle strength were examined as part of routine clinical care, patients unable to perform related tests due to reduced activities of daily living were not included in the present study. Fifth, there were few cases of severe sarcopenia, thus the 10 events per variable rule (54) could not be used when performing multivariate logistic regression for severe sarcopenia. A large-scale prospective interventional study that includes measurements of ROS, oxidative stress, and ATP levels in skeletal muscle and blood, as well as physical activity, and also includes analysis of dosage, duration, and XOR inhibitor indication in HD patients, including those with markedly reduced activities of daily living, is needed to clarify the role of XOR inhibitor treatment for prevention and treatment of sarcopenia in HD patients. Finally, the present study population consisted of nearly exclusively Japanese patients with HD, thus it is unclear whether the findings can generalized for other ethnic groups or non-HD subjects.

In conclusion, the present results showed that XOR inhibitor use in HD patients is significantly associated with reduced risk of sarcopenia and severe sarcopenia, as well as low muscle mass and low physical performance, based on the AWGS 2019 criteria.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to Masafumi Kurajoh, m1155129@med.osaka-cu.ac.jp.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Inoue Hospital Ethics Committee. The ethics committee waived the requirement of written informed consent for participation.

AUTHOR CONTRIBUTIONS

MK contributed to study design, interpretation, and writing of the manuscript. KM, MM, SM, and AK contributed to study design and interpretation. YT contributed to study design, data analysis, and interpretation. ME reviewed the manuscript. All authors have read and approved the final version of the manuscript.

REFERENCES

1. Rosenberg IH. Sarcopenia: origins and clinical relevance. *J Nutr.* (1997) 127(5 Suppl.):990S−1S. doi: 10.1093/jn/127.5.990S
2. Kim JK, Choi SR, Choi MJ, Kim SG, Lee YK, Noh JW, et al. Prevalence of and factors associated with sarcopenia in elderly patients with end-stage renal disease. *Clin Nutr.* (2014) 33:64–8. doi: 10.1016/j.clnu.2013.04.002
3. Mori K, Nishide K, Okuno S, Shoji T, Emoto M, Tsuda A, et al. Impact of diabetes on sarcopenia and mortality in patients undergoing hemodialysis. *BMC Nephrol.* (2019) 20:105. doi: 10.1186/s12882-019-1271-8
4. Goodkin DA, Bragg-Gresham JL, Koenig KG, Wolfe RA, Akiba T, Andreucci VE, et al. Association of comorbid conditions and mortality in hemodialysis patients in Europe, Japan, and the United States: the Dialysis Outcomes and Practice Patterns Study (DOPPS). *J Am Soc Nephrol.* (2003) 14:3270–7. doi: 10.1097/01.ASN.0000100127.54107.57
5. Inaba M, Karaboyas A, Akiba T, Akizawa T, Saito A, Fukuhara S, et al. Association of blood pressure with all-cause mortality and stroke in Japanese hemodialysis patients: the Japan Dialysis Outcomes and Practice Pattern Study. *Hemodial Int.* (2014) 18:607–15. doi: 10.1111/hdi.12156
6. Yamada S, Yamamoto S, Fukuma S, Nakano T, Tsuruya K, Inaba M. Geriatric nutritional risk index (GNRI) and creatinine index equally predict the risk of mortality in hemodialysis patients: J-DOPPS. *Sci Rep.* (2020) 10:5756. doi: 10.1038/s41598-020-62720-6
7. Ren H, Gong D, Jia F, Xu B, Liu Z. Sarcopenia in patients undergoing maintenance hemodialysis: incidence rate, risk factors and its effect on survival risk. *Ren Fail.* (2016) 38:364–71. doi: 10.3109/0886022X.2015.1132173
8. Hille R, Nishino T. Flavoprotein structure and mechanism. 4. Xanthine oxidase and xanthine dehydrogenase. *FASEB J.* (1995) 9:995–1003. doi: 10.1096/fasebj.9.11.7649415
9. Nomura J, Kobayashi T, So A, Busso N. Febuxostat, a xanthine oxidoreductase inhibitor, decreases NLRP3-dependent inflammation in macrophages by activating the purine salvage pathway and restoring cellular bioenergetics. *Sci Rep.* (2019) 9:17314. doi: 10.1038/s41598-019-53965-x
10. Kato S, Kato M, Kusano T, Nishino T. New strategy that delays progression of amyotrophic lateral sclerosis in G1H-G93A transgenic mice: oral administration of xanthine oxidoreductase inhibitors that are not substrates for the purine salvage pathway. *J Neuropathol Exp Neurol.* (2016) 75:1124–44. doi: 10.1093/jnen/nlw088
11. Johnson TA, Jinnah HA, Kamatani N. Shortage of cellular ATP as a cause of diseases and strategies to enhance ATP. *Front Pharmacol.* (2019) 10:98. doi: 10.3389/fphar.2019.00098
12. Fujii K, Kubo A, Miyashita K, Sato M, Hagiwara A, Inoue H, et al. Xanthine oxidase inhibitor ameliorates postischemic renal injury in mice by promoting resynthesis of adenine nucleotides. *JCI Insight.* (2019) 4:e124816. doi: 10.1172/jci.insight.124816
13. Fahal IH. Uraemic sarcopenia: aetiology and implications. *Nephrol Dial Transplant.* (2014) 29:1655–65. doi: 10.1093/ndt/gft070
14. Takemura K, Nishi H, Inagi R. Mitochondrial dysfunction in kidney disease and uremic sarcopenia. *Front Physiol.* (2020) 11:565023. doi: 10.3389/fphys.2020.565023
15. Del Canale S, Fiaccadori E, Ronda N, Soderlund K, Antonucci C, Guariglia A. Muscle energy metabolism in uremia. *Metabolism.* (1986) 35:981–3. doi: 10.1016/0026-0495(86)90032-6
16. Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, et al. Asian working group for sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *J Am Med Dir Assoc.* (2020) 21:300–7e2. doi: 10.1016/j.jamda.2019.12.012
17. Cheung YM, Roff G, Grossmann M. Precision of the Hologic Horizon A dual energy X-ray absorptiometry in the assessment of body composition. *Obes Res Clin Pract.* (2020) 14:514–8. doi: 10.1016/j.orcp.2020.10.005
18. Matsufuji S, Shoji T, Yano Y, Tsujimoto Y, Kishimoto H, Tabata T, et al. Effect of chair stand exercise on activity of daily living: a randomized controlled trial in hemodialysis patients. *J Ren Nutr.* (2015) 25:17–24. doi: 10.1053/j.jrn.2014.06.010

19. Ozden F, Coskun G, Bakirhan S. The test-retest reliability and concurrent validity of the five times sit to stand test and step test in older adults with total hip arthroplasty. *Exp Gerontol.* (2020) 142:111143. doi: 10.1016/j.exger.2020.111143
20. Nishimura T, Arima K, Okabe T, Mizukami S, Tomita Y, Kanagae M, et al. Usefulness of chair stand time as a surrogate of gait speed in diagnosing sarcopenia. *Geriatr Gerontol Int.* (2017) 17:659–61. doi: 10.1111/ggi.12766
21. Inaba M, Kurajoh M, Okuno S, Imanishi Y, Yamada S, Mori K, et al. Poor muscle quality rather than reduced lean body mass is responsible for the lower serum creatinine level in hemodialysis patients with diabetes mellitus. *Clin Nephrol.* (2010) 74:266–72. doi: 10.2379/CNX06500
22. Kurajoh M, Inaba M, Motoyama K, Kuriyama N, Ozaki E, Koyama T, et al. Inverse association of plasma leptin with cortical thickness at distal radius determined with a quantitative ultrasound device in patients with type 2 diabetes mellitus. *J Diabetes Invest.* (2019) 11:174–83. doi: 10.1111/jdi.13071
23. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2004) 27(Suppl. 1):S5–10. doi: 10.2337/diacare.27.2007.S5
24. Miyabe M, Kurajoh M, Mori K, Okuno S, Okada S, Emoto M, et al. Superiority of glycated albumin over glycated haemoglobin as indicator of glycaemic control and predictor of all-cause mortality in patients with type 2 diabetes mellitus receiving peritoneal dialysis. *Ann Clin Biochem.* (2019) 56:684–91. doi: 10.1177/0004563219873688
25. Yoowannakul S, Tangvoraphonkchai K, Vongsanim S, Mohamed A, Davenport A. Differences in the prevalence of sarcopenia in haemodialysis patients: the effects of gender and ethnicity. *J Hum Nutr Diet.* (2018) 31:689–96. doi: 10.1111/jhn.12555
26. Kittiskulnam P, Carrero JJ, Chertow GM, Kaysen GA, Delgado C, Johansen KL. Sarcopenia among patients receiving hemodialysis: weighing the evidence. *J Cachexia Sarcopenia Muscle.* (2017) 8:57–68. doi: 10.1002/jcsm.12130
27. Cha RH, Lee GS, Yoo JY, Rhee OB, Jeon YD. Hand grip and leg muscle strength in hemodialysis patients and its determinants. *J Korean Med Sci.* (2021) 36:e76. doi: 10.3346/jkms.2021.36.e76
28. Bakkal H, Dizdar OS, Erdem S, Kulakoglu S, Akcakaya B, Katircilar Y, et al. The relationship between hand grip strength and nutritional status determined by malnutrition inflammation score and biochemical parameters in hemodialysis patients. *J Ren Nutr.* (2020) 30:548–55. doi: 10.1053/j.jrn.2020.01.026
29. Studenski SA, Peters KW, Alley DE, Cawthon PM, McLean RR, Harris TB, et al. The FNIH sarcopenia project: rationale, study description, conference recommendations, and final estimates. *J Gerontol A Biol Sci Med Sci.* (2014) 69:547–58. doi: 10.1093/gerona/glu010
30. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. *Age Ageing.* (2010) 39:412–23. doi: 10.1093/ageing/afq034
31. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* (2019) 48:16–31. doi: 10.1093/ageing/afy169
32. Fielding RA, Vellas B, Evans WJ, Bhasin S, Morley JE, Newman AB, et al. Sarcopenia: an undiagnosed condition in older adults. Current consensus definition: prevalence, etiology, and consequences. International working group on sarcopenia. *J Am Med Dir Assoc.* (2011) 12:249–56. doi: 10.1016/j.jamda.2011.01.003
33. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc.* (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025
34. Gomez-Cabrera MC, Pallardo FV, Sastre J, Vina J, Garcia-del-Moral L. Allopurinol and markers of muscle damage among participants in the Tour de France. *JAMA.* (2003) 289:2503–4. doi: 10.1001/jama.289.19.2503-b
35. Sanchis-Gomar F, Pareja-Galeano H, Gomez-Cabrera MC, Candel J, Lippi G, Salvagno GL, et al. Allopurinol prevents cardiac and skeletal muscle damage in professional soccer players. *Scand J Med Sci Sports.* (2015) 25:e110–5. doi: 10.1111/sms.12213
36. Ferrando B, Gomez-Cabrera MC, Salvador-Pascual A, Puchades C, Derbre F, Gratas-Delamarche A, et al. Allopurinol partially prevents disuse muscle atrophy in mice and humans. *Sci Rep.* (2018) 8:3549. doi: 10.1038/s41598-018-21552-1
37. Beveridge LA, Ramage L, McMurdo ME, George J, Witham MD. Allopurinol use is associated with greater functional gains in older rehabilitation patients. *Age Ageing.* (2013) 42:400–4. doi: 10.1093/ageing/aft046
38. Linder N, Rapola J, Raivio KO. Cellular expression of xanthine oxidoreductase protein in normal human tissues. *Lab Invest.* (1999) 79:967–74.
39. Krenitsky TA, Tuttle JV, Cattau EL Jr, Wang P. A comparison of the distribution and electron acceptor specificities of xanthine oxidase and aldehyde oxidase. *Comp Biochem Physiol B.* (1974) 49:687–703. doi: 10.1016/0305-0491(74)90256-9
40. Saksela M, Lapatto R, Raivio KO. Xanthine oxidoreductase gene expression and enzyme activity in developing human tissues. *Biol Neonate.* (1998) 74:274–80. doi: 10.1159/000014034
41. Derbre F, Ferrando B, Gomez-Cabrera MC, Sanchis-Gomar F, Martinez-Bello VE, Olaso-Gonzalez G, et al. Inhibition of xanthine oxidase by allopurinol prevents skeletal muscle atrophy: role of p38 MAPKinase and E3 ubiquitin ligases. *PLoS ONE.* (2012) 7:e46668. doi: 10.1371/journal.pone.0046668
42. Ryan MJ, Jackson JR, Hao Y, Leonard SS, Alway SE. Inhibition of xanthine oxidase reduces oxidative stress and improves skeletal muscle function in response to electrically stimulated isometric contractions in aged mice. *Free Radic Biol Med.* (2011) 51:38–52. doi: 10.1016/j.freeradbiomed.2011.04.002
43. Muraoka S, Miura T. Inhibition by uric acid of free radicals that damage biological molecules. *Pharmacol Toxicol.* (2003) 93:284–9. doi: 10.1111/j.1600-0773.2003.pto930606.x
44. Molino-Lova R, Sofi F, Pasquini G, Vannetti F, Del Ry S, Vassalle C, et al. Higher uric acid serum levels are associated with better muscle function in the oldest old: results from the Mugello Study. *Eur J Intern Med.* (2017) 41:39–43. doi: 10.1016/j.ejim.2017.03.014
45. Macchi C, Molino-Lova R, Polcaro P, Guarducci L, Lauretani F, Cecchi F, et al. Higher circulating levels of uric acid are prospectively associated with better muscle function in older persons. *Mech Ageing Dev.* (2008) 129:522–7. doi: 10.1016/j.mad.2008.04.008
46. Floriano JP, Nahas PC, de Branco FMS, Dos Reis AS, Rossato LT, Santos HO, et al. Serum uric acid is positively associated with muscle mass and strength, but not with functional capacity, in kidney transplant patients. *Nutrients.* (2020) 12:2390. doi: 10.3390/nu12082390
47. Miller SG, Hafen PS, Brault JJ. Increased adenine nucleotide degradation in skeletal muscle atrophy. *Int J Mol Sci.* (2019) 21:88. doi: 10.3390/ijms21010088
48. Su Z, Klein JD, Du J, Franch HA, Zhang L, Hassounah F, et al. Chronic kidney disease induces autophagy leading to dysfunction of mitochondria in skeletal muscle. *Am J Physiol Renal Physiol.* (2017) 312:F1128–40. doi: 10.1152/ajprenal.00600.2016
49. Nishida A, Kubo K, Nihei H. Impaired muscle energy metabolism in uremia as monitored by 31P-NMR. *Nihon Jinzo Gakkai Shi.* (1991) 33:65–73.
50. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the health, aging, and body composition (health ABC) study. *Am J Clin Nutr.* (2008) 87:150–5. doi: 10.1093/ajcn/87.1.150
51. Hellsten Y, Hansson HA, Johnson L, Frandsen U, Sjodin B. Increased expression of xanthine oxidase and insulin-like growth factor I (IGF-I) immunoreactivity in skeletal muscle after strenuous exercise in humans. *Acta Physiol Scand.* (1996) 157:191–7. doi: 10.1046/j.1365-201X.1996.492235000.x
52. Hellsten Y, Frandsen U, Orthenblad N, Sjodin B, Richter EA. Xanthine oxidase in human skeletal muscle following eccentric exercise: a role in inflammation. *J Physiol.* (1997) 498(Pt 1):239–48. doi: 10.1113/jphysiol.1997.sp021855
53. Kosaki K, Kamijo-Ikemori A, Sugaya T, Tanahashi K, Akazawa N, Hibi C, et al. Habitual exercise decreases plasma xanthine oxidoreductase activity in middle-aged and older women. *J Clin Biochem Nutr.* (2018) 62:247–53. doi: 10.3164/jcbn.17-101
54. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression

analysis. *J Clin Epidemiol.* (1996) 49:1373–9. doi: 10.1016/S0895-4356(96)00236-3

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Plasma tumor necrosis factor-α is associated with sarcopenia in elderly individuals residing in agricultural and pastoral areas of Xinjiang, China

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Background: Inflammatory reactions play a significant role in the occurrence and development of sarcopenia. Determining the association between specific cytokines and sarcopenia may reveal the disease's pathophysiological mechanism(s). Accordingly, the present study aimed to investigate the association between sarcopenia and inflammatory cytokines among the elderly natural population in agricultural and pastoral areas of Xinjiang.

Methods: We conducted a cross-sectional epidemiological survey of the community-dwelling older people using a multi-stage random sampling method in Mulei County in northern Xinjiang and Luopu County in southern Xinjiang from September 2017 to May 2018. Of the 2,100 participants, the statistical analyses included 1,838 participants with complete data. Comparisons of living habits, disease status, biochemical indexes, and levels of interleukin (IL)-4, IL-6, IL-8, IL-10, and tumor necrosis factor (TNF)-α in sarcopenia and non-sarcopenia participants were made in this study.

Results: Our study revealed no significant differences (i.e., $P > 0.05$) in sex, age, ethnicity, smoking and drinking habits, serum renal function, total cholesterol, and diabetes in the elderly between the sarcopenia and non-sarcopenia groups in Xinjiang. However, triglyceride levels ($P = 0.004$), hypertension ($P = 0.019$), and abdominal obesity ($P < 0.001$) in the sarcopenia group were significantly higher than those in the non-sarcopenia group. Moreover, the levels of IL-10 ($P < 0.001$), IL-4 ($P < 0.001$), and TNF-α ($P < 0.001$) in the sarcopenia group were higher than those in the non-sarcopenia group after adjusting for sex, age, hypertension, blood lipid concentration, and obesity. Furthermore, after adjusting for sex, age, hypertension, obesity, and IL-10, IL-4, and IL-6 levels, an increased TNF-α level was also significantly associated with sarcopenia.

Conclusion: The results of the present study suggest that an increased plasma level of TNF-α is significantly associated with sarcopenia among elderly individuals residing in Xinjiang's agricultural and pastoral areas. Further study is still needed to determine the physiological role of "immune aging" in the pathogenesis of sarcopenia.

KEYWORDS

elderly population, inflammatory cytokines, plasma tumor necrosis factor-α, sarcopenia, Xinjiang

Introduction

China has gradually witnessed the emergence of an aging population since 2000; accordingly, the incidence of various chronic diseases has been increasing (1). Sarcopenia has become a significant public health problem. Sarcopenia is a systemic condition involving muscle mass reduction and/or decline in muscle strength and function. It seriously endangers the health of elderly individuals and significantly reduces their quality of life. Individuals with sarcopenia have more difficulty with daily activities and are at a higher risk of infection, fall(s), disability, and/or death (2).

Sarcopenia is characterized by lower levels of exercise tolerance and decreased neuromuscular function. Its etiology includes the following: age-related hormonal changes, increased levels of pro-inflammatory cytokines, myocyte apoptosis, genetic factors, and low nutrient intake (3, 4). Previous studies have reported that the prevalence of sarcopenia in elderly individuals is approximately 10–20% and increases with age, with a prevalence of 5–13% in those 60–70 years of age and 50% in the elderly, i.e., in people > 80 years of age (5–7). In addition, due to the close relationship between sarcopenia and common diseases, the difficulty of treatment increases for patients with comorbid diseases, the period of hospitalization is prolonged, and the burden of social care and medical expenses increases significantly (8, 9).

Therefore, discovering the potential pathophysiological mechanisms of sarcopenia has become increasingly vital, gaining consensus. Recently, some studies have reported that the occurrence and development of sarcopenia and the subsequent deterioration of individuals with this disease are all accompanied by different degrees of inflammatory reactions (10–12). More specifically, a variety of cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, and IL-10 play a role in the development of sarcopenia (13, 14). However, to our knowledge, the current literature on the relationship between sarcopenia and inflammatory cytokines remains unclear. Therefore, the present study explores the relationship between sarcopenia and IL-4, IL-6, IL-10, and TNF-α in the elderly population of agricultural and pastoral areas of Xinjiang, China, and is expected to lay a preliminary foundation for understanding its underlying mechanism.

Materials and methods

Study participants

The present investigation was a case-control study based on a cross-sectional epidemiological survey of sarcopenia in agricultural and pastoral areas of Xinjiang. The epidemiological survey was performed from September 2017 to May 2018 using a multi-stage random sampling method. In the first stage, two counties, Mulei County in Northern Xinjiang and Luopu County in southern Xinjiang, were selected; in the second stage, six towns were randomly selected from each county; in the third stage, five villages were randomly selected from each township; and, in the fourth stage, 35 elderly individuals were randomly selected using random number software from each village, which administrative village with household registration base books were provided by the relevant departments, followed by numbering the households in a particular order. A total of 2,100 subjects were included, of whom 1,838 completed the survey, corresponding to a response rate of 87.52%.

A total of 152 elderly individuals ≥ 60 years of age were randomly selected from a database established based on the above epidemiological survey using computer-generated balanced block randomization and randomized 1:1 to two groups: sarcopenia (n = 76) and non-sarcopenia (n = 76). All subjects provided informed consent to participate. The present study was reviewed and approved by the Medical Ethics Committee of Xinjiang Uygur Autonomous Region People's Hospital. The selection procedures of our study are as follows (**Figure 1**).

Inclusion/exclusion criteria

Individuals fulfilling the following criteria were included in the study: age ≥ 60 years; permanent

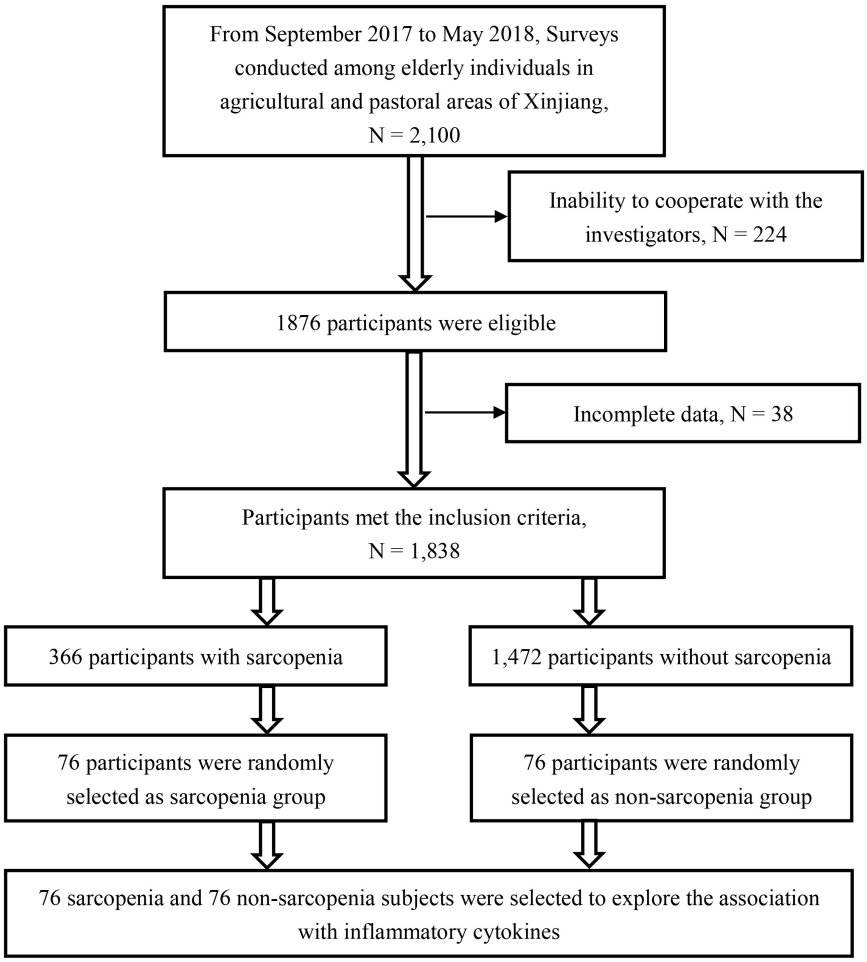

FIGURE 1
Selection procedure of the study participants.

residents with local household registration; able to walk independently without using assistive devices; and informed consent. Individuals with severe cognitive impairment, mental illness, a history of major organ failure (such as respiratory failure, heart failure, renal failure, and liver failure), recent surgical history, and consumptive disease(s) such as malignant tumor and/or tuberculosis were excluded.

Questionnaire survey

All subjects provided informed consent to participate and completed the questionnaire under the guidance of investigators. The questionnaire addressed age, sex, disease, medication, family and menstrual histories, regular exercise patterns and amount of exercise, fall(s), alcohol consumption, dietary habits, and smoking history.

Physical examination

The following parameters were measured by trained and qualified professionals in accordance with standard methods: blood pressure, weight, height, waist circumference, hip circumference, and body mass index (BMI). BMI was calculated as weight (kg) divided by height (m) squared (i.e., kg/m^2). Blood pressure levels were measured using an automatic blood pressure monitor. Readings were obtained after 5 min of seated rest. Three blood pressure measurements were obtained at 30-s intervals. The mean of all available measurements was used to define the systolic and diastolic blood pressure levels. In addition, the following indicators were measured.

Skeletal muscle strength

The grip strength test was performed using a handheld dynamometer (Jamar, Duluth, MN, United States), and the

results reflected skeletal muscle strength. Before the test, the grip distance was adjusted to within the proper range. During the test, the subjects were seated, with the upper arm and forearm angled at 90°, and the test arm was slightly extended but not > 30°. The instrument was gripped with maximum force using the right and left hands twice. Maximum values were recorded, and in cases where the data difference between the two sides was small, statistical analysis was conducted with data gathered for the right hand.

Skeletal muscle quality

A body composition analyzer (InBody720, InBody Co., Ltd., Cerritos, CA, United States) was used to assess skeletal muscle quality in the subjects' limbs. Subjects fasted for 2 h before measurement. During the test, the subjects were required to empty their bladders, rest for 1 h, and then stand still for 5 min. Subsequently, they removed their shoes and socks, and a small amount of alcohol was applied to the soles of their feet and fingers. Subjects stood on the foot electrodes of the test platform and were held by electrodes placed in the palms of both hands. Their arms were separated from the trunk by approximately 30°, and their bodies remained still during the test.

Skeletal muscle activity/function

Skeletal muscle activity was ascertained by measuring the maximum walking speed of the subjects. The colored tape was used to mark a 16-m straight line, with markings at the starting point, 3.00 m, 13.00 m, and the end. When subjects reached the 3.00-m point, the timing was started, and when they reached 13.00 m, the timing was ended. Three tests were conducted, and the fastest was included in the statistical analysis.

Collection of blood samples and laboratory testing

Blood was collected from all subjects using disposable blood collection equipment to extract 10 ml of sample (fasting at least 10 h). Sampling was performed in the morning, and all samples were centrifuged immediately to separate plasma (serum) and blood cells. Plasma and blood cells were stored at –80℃ until use.

A biochemical analysis system (Hitachi 7600 Clinical Series Analyzer, Hitachi, Tokyo, Japan) was used to assess blood lipid and glucose levels and other biochemical indicators, including fasting blood glucose (FBG), serum creatinine and urea nitrogen, total cholesterol (TC), total triglyceride (TG), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol.

Inflammatory cytokines

The levels of IL-6, IL-4, IL-10, and TNF-α were determined using commercially available ELISA kits [MULTISCIENCES (LIANKE) BIOTECH, CO., LTD, Hangzhou, China] as per the manufacturer's instructions.

Related diagnostic criteria

Sarcopenia

According to the diagnostic criteria for sarcopenia from the 2014 Asian Sarcopenia Working Group (AWG) (15), subjects meeting any two of the following criteria were considered sarcopenia patients: (i) decreased skeletal muscle mass (male < 7.0 kg/m^2, female < 5.7 kg/m^2); (ii) decreased grip strength (male < 26 kg, female < 18 kg); and (iii) decreased walking speed (<0.8 m/s).

Hypertension

Hypertension was diagnosed based on the Chinese Guidelines for the Prevention and Treatment of Hypertension (revised in 2018). For patients not using antihypertensive drugs, consultation room blood pressure was measured three times on different days, with systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg. Patients diagnosed with hypertension and taking antihypertensive drugs were also monitored.

Type II diabetes

According to the Chinese Guidelines for the Prevention and Treatment of Type II Diabetes Mellitus, 2017 Edition, patients with (i) typical diabetic symptoms (polydipsia, polyuria, overeating, unexplained weight loss) and random blood glucose ≥ 11.1 mmol/L; (ii) FBG ≥ 7.0 mmol/L; (iii) blood glucose at 2 h after glucose loading > 11.1 mmol/L; (iv) and patients diagnosed with type 2 diabetes and taking hypoglycemic drugs were monitored. Patients without typical symptoms of diabetes were re-examined on another day.

Abdominal obesity

According to the National Cholesterol Education Program (ATP-III), abdominal obesity was defined as waist circumference ≥ 102 cm (40 inches) for men and 88 cm (35 inches) for women.

Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, IL, United States) was adopted for data processing. Measurement data are expressed as mean ± standard deviation, and count data are expressed as the number of cases or percentages. Comparison

of clinical phenotypic measurement data between groups was performed using a *t*-test (between two groups), and that for the classification data was performed using a chi-squared test. Covariance analysis was used to explore the correlation between sarcopenia and inflammatory cytokines in the control and case groups after adjusting for sex, hypertension, blood lipids, and obesity. Binary logistics analysis was used to explore independent factors for sarcopenia in this population. To investigate the differences of four serum inflammatory cytokines between the sarcopenia group and the non-sarcopenia group, a partial least squares-discriminant analysis (PLS-DA) was initially performed using MetaboAnalyst (version 5.0).[1] Data of serum inflammatory cytokines were abundances normalized by median, log-transformed, and auto-scaled before PLS-DA. Then, a variable selection approach named variable importance in projection (VIP) was used to detect the essential inflammatory cytokines associated with case patients. In addition, the classification models were performed. Statistical significance was identified using an area under the receiver operating characteristic curve (AUROC). The discriminant Q2 under the null hypothesis was calculated through a 1,000-repetition permutation test. Differences with $P < 0.05$ were statistically significant.

Results

Clinical characteristics

There were no statistically significant differences in sex, age, ethnicity, smoking, drinking, serum renal function, TC, and diabetes between the sarcopenia and non-sarcopenia groups (i.e., $P > 0.05$); however, there were significant differences in BMI, FBG, hypertension, TG levels, and abdominal obesity (all $P < 0.05$) (**Table 1**).

Analysis of inflammatory factors

There was no significant difference in IL-6 levels between the sarcopenia and non-sarcopenia groups ($P = 0.983$); however, the levels of IL-10, IL-4, and TNF-α in the overall sarcopenia group were significantly higher than those in the non-sarcopenia group ($P < 0.001$, $P = 0.001$, and $P < 0.001$, respectively).

Regarding Han ethnicity, there was no significant difference in the level of IL-6 between the control group and the case group ($P = 0.806$). The levels of IL-10, IL-4, and TNF-α in the case group were higher than those in the control group ($P = 0.006$, $P = 0.016$, and $P < 0.001$, respectively). Regarding Uygur subjects, there was no significant difference in the level of IL-6 between the control group and the case group ($P = 0.807$), while the levels of IL-10, IL-4, and TNF-α in the case group were significantly higher than those in the control group ($P = 0.001$, $P = 0.013$, and $P < 0.001$, respectively) (**Table 2**).

After adjusting for sex, age, hypertension, blood lipid concentration, and obesity, the analysis of covariance revealed that the levels of IL-10, IL-4, and TNF-α in the case group were significantly higher than those in the control group ($P < 0.001$, $P = 0.011$, and $P < 0.001$, respectively) (**Table 3**).

Analysis of correlation between sarcopenia and clinical factors

Logistic analysis revealed that after adjusting for sex, age, hypertension, obesity, as well as IL-10, IL-4, and IL-6, an increased plasma level of TNF-α was significantly associated with sarcopenia ($P < 0.001$) (**Table 4**).

Partial least squares-discriminant analysis model of serum inflammatory cytokines for sarcopenia

Inflammatory cytokines profiles of all individuals with and without sarcopenia were compared using MetaboAnalyst. The results indicated that four normalized inflammatory cytokines levels differed significantly between the two groups using Wilcoxon rank-sum testing (**Figure 2**). In addition, the 2D and 3D score plots from the PLS-DA models for inflammatory cytokines showed clear and robust separation between sarcopenia and non- sarcopenia individuals (Q2 value: 0.498, R2Y value: 0.526, confirming that they had acceptable validity), suggesting a strong correlation between sarcopenia and inflammatory cytokines (**Figures 3A,B**). The VIP results indicated that TNF-α contributed the most to sarcopenia classification (**Figure 3C**). Two inflammatory cytokines, including TNF-α and IL-4, were selected by PLS-DA to construct a model, which had an overall AUROC of 0.896 (95%CI 0.816–0.954) for sarcopenia (**Figure 4**).

Discussion

We found that the plasma levels of IL-10, IL-4, and TNF-α in the case group were higher than in the control group. After adjusting for sex, age, hypertension, blood lipid concentration, and obesity, the level of TNF-α in the case group was higher than that in the control group, following that of IL-10 and IL-4, which indicated that an increased plasma level of TNF-α was significantly associated with sarcopenia.

Recently, exploring the potential role of inflammatory cytokines in sarcopenia has been a topic of great interest. A study

1 https://www.metaboanalyst.ca

TABLE 1 Clinical data analysis of subjects.

Variables	Sarcopenia ($N = 76$)	Non-sarcopenia ($N = 76$)	$t(\chi^2)$	P
Age (years)	71.0 ± 4.6	70.0 ± 3.8	1.443	0.151
Gender (male,%)	42.1	43.4	0.027	1.000
BMI (kg/m^2)	26.5 ± 3.0	20.9 ± 2.8	− 11.839	< 0.001
Nationality (Han/Uygur)	38/38	38/38	0.000	1.000
Diabetes (yes, %)	11.8	25.0	4.378	0.058
Hypertension (yes, %)	51.3	71.1	6.233	0.019
Coronary heart disease (yes, %)	17.1	10.5	1.381	0.240
Stoke (yes, %)	6.6	5.3	0.118	1.000
Smoking (yes, %)	14.5	14.5	0.000	1.000
Drinking (yes, %)	10.5	11.8	0.066	1.000
Abdominal obesity (yes, %)	51.3	88.2	24.440	< 0.001
Serum creatinine (mg/dl)	62.7 ± 17.0	65.4 ± 17.4	0.941	0.348
Serum urea nitrogen (mmol/L)	5.8 ± 1.5	6.2 ± 1.6	1.461	0.146
Fasting blood glucose (mmol/L)	6.5 ± 2.7	5.5 ± 1.2	− 2.779	0.006
Total cholesterol (mmol/L)	4.4 ± 0.9	4.6 ± 1.0	1.218	0.225
Triglyceride (mmol/L)	1.2 ± 0.6	1.7 ± 1.4	2.920	0.004
High-density lipoprotein cholesterol (mmol/L)	1.5 ± 1.3	1.5 ± 0.7	− 0.165	0.869
Hemoglobin (g/L)	143.1 ± 27.4	141.6 ± 20.0	− 0.288	0.774
Albumin (g/L)	43.4 ± 5.2	42.0 ± 3.5	− 1.945	0.054

TABLE 2 Inflammatory cytokines levels in difference nationality between sarcopenia and non-sarcopenia group.

Variables	Overall			Han nationality			Uygur nationality		
	Sarcopenia	Non-sarcopenia	P	Sarcopenia	Non-sarcopenia	P	Sarcopenia	Non-sarcopenia	P
IL-4 (pg/ml)	2.63 ± 0.66	2.27 ± 0.56	0.001	2.69 ± 0.74	2.34 ± 0.48	0.016	2.56 ± 0.57	2.21 ± 0.63	0.013
IL-6 (pg/ml)	1.85 ± 0.60	1.84 ± 0.53	0.983	1.89 ± 0.59	1.85 ± 0.62	0.806	1.81 ± 0.62	1.84 ± 0.44	0.807
IL-10 (pg/ml)	3.43 ± 0.92	2.72 ± 1.02	<0.001	3.27 ± 0.82	2.67 ± 1.03	0.006	3.59 ± 1.00	2.77 ± 1.03	0.001
TNF-α (pg/ml)	14.83 ± 3.40	6.76 ± 2.93	<0.001	14.12 ± 3.66	5.35 ± 2.91	<0.001	15.53 ± 3.00	8.16 ± 2.19	<0.001

TABLE 3 Analysis of inflammatory factors after adjusting for sex, age, hypertension, blood lipid concentration, and obesity.

Inflammatory cytokines	Groups	Mean	F	95% CI	P
IL-4	Non-sarcopenia	2.32	6.572	2.18–2.46	0.011
	Sarcopenia	2.59		2.45–2.73	
IL-6	Non-sarcopenia	1.87	0.225	1.73–2.00	0.636
	Sarcopenia	1.82		1.68–1.95	
IL-10	Non-sarcopenia	2.73	14.464	2.50–2.97	<0.001
	Sarcopenia	3.42		3.18–3.66	
TNF-α	Non-sarcopenia	7.16	160.948	6.40–7.91	<0.001
	Sarcopenia	14.41		13.66–15.16	

by Rong et al. found that the elderly population with sarcopenia in China was correlated with increased levels of inflammatory cytokine IL-10, IL-6, and IL-6/IL-10 ratios (16). Moreover, a cross-sectional study (17) indicated that higher plasma levels of TNF-α were significantly associated with a 7.6-fold increased risk of sarcopenia. However, levels of soluble receptors of tumor necrosis factor-alpha were lower for sarcopenic community-dwelling elderly women residents of Brazil (18). Taken together, these findings suggest a role of high-grade inflammation in developing sarcopenia.

Inflammatory cytokines are a type of "information material" produced by white blood cells and other cells (e.g., nerve

TABLE 4 Logistic analysis after adjusting for sex, age, hypertension, obesity, and interleukin (IL)-10, IL-4, and IL-6 levels.

	B	SE	*OR*	95% CI	*P*
Age	0.105	0.098	1.111	0.91–1.35	0.283
Gender	0.154	0.768	1.166	0.26–5.26	0.841
Hypertension	0.398	0.824	1.488	0.30–7.48	0.629
Triglyceride	0.206	0.503	1.228	0.46–3.29	0.682
Abdominal obesity	1.292	0.955	3.641	0.56–3.69	0.176
IL-4	1.140	0.630	3.127	0.91–10.75	0.070
IL-6	− 0.304	0.704	0.738	0.19–2.94	0.667
IL-10	0.554	0.420	1.741	0.76–3.96	0.187
TNF-α	0.981	0.203	2.667	1.79–3.97	< 0.001

cells and glial cells) in the body. An inflammatory cytokine is a protein or small molecular polypeptide with an immune regulation function. Inflammatory factors act on target cells via autocrine and paracrine pathways, causing extensive biological effects (19). Inflammatory cytokines can be divided into pro-inflammatory and anti-inflammatory factors. With an increase in age, cytokine imbalances in the elderly manifest as an increase in pro-inflammatory factors, a decrease in anti-inflammatory factors, and finally lead to a chronic low-level inflammatory state, also known as "immune aging" (20). Interleukins are multifunctional cytokines produced by many cells *in vivo,* such as inflammatory cells, vascular endothelial cells, fibroblasts, adipocytes, and muscle cells. IL-4, IL-10, and IL-6 are anti-inflammatory factors (21). The influence of inflammatory cytokines on the elderly is multifaceted and includes aspects such as bone quality, muscle metabolism, and nutrition balance. In addition, proinflammatory factors can also increase platelet brittleness and affect coagulation pathways by affecting the endothelium. All of these directly or indirectly lead to an increased risk for cardiovascular events in the elderly (22). Various cytokines involved in the pathogenesis of sarcopenia have been identified in foreign studies and include TNF-α, IL-1, IL-2, IL-4, IL-6, IL-8, and IL-10.

Wang et al. (23) found that the expression of the pro-inflammatory factors MCP-1, IL-8, and IL-6 increased significantly in young and older men after strenuous exercise, while the expression of the anti-inflammatory factors IL-4, IL-10, and IL-13 increased only slightly. Della Gatta et al. (24) found that elderly individuals with elevated serum IL-6 levels (> 5 pg/ml) were more likely to experience decreased muscle mass and muscle strength. They confirmed that the increase in the serum soluble IL-6 receptor was closely related to a decrease in muscle mass. However, in a study involving 4,252 elderly men aged 60–79 years, Conte et al. (25) came to a different conclusion: Their results revealed no correlation between IL-6 and arm muscle circumference and non-fat BMI. In the present study, there was no significant difference in plasma IL-6 concentration between the sarcopenia and control group, which may be related to the complex mechanism(s) of IL-6 and may be explained by the different populations investigated.

TNF-α is mainly produced by activated macrophages, natural killer cells, and T lymphocytes. It is vital in malignant consumptive disease, also known as "cachexia." As a typical proinflammatory cytokine, TNF-α is associated with many chronic diseases such as wasting syndrome, chronic infection, and metabolic disorder syndrome. However, the mechanism of TNF-α in the pathogenesis of sarcopenia remains unclear (26).

Lin and Yue (27) studied changes in muscle mass and muscle strength in 2,177 elderly patients during a 5-year follow-up. The authors found that TNF-α was negatively correlated with the cross-sectional area of the thigh muscle and with grip strength, suggesting that TNF-α negatively influenced muscle mass and grip strength in the elderly. A 4-year observation of individuals ≥ 85 years of age and 5-year observations of 70–79-year-old individuals demonstrated that plasma TNF-α levels were a predictor of a significant decrease in muscle strength. Grip strength decreased by 1.2–1.3 kg for each standard deviation increase in the TNF-α level (28). Similarly, Schaap et al. (29) found that mice with TNF-α gene transposition produced a large amount of TNF-α and exhibited thigh muscle fiber atrophy and growth restriction. The present study demonstrated that the concentration of TNF-α in the sarcopenia group was significantly higher than in the control group. Logistic analysis revealed that after adjusting for sex, age, hypertension, obesity, IL-10, IL-4, and IL-6, an increased plasma level of TNF-α was significantly associated with sarcopenia, which was consistent with the results of previous studies, suggesting that TNF-α may play an essential role in the pathogenesis of sarcopenia.

IL-10 is an anti-inflammatory cytokine derived from T-helper 2 and other cells. It acts on macrophages, down-regulates the expression of major histocompatibility complex II, weakens anti-inflammatory presentation, and reduces inflammatory reaction(s) (30). Coletti et al. (30) found no significant difference in the concentration of IL-10 between patients with sarcopenia and the control group. Another study reported that the number of anti-inflammatory

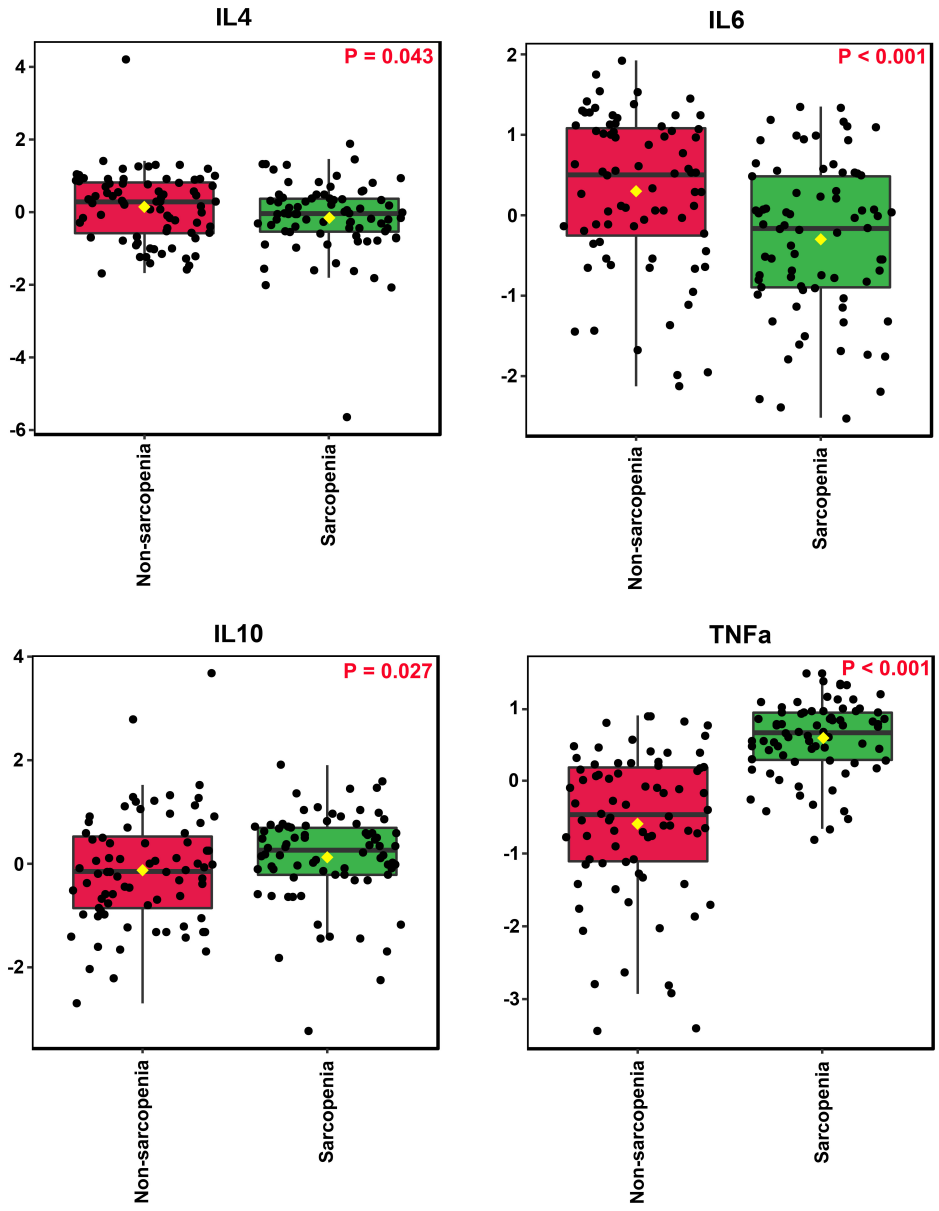

FIGURE 2
Comparison of the inflammatory cytokines of all individuals with and without sarcopenia.

cytokines decreased with increases in adipose tissue (31). The present study investigated the relationship between the plasma pro-inflammatory factors TNF-α and IL-6 and the anti-inflammatory factors IL-4 and IL-10 in the elderly population and sarcopenia. The results showed that increases in the concentration of the plasma pro-inflammatory factor TNF-α and the anti-inflammatory factors IL-4 and IL-10 were related to sarcopenia, which was contradictory to the definition of "immune aging." This may be explained by the chronically low level of inflammation in patients with sarcopenia. In response to stimulation of the proinflammatory factor TNF-α, the body secretes IL-4 and IL-10 to participate in the anti-inflammatory process. After further correction of plasma IL-10 and IL-4 levels, increased plasma TNF-α level remains associated with sarcopenia, suggesting that immune aging may be involved in the pathogenesis of sarcopenia.

Dysregulation of the cytokine network is deemed the major element speeding up the aging process and causing related illnesses (32). Cytokine interaction constitutes a cytokine network. It can be divided into two types: Pro-inflammatory cytokine network and anti-inflammatory cytokine network (33). Changes in inflammatory cytokine networks control the

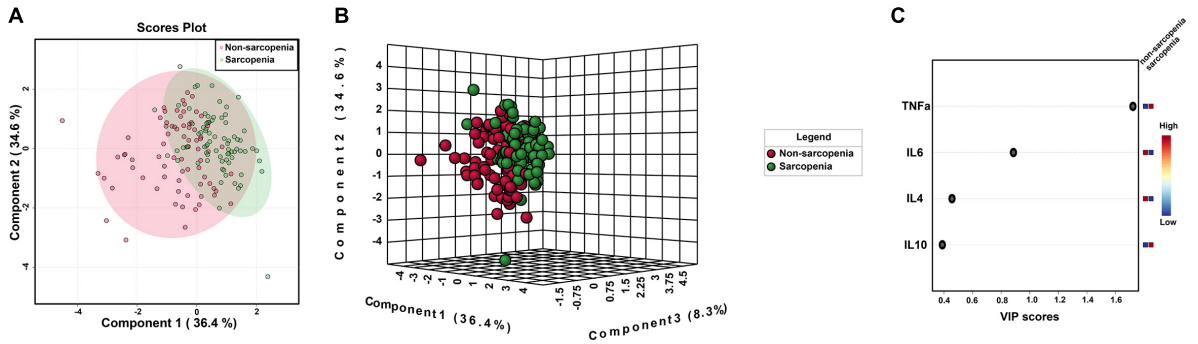

FIGURE 3
PLS-DA model of serum inflammatory cytokines for sarcopenia. **(A)** 2D clustering of sarcopenia vs. non-sarcopenia groups (n = 76 and n = 76, respectively) on PLS-DA; **(B)** 3D clustering of sarcopenia vs. non-sarcopenia groups (n = 76 and n = 76, respectively) on PLS-DA; **(C)** VIP scores of inflammatory cytokines between sarcopenia vs. non-sarcopenia groups.

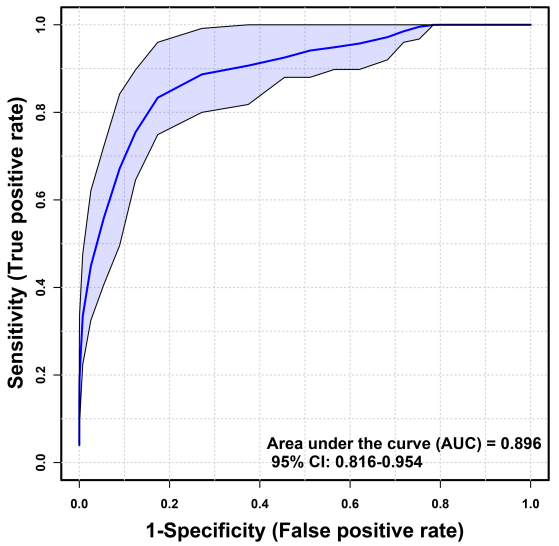

FIGURE 4
The area under the receiver operating characteristic curve performance for sarcopenia.

direction of inflammation. One of the main characteristics of aging is the chronic progressive increase of inflammatory response, which is called inflammatory aging and is closely related to immune aging. Increasing the level of serum inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the elderly are considered risk factors for cardiovascular and degenerative diseases (34). Our study found that the tumor necrosis factor α level in patients with sarcopenia was higher than that of patients without sarcopenia, which concludes that inflammatory factor network disorder is associated with sarcopenia. Inflammatory cytokines network disorder is mainly a response to tissue damage, which results from the age-related reduction in hemoglobin levels and consequent lower tissue oxygenation (35). Thus, IGF, growth hormone, testosterone, and nutritional supplements may be plausible strategies to improve inflammation and increase muscle mass. In addition, the exercise effect on anabolic hormone production has been well studied. Therefore, the optimal combination to treat sarcopenia must include personalized multicomponent exercise and supplementation with proteins and micronutrients to prevent deficiencies in the elderly (36).

A major limitation of our study is a relatively small sample size, which leads to our findings remaining exploratory. However, we have clearly shown an increased plasma level of TNF-α in individuals with sarcopenia with a clear separation compared to a healthy control using PLS-DA plus logistic regression analysis. In addition, using a cross-sectional rather than a longitudinal survey prevented us from disease progression and dietary consideration. Neither the amount of physical activity nor nutritional patterns were quantified in the present study. The study's strengths provide initial information on a unique inflammation profile in the elderly natural population with sarcopenia in a cross-sectional study in the agricultural and pastoral areas of Xinjiang of China. A large-scale, multicenter study will be indispensable for validating these findings and determining the role of inflammatory cytokines and whether the profile is altered before sarcopenia develops.

Conclusion

This study confirmed that an increased plasma level of TNF-α was associated with sarcopenia in elderly individuals residing in the agricultural and pastoral areas of Xinjiang, China. In the future, we intend to perform a cohort study to explore the causal relationship(s) between serum inflammatory markers and sarcopenia and the mechanism of sarcopenia development.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HW contributed to the study design, data collection and analysis, results interpretation, and provided critical manuscript revisions. AW and ZM wrote the article. WX, TM, JL, and SX involved in the data analysis and interpretation and also provided critical manuscript revisions. All authors read and approved the submitted manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Tan JP, Liu Y, Wang X, Wang LN. The development trend of aging population and the research status of geriatric health in China. *Chin J Gerontol.* (2012) 32:4335–7. doi: 10.3969/j.issn.1005-9202.2012.19.125

2. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* (2019) 48:16–31. doi: 10.1093/ageing/afy169

3. Rolland Y, Czerwinski S, Abellan Van Kan G, Morley JE, Cesari M, Onder G, et al. Sarcopenia: its assessment, etiology, pathogenesis, consequences and future perspectives. *J Nutr Health Aging.* (2008) 12:433–50. doi: 10.1007/bf02982704

4. Urso ML, Fiatarone Singh MA, Ding W, Evans WJ, Cosmas AC, Manfredi TG. Exercise training effects on skeletal muscle plasticity and IGF-1 receptors in frail elders. *Age (Dordr).* (2005) 27:117–25. doi: 10.1007/s11357-005-1629-7

5. Gariballa S, Alessa A. Sarcopenia: prevalence and prognostic significance in hospitalized patients. *Clin Nutr.* (2013) 32:772–6. doi: 10.1016/j.clnu.2013.01.010

6. Volpato S, Bianchi L, Cherubini A, Landi F, Maggio M, Savino E, et al. Prevalence and clinical correlates of sarcopenia in community-dwelling older people: application of the EWGSOP definition and diagnostic algorithm. *J Gerontol A Biol Sci Med Sci.* (2014) 69:438–46. doi: 10.1093/gerona/glt149

7. von Haehling S. The wasting continuum in heart failure: from sarcopenia to cachexia. *Proc Nutr Soc.* (2015) 74:367–77. doi: 10.1017/s0029665115002438

8. Lang T, Streeper T, Cawthon P, Baldwin K, Taaffe DR, Harris TB. Sarcopenia: etiology, clinical consequences, intervention, and assessment. *Osteoporos Int.* (2010) 21:543–59. doi: 10.1007/s00198-009-1059-y

9. Lexell J. Ageing and human muscle: observations from Sweden. *Can J Appl Physiol.* (1993) 18:2–18. doi: 10.1139/h93-002

10. Bano G, Trevisan C, Carraro S, Solmi M, Luchini C, Stubbs B, et al. Inflammation and sarcopenia: a systematic review and meta-analysis. *Maturitas.* (2017) 96:10–5. doi: 10.1016/j.maturitas.2016.11.006

11. Joanisse S, Parise G. Cytokine mediated control of muscle stem cell function. *Adv Exp Med Biol.* (2016) 900:27–44. doi: 10.1007/978-3-319-27511-6_2

12. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: the potential role of an aged immune system. *Ageing Res Rev.* (2017) 36:1–10. doi: 10.1016/j.arr.2017.01.006

13. Peake JM, Della Gatta P, Suzuki K, Nieman DC. Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. *Exerc Immunol Rev.* (2015) 21:8–25.

14. Zhou J, Liu B, Liang C, Li Y, Song YH. Cytokine signaling in skeletal muscle wasting. *Trends Endocrinol Metab.* (2016) 27:335–47. doi: 10.1016/j.tem.2016.03.002

15. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, et al. Sarcopenia in Asia: consensus report of the Asian working group for sarcopenia. *J Am Med Dir Assoc.* (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025

16. Rong YD, Bian AL, Hu HY, Ma Y, Zhou XZ. Study on relationship between elderly sarcopenia and inflammatory cytokine IL-6, anti-inflammatory cytokine IL-10. *BMC Geriatr.* (2018) 18:308. doi: 10.1186/s12877-018-1007-9

17. Li CW, Yu K, Shyh-Chang N, Li GX, Jiang LJ, Yu SL, et al. Circulating factors associated with sarcopenia during ageing and after intensive lifestyle intervention. *J Cachexia Sarcopenia Muscle.* (2019) 10:586–600. doi: 10.1002/jcsm.12417

18. Lustosa LP, Batista PP, Pereira DS, Pereira LSM, Scianni A, Ribeiro-Samora GA. Comparison between parameters of muscle performance and inflammatory biomarkers of non-sarcopenic and sarcopenic elderly women. *Clin Interv Aging.* (2017) 12:1183–91. doi: 10.2147/cia.S139579

19. Wang JX, Wang XF. *The Research on the Relationship Between Old People Sarcopania and Interleukin-6 and Tumor Necrosis Factor Alpha*. Tianjin: Tianjin University of Traditional Chinese Medicine (2016).

20. Zhang YH, Lin J. *Clinical Significance of Changes of Serum tnf-α and il-6 Levels in Senile Hypertensive Patients*. Liaoning: China Medical University (2006).

21. Miller RA. The aging immune system: primer and prospectus. *Science.* (1996) 273:70–4. doi: 10.1126/science.273.5271.70

22. Wang YD, Wang SS. Cytokine levels in peripheral blood of elderly patients with sarcopenia. *Chin J Osteopor Bone Miner Res.* (2019) 12:221–5.

23. Wang R, Fan L, Hu YX. Research progress of saropenia and inflammatory cytokines. *Chin J Healthcare Med.* (2014) 16:491–2, 496.

24. Della Gatta PA, Garnham AP, Peake JM, Cameron-Smith D. Effect of exercise training on skeletal muscle cytokine expression in the elderly. *Brain Behav Immun.* (2014) 39:80–6. doi: 10.1016/j.bbi.2014.01.006

25. Conte M, Vasuri F, Trisolino G, Bellavista E, Santoro A, Degiovanni A, et al. Increased Plin2 expression in human skeletal muscle is associated with sarcopenia and muscle weakness. *PLoS One.* (2013) 8:e73709. doi: 10.1371/journal.pone.0073709

26. Penninx BW, Kritchevsky SB, Newman AB, Nicklas BJ, Simonsick EM, Rubin S, et al. Inflammatory markers and incident mobility limitation in the elderly. *J Am Geriatr Soc.* (2004) 52:1105–13. doi: 10.1111/j.1532-5415.2004.52308.x

27. Lin TP, Yue JR. Study on the pathogenesis of sarcosis from serum inflammatory markers. *Pract Gerontol.* (2019) 33:833–5, 839.

28. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. *Proc Natl Acad Sci USA.* (1975) 72:3666–70. doi: 10.1073/pnas.72.9.3666

29. Schaap LA, Pluijm SM, Deeg DJ, Harris TB, Kritchevsky SB, Newman AB, et al. Higher inflammatory marker levels in older persons: associations with 5-year change in muscle mass and muscle strength. *J Gerontol A Biol Sci Med Sci.* (2009) 64:1183–9. doi: 10.1093/gerona/glp097

30. Coletti D, Moresi V, Adamo S, Molinaro M, Sassoon D. Tumor necrosis factor-alpha gene transfer induces cachexia and inhibits muscle regeneration. *Genesis.* (2005) 43:120–8. doi: 10.1002/gene.20160

31. Minton K. Immune regulation: IL-10 targets macrophage metabolism. *Nat Rev Immunol.* (2017) 17:345. doi: 10.1038/nri.2017.57

32. Marzetti E, Picca A, Marini F, Biancolillo A, Coelho-Junior HJ, Gervasoni J, et al. Inflammatory signatures in older persons with physical frailty and sarcopenia: the frailty "cytokinome" at its core. *Exp Gerontol.* (2019) 122:129–38. doi: 10.1016/j.exger.2019.04.019

33. Xia S, Jin TS. The study of inflamm-aging. *J Chengdu Med Coll.* (2012) 7:336–43. doi: 10.3969/j.issn.1674-2257.2012.03.001

34. Song BY. Research on pathogenesis of inflamm-aging. *J Xian Univ (Nat Sci Ed).* (2019) 22:85–7. doi: 10.3969/j.issn.1008-5564.2019.05.020

35. Picca A, Coelho-Junior HJ, Calvani R, Marzetti E, Vetrano DL. Biomarkers shared by frailty and sarcopenia in older adults: a systematic review and meta-analysis. *Ageing Res Rev.* (2022) 73:101530. doi: 10.1016/j.arr.2021.101530

36. Nascimento CM, Ingles M, Salvador-Pascual A, Cominetti MR, Gomez-Cabrera MC, Viña J. Sarcopenia, frailty and their prevention by exercise. *Free Radic Biol Med.* (2019) 132:42–9. doi: 10.1016/j.freeradbiomed.2018.08.035

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Using exergame-based exercise to prevent and postpone the loss of muscle mass, muscle strength, cognition, and functional performance among elders in rural long-term care facilities: A protocol for a randomized controlled trial

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Objective: Elderly individuals in long-term care facilities (LTCFs) have a higher prevalence of sarcopenia than those in the community. Exercise is the gold standard for preventing and treating sarcopenia. Regarding exercise, multicomponent exercises, including progressive resistance training (PRT), are beneficial. However, developing routine, structured exercise programs for the elderly in LTCFs is difficult because of a shortage of healthcare providers, particularly in rural regions. Exergame-based exercises can increase a player's motivation and reduce staff time for an intervention. Nintendo Switch RingFit Adventure (RFA) is a novel exergame that combines resistance, aerobic, and balance exercises. In this study, we aim to investigate the clinical effectiveness of RFA on muscle and functional performance parameters among the elderly in LTCFs.

Methods: The EXPPLORE (using **EX**ergame to **P**revent and **P**ostpone the **LO**ss of muscle mass, muscle strength, and functional performance in **R**ural **E**lders) trial is a single-center randomized controlled trial involving elderly individuals (≥60 years) living in LTCFs in rural southern Taiwan. The participants will be equally randomized to the intervention

group (exergame-based exercise plus standard care) or the control group (standard care alone). Both groups will receive standard care except that the intervention group will receive exergame-based exercises at the time previously scheduled for sedentary activities in the LTCFs. The exergame-based exercise will be performed using RFA in the sitting position with a specialized design, including arm fit skills and knee assist mode. Each session of the exercise lasts 30 mins and will be performed two times per week for 12 weeks. The primary outcomes will be the osteoporotic fracture index, appendicular skeletal muscle mass index, dominant handgrip strength, and gait speed. Meanwhile, the secondary outcomes will be the dexterity and agility, muscle strength and thickness, range of motion of the joints of the dominant upper extremity, Kihon checklist, Medical Outcomes Study 36-Item Short-Form Health Survey, and Brain Health Test.

Discussion: This trial will provide valuable knowledge on whether exergames using RFA can counteract physical decline and improve quality of life and cognition among the elderly in LTCFs.

Clinical trial registration: [www.ClinicalTrials.gov], identifier [NCT05360667].

KEYWORDS

exergame, sarcopenia, frailty, long term care, multicomponent training, RingFit Adventure

Background

Taiwan is now an aging society, and the population of elderly individuals aged ≥65 years constituted 15.95% of the total population in 2020. It is estimated that Taiwan will become a super-aged society as the number of individuals aged ≥65 years grow to be more than 20% of the total population by 2025 (1). Aging and inactivity are associated with declines in muscle mass, architecture, and strength (2, 3). The rate of muscle loss ranges from 1 to 2% per year for individuals more than 50 years old (4). Sarcopenia, defined as an age-related loss of skeletal muscle mass and a decline in muscle strength and physical performance (5), is thus emerging as an important issue in modern society. As reported by the Asian Working Group for Sarcopenia (AWGS), the prevalence of sarcopenia in community-dwelling older men and women in Taiwan was 9.3 and 4.1%, respectively (6). For mobility-impaired older adults, regular physical activity can prevent further disabilities and improve their overall health (7, 8), while physical inactivity is an important factor contributing to the development of sarcopenia (9). The prevalence of sarcopenia is much higher in individuals living in care facilities than those residing in the community, ranging between 17.7 and 73.3% in long-term nursing homes (10) and between 22 and 87.1% in daycare centers, because they are older and sicker and require more assistance with their activities of daily living (ADLs) after being admitted to care facilities (10–12).

The first-line strategies for preventing and treating sarcopenia focus on preserving skeletal muscle mass and maintaining muscle strength (13). A newly published network meta-analysis revealed that mixed exercises and physical activity with nutritional supplementation are the most effective interventions for sarcopenia (14). Resistance training (RT), particularly progressive RT (PRT) (15, 16), and multicomponent exercises have moderate- to high-quality evidence for their positive and significant effects on increasing muscle mass, muscle strength, and physical performance among elderly individuals with sarcopenia (17, 18). Moreover, some studies showed that low-intensity RT [≤50% 1 repetition maximum (RM)] is sufficient to induce strength gains (19, 20). However, the optimal exercise prescription, regarding intensity, type, frequency, duration, and progression, for preventing

Abbreviations: ASWG, Asian Working Group for Sarcopenia; ALDs, activities of daily living; RT, resistance training; PRT, progressive resistance training; RM, repetition maximum; LTC, long-term care; LTCFs, long-term care facilities; HRQoL, health-related quality of life; HGS, handgrip strength; ASMMI, appendicular skeletal muscle mass index; RFA, Nintendo Switch® RingFit Adventure; RCT, randomized controlled trial; Exergame-based exercise, exergame-based multicomponent exercise training *via* RFA; RPE, rate of perceived exertion; SOF index, Study of Osteoporotic Fractures Index; BIA, bioelectrical impedance analysis; MCID, minimal clinically important difference; BBT, Box and Block Test; MVIC, maximal voluntary isometric contraction; ROM, range of motion; KC-T, Kihon checklist-Taiwan; SF-36, Medical Outcomes Study 36-Item Short-Form Health Survey; BHT-Cog, Brain Health Test–brief cognitive test; AR, augmented reality.

and treating sarcopenia among elderly individuals is still controversial (14, 17).

Taiwan is one of the fastest-aging countries in the world (21). The Taiwanese government established a long-term healthcare system in 2010, which was reformed into The Long-Term Care 2.0 Plan (LTC 2.0) in 2017 to guarantee suitable services in response to the fast-growing care needs of older individuals (21). The LTC 2.0 aims to provide aging-in-place values into practice by delivering integrated home- or community-based primary healthcare and preventive services (22). Based on the consensus of the AWGS in 2019, community-based care facilities are important places for integrated LTC services to prevent or delay disability, particularly for individuals who are physically inactive or at risk of sarcopenia (23). These settings are essential, particularly in rural regions in Taiwan, where the proportion of elderly individuals is higher than that in urban areas (24) and the healthcare resources are limited (25).

The principles of PRT and multicomponent exercise programs include regular, mass-practiced, mildly overwhelming engagement (26). To achieve these principles, the devotion of time, workforce, and money are critical. Staffing constraints and resource shortages have made it challenging to promote regular exercise programs in long-term care facilities (LTCFs) (27, 28). Exergames are defined as any type of video game that requires the movement of the player's entire body, allowing real-time interaction (29). Exergames breakdown the barriers of repetitive and monotonous physical exercises since they contain attractive and multisensory game environments with an immersive environment in which the interaction occurs through whole-body movements (30). Moreover, the gamified approach and immersive scenarios motivate older individuals to acquire a greater commitment to the practice of physical and rehabilitative exercises (31). Therefore, playing exergames reduces staff time for intervention, encourages patients to perform relatively high-energy movements, and increases their motivation. Among community-dwelling older individuals with or without specific diseases (32), studies have proved the therapeutic application of exergames in improving lower limb strength (33), gait speed (34), balance (35), and cognitive function (34, 36). Few studies have evaluated the clinical effectiveness of exergames in LTCFs, and most outcomes of these studies pertained to health-related quality of life (HRQoL), cognition, and general functional status (37–39). Our team found that the handgrip strength (HGS) and walking speed of elderly individuals with sarcopenia living in care facilities improved significantly after 12 weeks of exergame-based progressive RT(40). However, it was a quasi-experimental study involving relatively few participants, and the platform to deliver exergames was difficult for older adults to use. Therefore, in this study, we will evaluate the feasibility and clinical application of a novel exergame-based multicomponent training program using Nintendo Switch® RingFit Adventure (RFA), which could deliver optimal exercise intensity for each player and perform fine-tuned upregulation and downregulation based on performance after each game, among elderly individuals living in rural care facilities.

Methods and analysis

Trial design and ethics

This randomized controlled trial (RCT) compares an exergame-based multicomponent exercise training (exergame-based exercise) to the standard care in older users of LTCFs in rural regions. The trial design adheres to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines for RCT (41) and the Consolidation Standards of Reporting Trials guidelines (42). The flowchart of this study is shown in **Figure 1**. This study will be conducted according to the Declaration of Helsinki, was approved by the Institutional Review Board of a medical center in Taiwan (approval number: B-ER-111-058), and was registered at www.ClinicalTrials.gov (registration number: NCT05360667).

Selection of participants

All individuals, who participate regularly in LTCFs, including daycare centers and nursing homes, in rural regions of Kaohsiung City, southern Taiwan, will be eligible for study participation. The inclusion criteria are as follows: individuals (a) aged ≥60 years, (b) those living or participating in LTCFs for at least 1 month, (c) those who can understand and speak Chinese or Taiwanese, (d) those with sufficient cognitive capacity (judged by the researchers) to give informed consent and participate in the exergame-based exercise and data collection, and (e) those who can sit for more than 50 min for training and can complete the measurement of gait speed. (a) Individuals who have significant cardiopulmonary diseases, (b) those who regularly receive oxygen supplementation, (c) those who have uncontrollable hypertension, and (d) those who had a recent infection or fracture or were diagnosed with other diseases that might prohibit them from participating in exercises according to the guidelines of the American College of Sports Medicine will be excluded from this study (43). Participants in the intervention group will receive standard care with the exergame-based exercise for 12 weeks, whereas those in the control group will receive standard care routinely applied in LTCFs as usual. We will replace the scheduled sedentary activities in the LTCFs, such as singing, table games, and gardening, with the exergame-based exercise in the intervention group. Meanwhile, participants in the control group will perform the aforementioned sedentary activities in the LTCFs as usual. Therefore, the time for activities in the two groups will be the same. In addition to the exergame-based exercise and

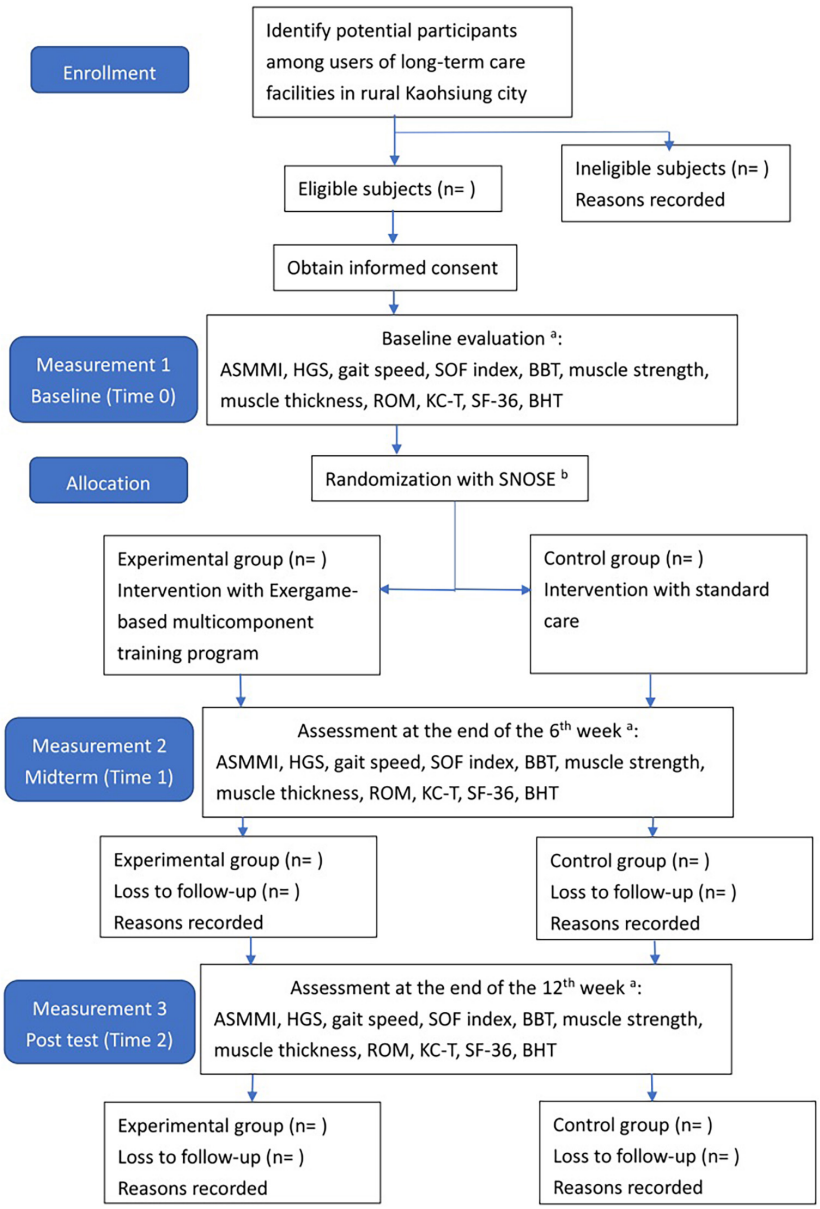


FIGURE 1
The flowchart of the participant inclusion and data collection process. [a]See **Table 1**; [b]sequentially numbered, opaque, and sealed envelopes. ASMMI, appendicular skeletal muscle mass index; HGS, handgrip strength; SOF index, study of osteoporosis index; BBT, box and block test; ROM, range of motion; KC-T, Kihon checklist Taiwan version; SF-36, short form 36 questionnaire; BHT, brain health test.

standard care, all participants will perform the usual traditional rehabilitation provided by therapists at the LTCFs.

Randomization

We will use sequentially numbered, opaque, sealed envelopes containing a group assignment number created by a person who is not clinically involved and, therefore, could be blinded to this study. Participants who fulfilled the inclusion and exclusion criteria will be equally randomized to the intervention and control groups with an allocation ratio of 1:1.

Exergame-based multicomponent training program (intervention group)

The program consists of PRT and functional movement of the four extremities but mainly the upper limbs. We will use RFA to deliver the program. RFA is a Nintendo Switch®-based

exergame. It requires one Nintendo Switch video game console, one Ring-Con (a Pilates ring that the user holds), one wireless controller Joy-Con (one should be placed on the Ring-Con and the other one should be affixed into a leg strap on the thigh of the player), and one display screen (**Figure 2**). The exergame-based exercise will be performed two times per week, at least 48 h apart from each training session, 50 min per session (10 min each for a warm-up and cool-down and 30 min for the main program), for 12 weeks, and will be supervised by a therapist.

RingFit Adventure is a fitness action role-playing game. The player advances the story while exercising as the movement of the player is linked to the main character on the screen. The movements of the player and battle actions are based on the performance of certain physical activities using the Ring-Con and leg strap (44). Ring-Con is a controller of the system with a built-in high-precision force and strain sensor that detects and digitizes the player's movements, such as stretching and squeezing. Joy-Con is equipped with a motion infrared-ray camera and is used to monitor the heart rate of the player. RFA could estimate the optimal exercise intensity for each player and perform fine-tuned upregulation and downregulation based on this physiological feedback (45). Therefore, providing an appropriate amount of exercise for all generations, from children to the elderly, has become possible (46, 47).

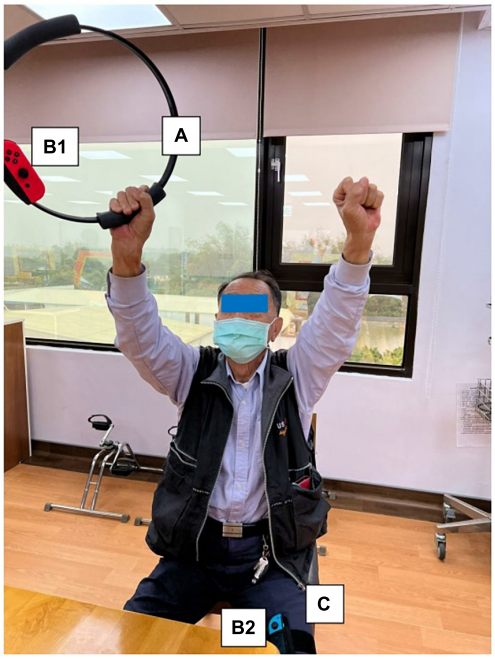


FIGURE 2
Components of Nintendo Switch. To play the RingFit Adventure, the player needs to use the Nintendo Switch, which includes the following devices: A. Ring-Con: A Pilates ring the user holds; B. Joy-Con: A wireless controller. One of the Joy-Con (B1) should be placed on the Ring-Con (A). The other Joy-Con (B2) should be affixed to a Leg Strap (C) on the thigh of the player.

Routinely, the player controls the character by jogging or squatting in the Adventure Mode. Each stage of the Adventure Mode consists of several battle scenes; in addition to aerobics training, the player must perform intensive RT and yoga exercises that exert stress on the muscles of the entire body to defeat the enemy and clear the stage. Players are rewarded based on the amount of exercise they perform and continue to advance while continuously improving their skills. Given that many individuals living in the LTCFs are becoming older and sicker and require more assistance with their ADLs, they have a higher potential risk of falls, and exergame-based exercise should be supervised to reduce unnecessary walking (40). Moreover, some individuals living in LTCFs use wheelchairs mainly for ambulation (28). Therefore, we will use the knee assist mode in RFA, which helps the player character jog and sprint when it is vital for level completion in the game. This means that traversal of the Adventure Mode is possible for players who only have reliable or pain-free use of their arms and trunk.

At the beginning of the game, we will enter the age, sex, and weight of each player, and they should stretch and squeeze the Ring-Con as hard as possible to measure their maximal intensity (this step could be considered the concept of 1 RM as in the traditional RT). Then, the Nintendo Switch sets the initial amount of exercise that must be performed in each stage and adjusts it progressively and automatically depending on the performance of the player. The ideal intensity of each session will set to be 13 (somewhat hard) on the Borg Rating of Perceived Exertion (RPE) scale (48). The higher the intensity, the more repetitions and the larger resistance of the Ring-Con to be stretched and squeezed. The supervisor could also adjust the intensity level manually based on the performance of the player; however, we will not adjust the intensity level manually in this study to ensure the consistency of the protocol. The Joy-Con on the Ring-Con could detect the heart rate of the player after the training session. RFA could provide a summary of the training results, such as exercising time, total calories burned, and total distance ran, after each training session. Warm-up and cool-down will last 10 min each for each training session. In addition to the data RFA records, we will measure the blood pressure, heart rate, heart rate reserve, and oxygen saturation using a pulse oximeter before and after each training session.

As for the skills required to complete the game, in addition to the regular stretching and squeezing that are mandatory for the character player to progress and earn rewards in the Adventure Mode, we have developed six fit skills that focus on training the upper extremities and trunk from the list provided by RFA, including overhead press, shoulder press, front press, bow pull, overhead arm spin, and triceps kickback. The player must choose three of the six aforementioned fit skills to defeat the monster in each beat mode. The flowchart of the exergame-based exercise and the corresponding trained muscle and joint movement is provided in **Table 1**.

Contents of standard care (control group)

The standard care in the control group will be applied as usual in our LTCFs, in the way of group activities, including calisthenics (that could be performed in the sitting position), horticultural therapy, and group static activities (e.g., tabletop games). The programs will be performed two times per week for approximately 30–60 min, depending on the activity, and will be led by a therapist.

Blinding

Because of the design of the study and the nature of the interventions, blinding the staff and participants of the LTCFs is impossible. The assessors, measuring the outcomes, and the interpreter, analyzing the data, will be blinded in this study.

Outcomes measured

All participants will be subjected to three evaluations. The first evaluation is at baseline (Time 0), which is before randomization. The second and third evaluations will be performed at the end of the 6^{th} (Time 1) and 12^{th} (Time 2) weeks after the intervention, respectively (**Table 2**).

Primary outcomes

The primary outcomes chosen for this study are the criteria for the diagnosis of sarcopenia proposed by the AWGS, including appendicular skeletal muscle mass index (ASMMI), dominant HGS, and usual gait speed. Moreover, the Study of Osteoporotic Fractures Index (SOF index), which indicates the frailty status, is also one of the primary outcomes.

Anthropometry and body composition: Appendicular skeletal muscle mass index

We will perform bioelectrical impedance analysis (BIA) to evaluate the participant's appendicular skeletal muscle mass. Compared with other established methods for measuring body composition, including dual-energy X-ray absorptiometry, computed tomography, and magnetic resonance imaging, BIA has been widely used in clinical settings because it is relatively simple, quick, and non-invasive (49). BIA of the tetrapolar eight-point electrode type (InBody S10 for the supine measure, InBody Co., South Korea) will be used in this study given that some participants will be difficult to measure in the standing position. This BIA model enables multifrequency impedance measurement of the arms, trunk, and legs using eight electrodes positioned at each hand and foot. Impedance parameters are measured with alternating currents of 80 and 100 mA at frequencies of 1, 5, 50, 250, 500, and 1,000 kHz for InBody S10 (50). The participants will be placed in the lying position for approximately 10–15 min before the test so that body water may be dispersed evenly inside the body. The four extremities should be spread naturally to a 15° angle away from the trunk to ensure that the extremities do not touch the trunk part of the body (51). The device will be calibrated in the morning using a standard control circuit supplied by the manufacturer. The ASMMI is defined as the appendicular skeletal muscle mass (in kg) divided by the height squared (in m^2) (52).

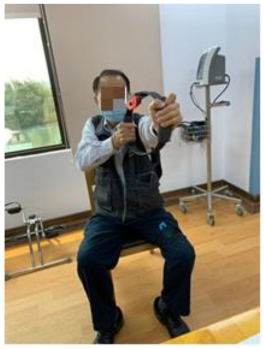
Dominant hand grip strength

The HGS will be measured using a JAMAR dynamometer (J A Preston Corporation, New York, NY, USA) using all five notches. A JAMAR dynamometer is a hydraulic instrument that measures isometric strength in kilograms. It has been proven to have good reliability in various older populations (53). The measurement will be performed under a standard position, and the instrument should be freely held. Each participant will be instructed to sit straight with their upper arm in a neutral position and their elbow flexed at 90°C, their forearm in a neutral position, and their wrist at 0–30°C extension (54). The measurement should be performed three times, and the highest of the three measurements will be recorded. The participants will be allowed to rest for 1 min between measurements. The minimal clinically important difference (MCID), defined as the minimal amount of change required to distinguish a true performance change due to variability in performance or measurement error, is also measured in this study. No available studies on the MCID of the HGS in older adults so far, whereas, in healthy individuals, the MCID of the HGS is 2.44–2.69 kg (55).

Gait speed

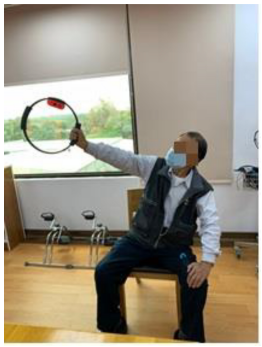
The participants will be instructed to walk at a normal speed on a 6-m-long corridor without a barrier, and the usual gait speed is calculated by measuring the time spent to reach the end of the corridor by a participant as suggested by the AWGS (52). The participants could walk with or without assistive devices during the measurement. The time will be initially recorded once the participants start walking and stop at the point when they reach a distance of 6 m. Gait speed should be measured two times, and the average of the two speeds will be recorded. The participants will be allowed to rest for 10 min between measurements. The MCID of gait speed across multiple patient groups is 0.10–0.20 m/s (56).

TABLE 1 The flowchart of exergame-based multicomponent training program.

Timeframe	Activity	Activity description	Mainly target muscles and joints
0–10 min	Warm-up	Flexibility exercise.	Progressive static stretch of the neck, chest, arm, thighs, and legs.
11–50 min	Main training	Ringfit Adventure with knee assist mode. The player controls the character in the Adventure Mode by squeezing and stretching the Ring-Con.	**Muscles:** trapezius, triceps, pectoralis major and minor, and core muscles. **Joints:** shoulder horizontal adduction/abduction, shoulder external/internal rotation, and elbow flexion
		To defeat the monsters in each stage, the players has to use the combination of the following six arm fit skills.	Not applicable
		Overhead press: Hold the Ring-Con overhead and squeeze it.	**Muscles:** deltoid, and biceps brachii **Joints:** shoulder flexion, and elbow flexion/extension
			
		Shoulder press: Hold the Ring-Con on each side of the shoulder and squeeze it.	**Muscles:** biceps/triceps brachii **Joints:** elbow flexion/extension
			
		Front press: Hold the Ring-Con below umbilicus with anterior-tilting of the trunk slightly.	**Muscles:** trapezius, triceps, pectoralis major and minor, and core muscles. **Joints:** shoulder horizontal adduction/abduction, and elbow flexion
			
		Bow pull: Put the Ring-Con in front of the chest and pull it like it is a bow.	**Muscles:** biceps/triceps brachii, and latissimus dorsi **Joints:** shoulder adduction/abduction, elbow flexion/flexion, and wrist flexion/extension
			

(Continued)

TABLE 1 (Continued)

Timeframe	Activity	Activity description	Mainly target muscles and joints
		**Overhead arm spin:** raise both arms straight up and twist the wrists.	**Muscles:** deltoid and shoulder girdles **Joints:** shoulder flexion, elbow pronation/supination, and wrist flexion
		**Tricep kickback:** With elbow locked, move the Ring-Con up-and-down	**Muscles:** triceps, and spinal erectors **Joints:** shoulder horizontal adduction/abduction, and elbow flexion/extension
51–60 min	Cool-down	Flexibility exercise.	Progressive static stretch of the neck, chest, arm, thighs, and legs.

Study of osteoporotic fractures index

The SOF index consists of the following three components: (a) a weight loss of ≥5% during the preceding year (regardless of the intention to lose weight), (b) the inability to rise from a chair five times without using the arms, and (c) an answer of "no" to the question "Do you feel full of energy?" The participants will be identified to be frail if they have two or more of the aforementioned components; those with one disability are considered in pre-frailty status, and those with none of the aforementioned impairments are considered robust (57). The SOF index has been proven to be a valid tool to evaluate frailty, particularly for community-dwelling older adults in Taiwan (58).

Secondary outcomes

Manual dexterity

The box and block test (BBT) can be used to measure the unilateral gross manual dexterity in various populations with high test–retest reliability and validity (59). The setup of the BBT consists of a wooden box divided into two compartments, with 100 wooden blocks inside one compartment. The participants will be instructed to transfer the wooden blocks one by one from one compartment to the other in the sitting position. The score is based on the number of blocks the participants transferred in 60 s. Most studies on the MCID of the BBT have involved patients with stroke, and the MCID was 5.5 cubes/min for the most affected side and 7.8 cubes/min for the least affected side (60).

Biceps and triceps brachii muscle strength of the dominant side

We will use the microFET® 3 (Hoggan Health Industries, West Jordan, UT, USA) to measure the maximal voluntary isometric contraction (MVIC) of the biceps and triceps brachii of the dominant side. The microFET® 3 is an electronic handheld dynamometer that can detect 0–150 lb of force with high reliability and validity (61). The participants will be instructed to lie on the treatment table with their elbows forming a 90° angle to the horizontal such that the arm is perpendicular to the limb. The device will be placed on the ventral (for the biceps brachii) or dorsal (for the triceps brachii) side of the arm and aligned with the ulnar styloid. The participants will be encouraged to go against the force, which is exerted toward the device, with their

TABLE 2 Instruments and measures to be implemented for data collection.

Instrument or measure	Outcome	Description	Time point	References
Appendicular skeletal muscle mass index	Diagnostic criterion of sarcopenia. Primary outcome	Measured by bioelectrical impedance analysis. Defined as the appendicular skeletal muscle mass (Kg) divided by the height squared (m^2)	T0, T1, T2	(49, 52)
Dominant handgrip strength	Diagnostic criterion of sarcopenia. Primary outcome	Measured by JAMAR dynamometer under standard position.	T0, T1, T2	(54)
Gait speed	Diagnostic criterion of sarcopenia. Primary outcome	The participants are asked to walk at a normal speed on a 6-m long corridor without a barrier and the usual gait speed calculated by measuring the time spent by a participant.	T0, T1, T2	(52)
Study of osteoporotic fracture index	Indicator of frailty. Primary outcome	Including three components: (a) a weight loss of $\geq$ 5% during the preceding year, (b) an inability to rise from a chair five times without using the arms, and (c) an answer of "no" to the question "Do you feel full of energy?"	T0, T1, T2	(57)
Box and block test	Indicator of hand dexterity. Secondary outcome	The number of blocks the participants transferred in 60 seconds from one compartment to the other compartment of the wooden box.	T0, T1, T2	(59)
Biceps and triceps brachii muscle strength of the dominant side	Indicator of muscle strength. Secondary outcome	Using the microFET® 3 to measure the maximal voluntary isometric contraction under standard positions.	T0, T1, T2	(62)
Sonographic thickness of biceps and triceps brachii, quadriceps, and gastrocnemius muscles	Indicator of muscle morphology. Secondary outcome	Using a portable LOGIQ e ultrasound, equipped with a 5-12 MHz linear array transducer, to measure the muscle thickness under standard positions.	T0, T1, T2	(63, 64)
Range of motion of the joints of upper extremity	Indicator of functional movement. Secondary outcome	Using a goniometer under standard positions to measure shoulder flexion, abduction, and external rotation; elbow flexion and extension; forearm supination and pronation and wrist flexion and extension.	T0, T1, T2	(65)
Kihon checklist-Taiwan	Indicator of general function. Secondary outcome	A self-reported questionnaire, consisting of 25 items divided into 7 sub-categories. Each item is rated as pass (0) or fail (1). A higher total score indicates a lower level of function.	T0, T1, T2	(66)
Medical outcomes study 36-Item short-form health survey	Indicator of health-related quality of life. Secondary outcome	A self-assessment containing 36 items, divided into 8 subscales. Responses to each question were transformed to a scale ranging from 0–100. The higher the scores, the better the quality of life.	T0, T1, T2	(70)
Brain health test brief cognitive test	Indicator of cognitive function. Secondary outcome	A clinical assessment tool, including orientation to time, immediate and delayed recall of five items, categorical verbal fluency test (listing four-legged animals in one minute), and the Clock Drawing Test. The higher the scores, the better the cognitive function.	T0, T1, T2	(71)

T0, baseline measurement; T1, the end of the 6^{th} week after the exergame-based exercise; T2, the end of the 12^{th} week after the exergame-based exercise.

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maximum strength (62). The MVIC will be measured two times, and the average of the two measurements is recorded. The participants will be allowed to rest for 1 min between measurements.

Sonographic thickness of biceps and triceps brachii muscles

A single-experienced operator, who is not involved in any further data analysis and is blinded to clinical symptoms, will use a portable LOGIQ e-ultrasound (General Electric Company, USA, 2010) equipped with a 5–12-MHz linear array transducer to measure the muscle thickness under sonography. Measurements should be performed by gently applying the transducer onto the skin that is coated by a thin layer of water-soluble gel. The transducer should be held orthogonal to the skin surface to ensure precise depth analyses and avoid transmission parallax error. All participants will undergo measurements in the afternoon. All measurements will be performed once on each side and recorded, respectively. The position used to measure each muscle is as follows:

Biceps brachii muscle

The thickness of the brachial biceps will be obtained at two-third of the way between the acromion and the antecubital crease of the examined upper limb with the transducer placed perpendicular while exerting minimum pressure. The examined upper limb should be extended fully. The thickness is measured in the transverse plane (63).

Triceps brachii muscle

The thickness of the brachial triceps will be obtained at the proximal one-third of the way between the acromion and the olecranon of the examined upper limb with the transducer placed perpendicular while exerting minimum pressure. The examined upper limb should be overhead and extended fully. The thickness is measured in the transverse plane (64).

Measurement of the range of motion of the joints of the dominant upper extremity

The range of motions (ROMs) of the dominant upper extremity, including shoulder flexion, abduction, and external rotation; elbow flexion and extension; forearm supination and pronation; and wrist flexion and extension, will be measured. The ROMs will be measured using a goniometer under standard positions (65).

General function

We will use the Kihon checklist-Taiwan (KC-T) to indicate the ADLs of the participants in this study. The KC-T is a self-reported questionnaire consisting of 25 items divided into seven subcategories: general independence, physical strength, nutrition, oral function, level of social activities outside the home, cognitive function, and risk of depression. Each item is rated as a pass (0) or fail (1); therefore, a higher total score indicates a lower level of function (66). The KC-T is used by the Ministry of Health and Welfare in Taiwan as an outcome indicator for community-based programs that delay and prevent disability (67), and its usability in practice in real-world settings in Taiwan has been proven (68).

Health-related quality of life

We will use the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) to indicate the HRQoL of the participants in this study. The SF-36 is a self-assessment validated generic health survey containing 36 items divided into eight subscales: physical functioning, role limitation due to physical problems, bodily pain, general health, vitality, social functioning, role limitation due to emotional problems, and mental health. The first four subscales represent the physical function, and the subsequent four subscales represent the mental function. Responses to each question will be transformed into a scale ranging from 0 to 100. The higher the scores, the better the HRQoL (69). All subscales have high levels of validity and reliability. The SF-36 has an acceptable internal consistency (Cronbach's α value ranged from 0.74 to 0.95) among the Taiwanese population (70). We will record the scores of the entire questionnaire, physical function, and mental function in this study.

Cognitive level

We will use the Brain Health Test (BHT)–brief cognitive test (BHT-Cog) to measure the cognitive level of the participants in this study. The BHT, developed by the Taiwan Dementia Society, is a simple dementia screening tool with high validity that can help primary care physicians identify patients with cognitive impairment among subjects with memory complaints or those at a high risk of dementia. It consists of risk factors and a brief cognitive test. The BHT-Cog includes orientation to time, immediate and delayed recall of five items, a categorical verbal fluency test (listing four-legged animals in 1 min), and the Clock Drawing Test (10:10) (71). The Ministry of Health and Welfare also uses the BHT in Taiwan as an outcome indicator for community-based programs that delay and prevent disability (67).

Statistical analysis

Sample size calculation

Since a high-quality study evaluating the effects of a similar intervention on muscle strength, physical activity, and function was unavailable when we wrote our protocol, we used previous results from one study using augmented reality (AR)-based exercise for one of our primary outcome measures (ASMMI) (72) for this power calculation. The effect size of a similar study for the AR-based intervention is high (0.71). Based on G*Power (version 3.1.9.2, for Windows), at least 18 observations in each group should be recruited by detecting a difference of 2 standard deviations (SD) of the ASMMI between the groups with a power of 80% and alpha of 5%, and the effect size is determined to be high (*F*-test family, 0.4) (73). To account for an expected dropout rate of 40%, given that most participants in the LTCFs have comorbidities, we decided to increase this number to a group size of 26. Thus, the aim will be to include at least 52 patients in the study.

Quantitative data

We will use Statistical Package for the Social Sciences for Windows (version 19.0; Released 2010; IBM Corp., Armonk, NY, USA) for all statistical analyses. Continuous data are expressed as means with SDs, and categorical variables are presented as absolute numbers or percentages. The normality and homoscedasticity of the data will be checked before each analysis. For comparisons of demographic data between the experimental and control groups, the chi-square test, independent *t*-test, and Mann–Whitney *U*-test will be used as appropriate depending on the features of distributions of the data. As for the training effects on outcomes, a mixed analysis of variance, with time as a within-subject factor and intervention as a between-subject factor, will be performed. *Post hoc* analysis will be performed using the Bonferroni test. *P*-values < 0.05 will be used to indicate statistical significance. If both the data and the residuals are not normally distributed for ANOVA, we will use bootstrapping to get confidence intervals and use those to determine whether effects are statistically significant, rather than using *p*-values directly.

Anticipated results and discussion

This study protocol describes the design of an RCT that evaluates the clinical effect of the exergame-based exercise delivered *via* RFA among older adults in LTCFs. Individuals around the world live longer nowadays, and many countries will become a super-aged society soon. Preventing and delaying the loss of intrinsic capacity and functional ability of older adults and helping them age successfully are crucial for us (74). Elderly individuals living in LTCFs are more prone to geriatric syndromes, such as frailty and sarcopenia, than those living in the community. Moreover, many LTCFs lack healthcare professionals, particularly those in rural regions. This project aims to study whether we can use exergame-based exercise as an alternative to previous manpower-consuming therapies in LTCFs. Given that the concepts of frailty and sarcopenia are multifactorial and their definitions vary, this study focuses on the parameters of muscle and functional performance.

This project is unique as the exergame-based exercise is delivered *via* RFA. Similar to other exergames, RFA uses a gamified approach and immersive scenarios to motivate the player by role-playing (31). It could provide both visual feedback from the screen and sensory feedback from the Ring-Con. Apart from other exergames, with its featured Ring-Con, RFA could be considered a multicomponent exercise, combining PRT and aerobic exercises for strength, balance, and muscle stretching. Studies have evaluated the effectiveness of PRT among community-dwelling older adults and proven that it is easily available at a low cost and effective in improving physical function and strength (10, 75). Therefore, we are looking forward to the clinical effectiveness in improving the muscle parameters after this study.

Another feature of using RFA to deliver exergames is that it could set up the initial amount of exercise that must be performed in each stage and adjust it progressively and automatically depending on the performance of the player. Therefore, the progression for the exercise prescription might be individualized, and it is time- and manpower-saving given that it could be modified by the machine. The ACSM suggests increasing intensity over time to maintain the intensity of the exercises at moderate levels (41–60% of 1 RM for resistance exercise and Borg RPE 12–14 for aerobic exercise) (76). Therefore, the study protocol sets the target intensity of the exergame-based exercise at Borg RPE 13 and leaves the weight intensity to Nintendo Switch itself. Although it is practical to do so in real-world settings, and it is safer for the elderly to perform PRT by squeezing or stretching the Ring-Con, one limitation is that we cannot confirm whether the weight intensity given by the Ring-Con is sufficient at moderate levels.

Given that many elderly individuals in LTCFs use a wheelchair for community ambulation because of poor muscle endurance even though they can walk for a short distance to complete gait measurements (40), the exergame-based exercise of this project focuses on training the upper extremities and trunk. By doing so, the participants could be trained in the sitting position to avoid the potential risk of falls. Therefore, most outcomes of this project, including the HGS, MVIC, sonographic thickness of the biceps and triceps brachii muscles, ROMs of the joints of the dominant upper extremity, and BBT, are measured to evaluate the training effects. A study has proven that aging can attenuate the hypertrophic response of muscle groups to RT (77). Contrary to the findings from healthy young adults, in whom neural factors account for a larger proportion of

the initial strength increment and muscle hypertrophy becomes the dominant factor after the first 3–5 weeks (78), the effect of muscle training may entirely depend on the neuromuscular adaptation among older adults after an 8-week training course (79). Given the results from the classical studies by Moritani and deVries and considering that the project lasted only 3 months, we measured not only the MVIC but also the sonographic thickness to see whether some early morphological changes in the trained muscles could be detected earlier.

The primary outcomes in this protocol are the criteria to diagnose frailty and sarcopenia. Therefore, this project will measure the gait speed through the exergame-based exercise, which focuses on the upper extremities and trunk only. Walking is a complex movement that requires several functional tasks, such as ROMs, velocity, position, and trained muscles (80). The participants must balance in different positions, such as leaning forward, forward reaching, side shuffle, and lateral shifting, to use the RFA. Those movements might occur at the trunk/hips/knees, although the protocol does not allow the performance of PRT on the lower extremities. Granacher et al. demonstrated that the ability of older adults to rise from a chair, ambulate, and make turns improves after 9 weeks of core muscle strength training (81). Park et al. observed that walking speed increased after a sitting boxing program focusing on upper extremity stretching and strengthening for 6 weeks (82). Both studies have proven that strengthening programs can also induce adaptive processes, particularly in the neuromuscular system, thereby enhancing balance performance and functional mobility. Moreover, interlimb and intralimb segment coordination are important for bipedal human gait. Arm swing in the human gait cycle plays an active role in maintaining body posture. The gait speed increases when the amplitude of the arm and leg is increased (83). Given the aforementioned three main reasons, the exergame-based exercise in this study might increase gait speed.

By presenting with heterogeneity, one recent systematic review with the recruitment of 15 eligible RCTs found that exercise not only has a positive effect on physical outcomes but also improves QoL and ADLs in elderly individuals (84). Therefore, in addition to measuring the clinical effects of the exergame-based exercise on parameters of muscle and functional performance, this protocol also includes the QoL and ADL as secondary outcomes. The SF-36 is used to indicate the HRQoL because it is a validated self-assessment generic health survey with a reliable intraclass correlation coefficient (70). KC-T will be used to indicate the ADL since its usability in practice in real-world settings in Taiwan has been proven (68), and it is widely used in LTCFs. Moreover, both the SF-36 and Kihon checklist have Taiwanese versions, which are important when measuring the QoL and ADL because the values toward QoL and ADL vary from culture to culture.

No cutoff value of available cognitive measurements allows us to screen who is suitable for exergames. Most studies on exergames in the elderly recruited participants with sufficient cognition levels to understand the orders and procedures required in the game (36). Many older adults in LTCFs have some cognitive deficits, though the degree varies in Taiwan (68). Exergames have therapeutic effects on cognition among older adults (34, 36). Although the manner of play varies among exergames, there are some commonly shared concepts. Exergame environments provide an extra spatial feature. Players confront challenging tasks with visual and auditory stimuli, cues, and feedback. Immersion into a virtual environment also redirects the player's experiences to improve attention restoration, reduce stress, and promote cognitive rehabilitation. Furthermore, exergames require players to make decisions during the game. Exergames delivered *via* RFA provide all aforementioned features, and we expect that the cognitive level of the elderly in LTCFs improves after the training.

Supervised exercise is safe for frail older adults (85). Only one well-trained assistant will be required to supervise the entire exergame-based exercise procedure in this project. Many participants can play the game simultaneously if the number of Nintendo Switch and RFA is sufficient, which is time- and manpower-consuming. However, this study protocol has some limitations. First, this study used the convenience sampling method because recruiting older adults who regularly participate in LTCFs publicly is difficult and impractical. This project might be less representative, even though the minimum estimated sample size can be met. The results of this study should be applied with caution and could only be generalizable to similar populations. Second, the exergame-based exercise and outcome measures only lasted 3 months. This duration might be insufficient to observe an increase in the muscle mass of older patients. However, given that an intervention period of more than 3 months might lead to a higher attrition rate of the participants, particularly for those in LTCFs with several comorbidities, we will also measure muscle morphology using sonography to detect any earlier change in muscle parameters, and we still suggest a 3-month protocol. Third, to our knowledge, this project is the first study to evaluate the effectiveness of using RFA among elderly individuals living in LTCFs. No established protocol for training with RFA could be followed. Although the entire exercise prescription is based on the guidelines for older adults by the ACSM, we cannot ensure that the protocol for the current exergame-based exercise is ideal. Fourth, individualized setting and progression of the exercises automatically are the features of using RFA to train elderly individuals. However, this project can only measure the exercise intensity by RPE. The investigators cannot confirm the exact resistance provided by the Ring-Con during each training session.

If the findings of this study show that the exergame-based exercise *via* RFA could improve the parameters of muscle and functional performance, the training method should be implemented as a treatment option for all older individuals living in LTCFs, particularly for those in rural

regions with lesser healthcare resources. A successful exergame-based exercise would provide benefits beyond muscle strength, muscle mass, and ADLs of the elderly because it would also enhance the HRQoL or even help the elderly age gracefully. We expect that the protocol will be useful and practical to implement in real-world settings. With the positive results of this protocol, further larger studies could be conducted to examine whether the exergame-based exercise *via* RFA could be a treatment alternative to time- and manpower-consuming therapies currently applied in LTCFs.

Ethics statement

Written informed consent was obtained from the individual for the publication of any potentially identifiable images or data included in this article.

Author contributions

S-HT and Y-JT planned the project and developed the research design. S-HT and S-FS calculated the sample size. L-HC and K-LL were responsible for literature reviewing and evaluating the appropriate measurement tools. S-HT wrote the first draft. Y-JT, L-HC, and S-FS were responsible for the revisions of the manuscript. All authors read and approved the final manuscript version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Ministry of Health and Family Welfare. *Ageing and Long-Term Care Statistics*. New Delhi: Ministry of Health and Family Welfare (2021).

2. Evans W. Skeletal muscle loss: cachexia, sarcopenia, and inactivity. *Am J Clin Nutr.* (2010) 91:1123S–7S. doi: 10.3945/ajcn.2010.28608A

3. Seene T, Kaasik P, Riso E. Review on aging, unloading and reloading: changes in skeletal muscle quantity and quality. *Arch Gerontol Geriatr.* (2012) 54:374–80. doi: 10.1016/j.archger.2011.05.002

4. Marzetti E, Leeuwenburgh C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. *Exp Gerontol.* (2006) 41:1234–8. doi: 10.1016/j.exger.2006.08.011

5. Cruz-Jentoft A, Baeyens J, Bauer J, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European Working Group on Sarcopenia in Older People. *Age Ageing.* (2010) 39:412–23. doi: 10.1093/ageing/afq034

6. Kuo Y, Wang T, Liu L, Lee W, Peng L, Chen L. Epidemiology of sarcopenia and factors associated with it among community-dwelling older adults in Taiwan. *Am J Med Sci.* (2019) 357:124–33. doi: 10.1016/j.amjms.2018.11.008

7. Cesari M, Vellas B, Hsu F, Newman A, Doss H, King A, et al. A physical activity intervention to treat the frailty syndrome in older persons—results from the LIFE-P study. *J Gerontol A Biol Sci Med Sci.* (2015) 70:216–22. doi: 10.1093/gerona/glu099

8. Thomas E, Battaglia G, Patti A, Brusa J, Leonardi V, Palma A, et al. Physical activity programs for balance and fall prevention in elderly: a systematic review. *Medicine.* (2019) 98:e16218.

9. Cruz-Jentoft A, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* (2019) 48:16–31.

10. Liu C, Latham N. Progressive resistance strength training for improving physical function in older adults. *Cochrane Database Syst Rev.* (2009) 2009:Cd002759.

11. Shiota A, Nakayama N, Saito Y, Maeda T, Maeda Y, Nakayama K. Prevalence and associated factors of malnutrition and sarcopenia in a daycare facility: a cross-sectional study. *Healthcare.* (2020) 8:576. doi: 10.3390/healthcare8040576

12. Chang C, Yeh Y, Chang H, Yi-Ju Tsai S, Wang J. Prevalence and risk factors of sarcopenia among older adults aged =65 years admitted to daycare centers of Taiwan: using AWGS 2019 guidelines. *Int J Environ Res Public Health.* (2021) 18:8299. doi: 10.3390/ijerph18168299

13. Lo Y, Wahlqvist M, Huang Y, Chuang S, Wang C, Lee M. Medical costs of a low skeletal muscle mass are modulated by dietary diversity and physical activity in community-dwelling older Taiwanese: A longitudinal study. *Int J Behav Nutr Phys Act.* (2017) 14:31. doi: 10.1186/s12966-017-0487-x

14. Negm A, Lee J, Hamidian R, Jones C, Khadaroo R. Management of sarcopenia: a network meta-analysis of randomized controlled trials. *J Am Med Dir Assoc.* (2022) 23:707–14. doi: 10.1016/j.jamda.2022.01.057

15. Daly M, Vidt M, Eggebeen J, Simpson W, Miller M, Marsh A, et al. Upper extremity muscle volumes and functional strength after resistance training in older adults. *J Aging Phys Act.* (2013) 21:186–207. doi: 10.1123/japa.21.2.186

16. Papa E, Dong X, Hassan M. Resistance training for activity limitations in older adults with skeletal muscle function deficits: a systematic review. *Clin Interv Aging.* (2017) 12:955–61. doi: 10.2147/cia.S104674

17. Beckwée D, Delaere A, Aelbrecht S, Baert V, Beaudart C, Bruyere O, et al. Exercise interventions for the prevention and treatment of sarcopenia. a systematic

umbrella review. *J Nutr Health Aging.* (2019) 23:494–502. doi: 10.1007/s12603-019-1196-8

18. Zhang Y, Zou L, Chen S, Bae J, Kim D, Liu X, et al. Effects and moderators of exercise on sarcopenic components in sarcopenic elderly: a systematic review and meta-analysis. *Front Med.* (2021) 8:649748. doi: 10.3389/fmed.2021.649748

19. Martins W, de Oliveira R, Carvalho R, de Oliveira Damasceno V, da Silva V, Silva M. Elastic resistance training to increase muscle strength in elderly: a systematic review with meta-analysis. *Arch Gerontol Geriatr.* (2013) 57:8–15. doi: 10.1016/j.archger.2013.03.002

20. Csapo R, Alegre L. Effects of resistance training with moderate vs heavy loads on muscle mass and strength in the elderly: a meta-analysis. *Scand J Med Sci Sports.* (2016) 26:995–1006. doi: 10.1111/sms.12536

21. Hsu H, Chen CF. LTC 2.0: the 2017 reform of home- and community-based long-term care in Taiwan. *Health Policy.* (2019) 123:912–6. doi: 10.1016/j.healthpol.2019.08.004

22. Liang C, Change Q, Hung Y, Chen C, Lin C, Wei Y, et al. Effects of a community care station program with structured exercise intervention on physical performance and balance in community-dwelling older adults: a prospective 2-year observational study. *J Aging Phys Activity.* (2017) 25:596–603. doi: 10.1123/japa.2015-0326

23. Chen L, Woo J, Assantachai P, Auyeung T, Chou M, Iijima K, et al. Asian Working Group for Sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *J Am Med Dir Assoc.* (2020) 21:300–(307.e2). doi: 10.1016/j.jamda.2019.12.012

24. Lin Y, Huang C. Aging in Taiwan: building a society for active aging and aging in place. *Gerontologist.* (2015) 56:176–83. doi: 10.1093/geront/gnv107

25. Yu T, Chung K, Wei C, Chien K, Hou Y. Do the preferences of healthcare provider selection vary among rural and urban patients with different income and cause different outcome? *PLoS One.* (2016) 11:e0152776. doi: 10.1371/journal.pone.0152776

26. Winstein C, Wolf S, Dromerick A, Lane C, Nelsen M, Lewthwaite R. Effect of a task-oriented rehabilitation program on upper extremity recovery following motor stroke: the ICARE randomized clinical trial. *JAMA.* (2016) 315:571–81. doi: 10.1001/jama.2016.0276

27. Benjamin K, Edwards N, Guitard P, Murray M, Caswell W, Perrier M. Factors that influence physical activity in long-term care: perspectives of residents, staff, and significant others. *Can J Aging.* (2011) 30:247–58. doi: 10.1017/S0714980811000080

28. Chen K, Li C, Huang H, Cheng Y. Feasible modalities and long-term effects of elastic band exercises in nursing home older adults in wheelchairs: a cluster randomized controlled trial. *Int J Nurs Stud.* (2016) 55:4–14. doi: 10.1016/j.ijnurstu.2015.11.004

29. Meekes W, Stanmore E. Motivational determinants of exergame participation for older people in assisted living facilities: mixed-methods study. *J Med Internet Res.* (2017) 19:e6841. doi: 10.2196/jmir.6841

30. Adcock M, Thalmann M, Schättin A, Gennaro F, De Bruin E. A pilot study of an in-home multicomponent exergame training for older adults: feasibility, usability and pre-post evaluation. *Front Aging Neurosci.* (2019) 11:304. doi: 10.3389/fnagi.2019.00304

31. Jahouh M, González-Bernal J, González-Santos J, Fernández-Lázaro D, Soto-Cámara R, Mielgo-Ayuso J. Impact of an intervention with Wii video games on the autonomy of activities of daily living and psychological–cognitive components in the institutionalized elderly. *Int J Environ Res Public Health.* (2021) 18:1570. doi: 10.3390/ijerph18041570

32. Ismail N, Hashim H, Ahmad Yusof H. Physical activity and exergames among older adults: a scoping review. *Games Health J.* (2022) 11:1–17. doi: 10.1089/g4h.2021.0104

33. Willaert J, De Vries A, Tavernier J, Van Dieen J, Jonkers I, Verschueren S. Does a novel exergame challenge balance and activate muscles more than existing off-the-shelf exergames? *J Neuroeng Rehabil.* (2020) 17:6. doi: 10.1186/s12984-019-0628-3

34. Liao Y, Chen I, Lin Y, Chen Y, Hsu W. Effects of virtual reality-based physical and cognitive training on executive function and dual-task gait performance in older adults with mild cognitive impairment: a randomized control trial. *Front Aging Neurosci.* (2019) 11:162. doi: 10.3389/fnagi.2019.00162

35. Afridi A, Rathore F, Nazir S. Wii fit for balance training in elderly: a systematic review. *J Coll Physicians Surg Pak.* (2021) 30:559–66. doi: 10.29271/jp.2021.05.559

36. Yen H, Chiu H. Virtual reality exergames for improving older adults' cognition and depression: a systematic review and meta-analysis of randomized control trials. *J Am Med Dir Assoc.* (2021) 22:995–1002. doi: 10.1016/j.jamda.2021.03.009

37. Gunst M, De Meyere I, Willems H, Schoenmakers B. Effect of exergaming on wellbeing of residents in a nursing home: a single blinded intervention study. *Aging Clin Exp Res.* (2022) 34:151–7. doi: 10.1007/s40520-021-01903-1

38. Chao Y, Scherer Y, Montgomery C, Lucke K, Wu Y. Exergames-based intervention for assisted living residents: a pilot study. *J Gerontol Nurs.* (2014) 40:36–43. doi: 10.3928/00989134-20140407-04

39. Chu C, Biss R, Cooper L, Quan A, Matulis H. Exergaming platform for older adults residing in long-term care homes: user-centered design, development, and usability study. *JMIR Serious Games.* (2021) 9:e22370. doi: 10.2196/22370

40. Chen G, Lin C, Huang H, Wu Y, Su H, Sun S, et al. Using virtual reality-based rehabilitation in sarcopenic older adults in rural health care facilities-a quasi-experimental study. *J Aging Phys Activity.* (2021) 29:866–77. doi: 10.1123/japa.2020-0222

41. Chan A, Tetzlaff J, Gøtzsche P, Altman D, Mann H, Berlin J, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ.* (2013) 346:e7586. doi: 10.1136/bmj.e7586

42. Schulz K, Altman D, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* (2010) 340:c332. doi: 10.1136/bmj.c332

43. Garber C, Blissmer B, Deschenes M, Franklin B, Lamonte M, Lee I, et al. American College of Sports Medicine position stand. quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc.* (2011) 43:1334–59. doi: 10.1249/MSS.0b013e318213fefb

44. Koivisto J, Hamari J. The rise of motivational information systems: a review of gamification research. *Int J Inf Manage.* (2019) 45:191–210. doi: 10.1016/j.ijinfomgt.2018.10.013

45. Lu C, Hassan L, Buruk O, Nummenmaa T, Peltonen J. "Switch" Up Your Exercise: An Empirical Analysis of Online User Discussion of the Ring Fit Adventure Exergame. *Proceedings of the 5th International GamiFIN Conference.* Liverpool (2021).

46. Comeras-Chueca C, Villalba-Heredia L, Pérez-Llera M, Lozano-Berges G, Marín-Puyalto J, Vicente-Rodríguez G, et al. Assessment of active video games' energy expenditure in children with overweight and obesity and differences by gender. *Int J Environ Res Public Health.* (2020) 17:6714. doi: 10.3390/ijerph17186714

47. Sato T, Shimizu K, Shiko Y, Kawasaki Y, Orita S, Inage K, et al. Effects of nintendo ring fit adventure exergame on pain and psychological factors in patients with chronic low back pain. *Games Health J.* (2021) 10:158–64. doi: 10.1089/g4h.2020.0180

48. Williams N. The Borg Rating of Perceived Exertion (RPE) scale. *Occup Med.* (2017) 67:404–5.

49. Heymsfield S, Adamek M, Gonzalez M, Jia G, Thomas D. Assessing skeletal muscle mass: Historical overview and state of the art. *J Cachexia Sarcopenia Muscle.* (2014) 5:9–18. doi: 10.1007/s13539-014-0130-5

50. Buckinx F, Reginster J, Dardenne N, Croisiser J, Kaux J, Beaudart C, et al. Concordance between muscle mass assessed by bioelectrical impedance analysis and by dual energy X-ray absorptiometry: a cross-sectional study. *BMC Musculoskeletal Disord.* (2015) 16:60. doi: 10.1186/s12891-015-0510-9

51. Choi H, Ko C, Chang Y, Kim G, Choi K, Kim C. Development and validation of bioimpedance prediction equations for fat-free mass in unilateral male amputees. *PeerJ.* (2021) 9:e10970. doi: 10.7717/peerj.10970

52. Chen L, Liu L, Woo J, Assantachai P, Auyeung T, Bahyah K, et al. Sarcopenia in Asia: consensus report of the Asian working group for sarcopenia. *J Am Med Dir Assoc.* (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025

53. Vermeulen J, Neyens J, Spreeuwenberg M, van Rossum E, Hewson D, de Witte L. Measuring grip strength in older adults: comparing the grip-ball with the Jamar dynamometer. *J Geriatr Phys Ther.* (2015) 38:148–53. doi: 10.1519/jpt.0000000000000034

54. Trampisch U, Franke J, Jedamzik N, Hinrichs T, Platen P. Optimal jamar dynamometer handle position to assess maximal isometric hand grip strength in epidemiological studies. *J Hand Surg.* (2012) 37:2368–73. doi: 10.1016/j.jhsa.2012.08.014

55. Bobos P, Nazari G, Lu Z, MacDermid J. Measurement properties of the hand grip strength assessment: A systematic review with meta-analysis. *Arch Phys Med Rehabil.* (2020) 101:553–65. doi: 10.1016/j.apmr.2019.10.183

56. Bohannon R, Glenney S. Minimal clinically important difference for change in comfortable gait speed of adults with pathology: a systematic review. *J Eval Clin Pract.* (2014) 20:295–300. doi: 10.1111/jep.12158

57. Ensrud K, Ewing S, Cawthon P, Fink H, Taylor B, Cauley J, et al. A comparison of frailty indexes for the prediction of falls, disability, fractures, and mortality

in older men. *J Am Geriatr Soc.* (2009) 57:492–8. doi: 10.1111/j.1532-5415.2009.02137.x

58. Hu B, Hsiao-Wei Y, Chiu T, Li-Ling L, Chen Y. The validity of the study of osteoporotic fractures (sof) index for assessing community-based older adults in Taiwan. *Innov Aging.* (2018) 2(Suppl 1):1015–1015. doi: 10.1093/geroni/igy031.3746

59. Desrosiers J, Bravo G, Hebert R, Dutil E, Mercier L. Validation of the box and block test as a measure of dexterity of elderly people: reliability, validity, and norms studies. *Arch Phys Med Rehabil.* (1994) 75:751–5. doi: 10.1016/0003-9993(94)90130-9

60. Chen H, Chen C, Hsueh I, Huang S, Hsieh C. Test-retest reproducibility and smallest real difference of 5 hand function tests in patients with stroke. *Neurorehabil Neural Repair.* (2009) 23:435–40. doi: 10.1177/1545968308331146

61. Buckinx F, Croisier J, Reginster J, Dardenne N, Beaudart C, Slomian J, et al. Reliability of muscle strength measures obtained with a hand-held dynamometer in an elderly population. *Clin Physiol Funct Imaging.* (2017) 37:332–40. doi: 10.1111/cpf.12300

62. Stark T, Walker B, Phillips J, Fejer R, Beck R. Hand-held dynamometry correlation with the gold standard isokinetic dynamometry: a systematic review. *PM R.* (2011) 3:472–9. doi: 10.1016/j.pmrj.2010.10.025

63. Sanz-Paris A, González-Fernandez M, Hueso-Del Río L, Ferrer-Lahuerta E, Monge-Vazquez A, Losfablos-Callau F, et al. Muscle thickness and echogenicity measured by ultrasound could detect local sarcopenia and malnutrition in older patients hospitalized for hip fracture. *Nutrients.* (2021) 13:2401. doi: 10.3390/nu13072401

64. De Toni L, Petre G, Garolla A, De Santis I, Valente U, Foresta C, et al. Prognostic value of ultrasound stratigraphy in long-term weight loss: results from a nutritional counseling program. *Obesity Facts.* (2019) 12:606–17. doi: 10.1159/000502119

65. Norkin C, White D. *Measurement of Joint Motion: A Guide to Goniometry*. Philadelphia, PA: F.A. Davis (2009).

66. Satake S, Senda K, Hong Y, Miura H, Endo H, Sakurai T, et al. Validity of the kihon checklist for assessing frailty status. *Geriatr Gerontol Int.* (2016) 16:709–15. doi: 10.1111/ggi.12543

67. Tsay S, Chen S, Lu S, Liu L. [The policy and practice of preventive care for the elderly]. *Hu Li Za Zhi.* (2018) 65:13–9. doi: 10.6224/jn.201804_65(2)0.03

68. Mao H, Tsai A, Chang L, Tsai I. Multi-component cognitive intervention for older adults with mixed cognitive levels: implementation and preliminary effectiveness in real-world settings. *BMC Geriatrics.* (2021) 21:543. doi: 10.1186/s12877-021-02489-z

69. Chang C, Huang C, Chang Y, Tai C, Lin J, Wang J. Cross-validation of the Taiwan version of the moorehead-ardelt quality of life questionnaire II with WHOQOL and SF-36. *Obesity Surg.* (2010) 20:1568–74. doi: 10.1007/s11695-009-9813-y

70. Tseng H, Lu J, Gandek B. Cultural issues in using the SF-36 Health Survey in Asia: results from Taiwan. *Health Qual Life Outcomes.* (2003) 1:72. doi: 10.1186/1477-7525-1-72

71. Tsai P, Liu J, Lin K, Chang C, Pai M, Wang W, et al. Development and validation of a dementia screening tool for primary care in Taiwan: brain health test. *PLoS One.* (2018) 13:e0196214. doi: 10.1371/journal.pone.0196214

72. Jeon S, Kim J. Effects of augmented-reality-based exercise on muscle parameters, physical performance, and exercise self-efficacy for older adults. *Int J Environ Res Public Health.* (2020) 17:3260. doi: 10.3390/ijerph17093260

73. Faul F, Erdfelder E, Lang A, Buchner A. G* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* (2007) 39:175–91.

74. Michel J, Leonardi M, Martin M, Prina M. WHO's report for the decade of healthy ageing 2021–30 sets the stage for globally comparable data on healthy ageing. *Lancet Healthy Longevity.* (2021) 2:e121–2. doi: 10.1016/S2666-7568(21)00002-7

75. Bårdstu H, Andersen V, Fimland M, Aasdahl L, Raastad T, Cumming K, et al. Effectiveness of a resistance training program on physical function, muscle strength, and body composition in community-dwelling older adults receiving home care: a cluster-randomized controlled trial. *Eur Rev Aging Phys Act.* (2020) 17:11. doi: 10.1186/s11556-020-00243-9

76. McDermott A, Mernitz H. Exercise and older patients: prescribing guidelines. *Am Family Phys.* (2006) 74:437–44.

77. Brook M, Wilkinson D, Phillips B, Perez-Schindler J, Philp A, Smith K, et al. Skeletal muscle homeostasis and plasticity in youth and ageing: impact of nutrition and exercise. *Acta Physiol.* (2016) 216:15–41. doi: 10.1111/apha.12532

78. Moritani T, deVries H. Neural factors versus hypertrophy in the time course of muscle strength gain. *Am J Phys Med.* (1979) 58:115–30.

79. Moritani T, Devries H. Potential for gross muscle hypertrophy in older men. *J Gerontol.* (1980) 35:672–82. doi: 10.1093/geronj/35.5.672

80. Eng J. Strength Training in Individuals with Stroke. *Physiother Can.* (2004) 56:189–201. doi: 10.2310/6640.2004.00025

81. Granacher U, Lacroix A, Muehlbauer T, Roettger K, Gollhofer A. Effects of core instability strength training on trunk muscle strength, spinal mobility, dynamic balance and functional mobility in older adults. *Gerontology.* (2013) 59:105–13. doi: 10.1159/000343152

82. Park J, Gong J, Yim J. Effects of a sitting boxing program on upper limb function, balance, gait, and quality of life in stroke patients. *NeuroRehabilitation.* (2017) 40:77–86. doi: 10.3233/NRE-161392

83. Bovonsunthonchai S, Hiengkaew V, Vachalathiti R, Vongsirinavarat M, Tretriluxana J. Effect of speed on the upper and contralateral lower limb coordination during gait in individuals with stroke. *Kaohsiung J Med Sci.* (2012) 28:667–72. doi: 10.1016/j.kjms.2012.04.036

84. Campbell E, Petermann-Rocha F, Welsh P, Celis-Morales C, Pell J, Ho F, et al. The effect of exercise on quality of life and activities of daily life in frail older adults: a systematic review of randomised control trials. *Exp Gerontol.* (2021) 147:111287. doi: 10.1016/j.exger.2021.111287

85. De Labra C, Guimaraes-Pinheiro C, Maseda A, Lorenzo T, Millán-Calenti J. Effects of physical exercise interventions in frail older adults: a systematic review of randomized controlled trials Physical functioning, physical health and activity. *BMC Geriatrics.* (2015) 15:154. doi: 10.1186/s12877-015-0155-4

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Caloric restriction delays age-related muscle atrophy by inhibiting 11β-HSD1 to promote the differentiation of muscle stem cells

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Introduction: Calorie restriction (CR) is an important direction for the delay of sarcopenia in elderly individuals. However, the specific mechanisms of CR against aging are still unclear.

Methods: In this study, we used a CR model of elderly mice with muscle-specific 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) knockout mice and 11β-HSD1 overexpression mice to confirm that CR can delay muscle aging by inhibiting 11β-HSD1 which can transform inactive GC(cortisone) into active GC(cortisol). The ability of self-proliferation and differentiation into muscle fibers of these mouse muscle stem cells (MuSCs) was observed *in vitro*. Additionally, the mitochondrial function and mitochondrial ATP production capacity of MuSCs were measured by mitochondrial oxygen consumption.

Results: It was found that the 11β-HSD1 expression level was increased in age-related muscle atrophy. Overexpression of 11β-HSD1 led to muscle atrophy in young mice, and 11β-HSD1 knockout rescued age-related muscle atrophy. Moreover, CR in aged mice reduced the local effective concentration of glucocorticoid (GC) through 11β-HSD1, thereby promoting the mitochondrial function and differentiation ability of MuSCs.

Conclusions: Together, our findings highlight promising sarcopenia protection with 40% CR in older ages. Furthermore, we speculated that targeting an 11β-HSD1-dependent metabolic pathway may represent a novel strategy for developing therapeutics against age-related muscle atrophy.

KEYWORDS

caloric restriction (CR), 11β-HSD1, muscle atrophy, muscle stem cells (MuSCs), mitochondrial function

Highlights

- 11β-HSD1 expression levels were increased in age-related muscle atrophy.
- Overexpression of 11β-HSD1 led to muscle atrophy in young mice, and muscle-specific 11β-HSD1 knockout rescued age-related muscle atrophy.
- CR in aged mice reduced the local effective concentration of glucocorticoid (GC) through 11β-HSD1, thereby promoting the mitochondrial function and differentiation ability of MuSCs.
- Our findings highlight promising sarcopenia protection with 40% CR in older ages.
- We speculated that targeting an 11β-HSD1-dependent metabolic pathway may represent a novel strategy for developing therapeutics against age-related muscle atrophy.

1. Introduction

Progressive age-related loss of skeletal muscle mass and function is known as sarcopenia and increases an adult's individual's susceptibility to adverse clinical outcomes (1). Currently, there is no effective treatment for sarcopenia, and the treatment is limited to improving nutrition and strengthening exercise. It is well known that muscle stem cells (MuSCs) are mainly responsible for skeletal muscle regeneration (2). With increasing age, the proliferation and differentiation abilities of MuSCs decrease significantly, resulting in impaired muscle regeneration in senescent individuals (3). Thus, improving the function of MuSCs is an effective way to delay sarcopenia.

It is well established that calorie restriction (CR) cannot only reduce fat accumulation and improve insulin resistance but can also maintain organ function and has anti-aging effects (4). Recently, Sharples et al. also found that CR is an effective intervention method to reduce progressive muscle loss in elderly individuals, which is of great significance to prolong life and promote healthy aging (5). Yang et al. found that CR maintained muscle homeostasis and improved muscle protein quality by enhancing autophagy and reducing inflammation, indicating that CR is an important regulator of sarcopenia (6). In addition, studies have found that CR cannot only increase the expression of Pax7, a specific gene of muscle stem cells, but can also increase the number of mitochondria, oxidative respiratory chain enzymes, and aerobic utilization in stem cells (7). Thus, CR is an important direction for the delay of sarcopenia in elderly individuals. However, the specific mechanisms of CR against aging are still unclear.

Numerous studies have shown that long-term stress accelerates aging (8, 9). In fact, glucocorticoid (GC) is a stress hormone, and endogenous GC levels increase by 20–50% with aging (10). The GC activity is mainly regulated by 11β-hydroxylated steroid dehydrogenase1 (11β-HSD1) which can transform inactive GC(cortisone) into active GC(cortisol). So 11β-HSD1 is known as the local amplifier of GC and plays a key role in regulating organ aging (11). Some studies have found that the expression of 11β-HSD1 increases with aging in brain, skin, and muscle tissues, which is closely related to impaired memory, skin aging and decreased muscle strength (12–14). It has been confirmed that CR can affect the expression level of 11β-HSD1 in fat, liver, muscle, and other organs of mice and pigs (15). Therefore, there may be a potential relationship between 11β-HSD1, CR and skeletal muscle aging. The study of 11β-HSD1 is helpful to clarify the mechanism of the anti-aging action of CR.

Thus, we used a CR model of elderly mice, combined with muscle-specific 11β-HSD1 knockout mice and 11β-HSD1 overexpression mice, to confirm that CR can delay muscle aging by inhibiting 11β-HSD1. Moreover, the proliferation and differentiation ability of MuSCs from these mice were observed *in vitro*. Together, we aimed to propose an effective regimen to improve the function of MuSCs that may be promising in sarcopenia protection during aging.

2. Materials and methods

2.1. Human samples

Circulating blood samples were taken from 440 people aged from 23 to 94 years. We collected blood samples from participants who had fasted overnight, centrifuged the samples at 4.0°C for 10 min at 1,000 rpm, and then analyzed the samples. Muscle biopsies from 20 adults aged 15 to 65 years were obtained from the vastus lateralis under general anesthesia during fracture surgery. The exclusion criteria were as follows: (1) Adults with musculoskeletal disorders, autoimmune disorders, thyroid dysfunction, other endocrine disorders (e.g., pituitary, adrenal, and parathyroid disorders), and other disorders; and (2) patients who took gonadal hormone, glucocorticoids, thyroid hormones, anti-seizure medication, anti-depressants or other drugs that affect muscle metabolism. All subjects and samples in this study were collected in accordance with the protocol approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (No. 2019-SR-481). Informed consent of all subjects was obtained.

2.2. Muscle mass and function assessment

A dual-energy X-ray absorptiometry scanner (Hologic, Bedford, Massachusetts, USA) was used to measure total skeletal muscle mass as well as local muscle mass, such as that of the arms and legs. All scans were obtained by the same certified

technician. The instrument used in this study has stable long-term performance (coefficient of variation <0.5%) and high-confidence *in vivo* precision.

The electronic grasping force instrument measures grip strength of the mice:mice were placed in the laboratory for 20 min to acclimate. Remove the mice from the cage and grasp the middle part of the mouse's tail with the thumb and forefinger. After the mice forelimb grasps the sensing rod of the instrument, drag the mouse tail parallel to the rear until the forelimb is released. The instrument automatically records the maximum grip during the process. This process was repeated three times and the maximum grip value was recorded.

2.3. Animal model

2.3.1. Calorie restricted (CR) mice

19-month-old C57BL/6J male mice (Model Animal Research Center of Nanjing University) were reared on a 12-hour light/dark cycle. They were housed individually in cages and fed *ad libitum* with standard laboratory chow for 4 days. Mean intakes were estimated as 4-day daily food intakes. Then, they were randomly divided into two groups: normal diet (ND) and calorie restricted (CR). For the next 12 weeks, ND mice were maintained on an *ad libitum* diet. Gradually increase the degree of CR, starting with a 10% restriction in the first week, increasing to 25% in the second week and 40% in the remaining 10 weeks. Both groups had unlimited access to water. Food intake was monitored daily, and the animals were weighed weekly.

2.3.2. 11β-HSD1 knock-in mice

The strategy we chose recommended to select Transcript Hsd11b1-201 (NM_017080) for presentation. The Hsd11b1-201 gene had six exons, the ATG start codon is in exon 1, and the exon 6 contains the TAG stop codon. We generated H11-CAG-Hsd11b1-flag-PolyA knock-in mice by the CRISPR/Cas9 system. Co-inject Cas9 mRNA, sgRNA, and donor into fertilized eggs. The sgRNA directs Cas9 endonuclease to cut at the H11 locus and creates a DSB (double-strand break). This breaks were repaired and leads to the insertion of in CAG-Hsd11b1-flag-PolyA into the H11 locus.

2.3.3. 11β-HSD1 knockout mice

The strategy we chose recommended to select Transcript Hsd11b1-001 for presentation. Approximately 0.7 kb of the genomic region was removed *via* Cre/LoxP excision. The absence of exon 3 caused a frame shift that ultimately disrupted the protein domain that followed. Approximately 29 N-terminal residues remained intact.

2.3.4. MyoD-cre 11β-HSD1 knockout mice

11β-HSD1$^{flox/flox}$ mice were crossed with MyoD-Cre mice obtained from Shanghai Institute of Biochemistry and Cell Biology. Offspring were intercrossed to generate MyoD-cre 11β-HSD1$^{-/-}$ mice. 11β-HSD1$^{flox/flox}$ littermates were used as controls.

All experimental protocols for animals in this study were reviewed and approved by the Animal Care Committee of the Model Animal Research Center of Nanjing University and in compliance with the Institutional Animal Care and Use Committee guidelines.

2.4. Muscle satellite cell isolation and differentiation

Mouse muscle stem cells (MuSCs) were isolated by a preliminary research protocol (16). Briefly, All hindlimb muscles of mice were dissected and scratched to pieces. Muscles were digested in wash medium (Ham's F10 with 10% horse serum) containing collagenase II (800 units/ml) for 60 min at 37°C. Digested muscles were washed in washing medium, then collagenase II (80 units/ml) and dispase (1 unit/ml) were added for further digestion for 30 min. The resulting suspensions were pipetted 15 times with a 20G needle of a syringe and then filtered with a 40-μm cell strainer. FACS analysis was performed, and cells with Sca1-/CD11-/CD31-/CD45-/VCAM1 + signals represent the population of MuSCs. All antibodies used in FACS analysis were used at a dilution of 1:75. MuSC sorting was performed by using the BD Influx cell sorter (BD Biosciences). MuSCs were cultured on collagen-coated dishes in Ham's F-10 Nutrient Mixture (Gibco) [20% FBS, 2.5 ng/ml bFGF (Invitrogen)] and T-cell conditioned medium (F10 medium with 20% FBS:T-cell medium = 50:50). Cultures were passaged when they reached 60–70% confluence.

The medium was replaced to differentiation medium (DMEM) containing 2% horse serum to induce cells differentiation. Cells could be maintained in differentiation medium for 72 h. BVT.2733 is a 11β-HSD1 selective inhibitor synthesized by China Pharmaceutical University based on patent information.

2.5. MTT assay

The Cell vaccination in 96-well plates (with equal number of cells in each well), and the cell viability was measured by MTT assay daily for consecutive 5 days. Briefly, add 20 μl MTT working solution (5 mg/ml) to each well, and incubate the plates at 37°C for 4 h. Then, remove the supernatants, and the resultant

MTT formazan was dissolved in 100 μl of DMSO. Absorbance measurements at 595 and 630 nm wavelengths.

2.6. Myofiber diameter and cross-sectional area measurement

Using Adobe Acrobat 9 pro software (Adobe) to measure the myofiber diameter. Three independent visual fields were randomly selected in each sample. Each treatment measured 300 fibers from each field of view. Measure the myofiber cross-sectional area by ImageJ software (NIH). Three independent visual fields in each sample were chosen randomly. Three hundred myofibers from each visual field were measured for analysis. The person who performed the measurement was blinded to the identity of the sample.

2.7. Gene expression analysis

Total RNA was isolated using RNeasy kits (Qiagen). 1 μg of total RNA per sample was reverse transcribed to cDNA by MuLV transcriptase (NEB) and oligo dT primers following the manufacturer's instructions. Briefly, RNA was denatured at 85°C for 3 min. MuLV reverse transcriptase was subsequently added to the mixture and incubation under 42°C for 1 h. Three replicate quantitative PCR (qPCR) reactions were performed in a Bio-Rad thermocycler system (Bio-Rad) by using SYBR Green PCR master mix (DBI). Data were analyzed with iQ5 optical system software (Bio-Rad).

2.8. Western blot method

Four mice were decapitated with 1% sodium pentobarbital and muscle was immediately collected. Tissues and cells were homogenized in a lysis buffer containing a protease inhibitor mixture, centrifuged at 12,000 × *g* for 15 min, and supernatant was collected. The proteins were isolated using SDS-PAGE gel (10% separation gel) and transferred to NC membranes. The membranes were blocked at room temperature with 5% degreased emulsion for 1 h and incubated with the respective master antibody at 4°C overnight:11β-HSD1 (ab169785, 1:1000, Abcam, Cambridge, United Kingdom), MuRF1 [MuRF1 (C-11): sc-398608, 1:200; SANTA CRUZ BIOTECHNOLOGY, INC.], Atrogin1 [MAFbx (F-9): SC-166806, 1:200; SANTA CRUZ BIOTECHNOLOGY, INC.], and GAPDH (MC4, #RM2002, 1:10000; Ray Antibody Biotech). After three washes with TBST, Horseradish peroxidase (HRP) is combined with goat anti-rabbit Antibody (#RM3002, 1:10000, Ray Antibody Biotech) or goat anti-mouse antibody (#RM3001, 1:10000, Ray Antibody Biotech). The protein was detected by ECL (Bio-Rad) method.

2.9. Immunofluorescent staining

Cells or sections were fixed with 4% paraformaldehyde and then permeabilized with cold methanol, along with anti-MYHC (Merck Millipore, clone 05-716, 1:1000) and anti-lamnin (Abcam, clone B00648, 1:1000) antibodies as primary antibodies. Cells and sections were subsequently stained with Alexa 488-, 561- or 647-labeled anti-mouse or -rabbit antibodies (Invitrogen). All images were obtained by confocal microscopy (Leica).

2.10. Measurement of oxygen consumption

Differentiated cell's oxygen consumption rates (OCRs) were measured using an XF24 respirometer (Seahorse Bioscience, Santa Clara, California, USA). Basal oxygen consumption as well as oxygen consumption in the presence of drugs that disrupt the mitochondrial respiratory chain were measured: oligomycin (OL, ATP synthase inhibitor, 1 mM) and carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP, uncoupler, 1 mM). Finally, mitochondrial respiration was blocked with rotenone (Rot, 1 mM) (Sigma–Aldrich).

2.11. Statistical analysis

All data are expressed as the mean ± SEM. Comparisons between groups were analyzed using an unpaired Student's *t*-test by GraphPad Prism. One-way ANOVA with Tukey *post-hoc* analysis was used for comparisons between multiple groups. The results of the analysis and statistical significance are presented in the Figures and Figure Legends. A *P*-value < 0.05 was considered significant. Every experiment was repeated independently.

3. Results

3.1. The 11β-HSD1 expression level is up-regulated in human muscles with age-related atrophy

Total and regional lean mass (arms and legs) were obtained from the DXA measurement. Pearson correlation analysis showed that total lean mass was negatively correlated with age ($r = -0.328$, $P < 0.0001$) (**Figure 1A**). In addition, as shown in **Figure 1B**, the decline rate of sarcopenia caused by aging in all regions of the body was not consistent. With aging, the muscles of the lower limbs declined faster than those of the upper limbs (legs: $r = -0.411$, $P < 0.0001$; arms: $r = -0.345$, $P < 0.0001$)

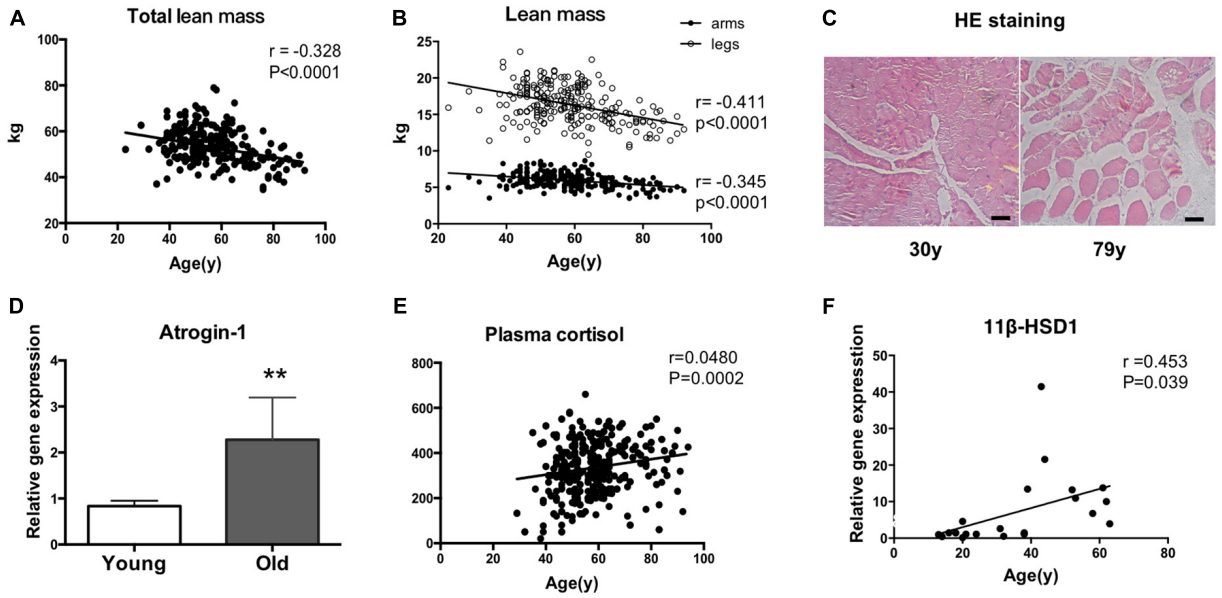

FIGURE 1
11β-HSD1 expression is up-regulated in human muscles with age-related atrophy. **(A)** Total and regional lean mass (arms and legs) were obtained from the DXA measurement. Total lean mass was negatively correlated with age ($r = -0.328$, $P < 0.0001$). **(B)** With aging, the muscles of the lower limbs decline faster than those of the upper limbs (legs: $r = -0.411$, $P < 0.0001$; arms: $r = -0.345$, $P < 0.0001$). **(C)** Representative HE staining images of muscle fibers in a 79-year-old man and a 30-year-old man. Scale bars, 50 μm. **(D)** RT–PCR assays were performed with vastus lateralis muscles obtained from young (aged < 30 years, $n = 7$) or old people (aged > 60 years, $n = 8$), and the results were normalized to GAPDH. $^{**}p < 0.01$. **(E)** Plasma cortisol levels were examined by ELISA in blood samples taken from recruited people ($n = 440$). **(F)** The mRNA level of 11β-HSD1 in vastus lateralis muscles was positively correlated with age ($r = 0453$, $p = 0.039$) ($n = 20$).

(Figure 1B). The muscle fiber size was also examined. Muscle fiber size seemly was reduced in a 79-year-old man compared with a 30-year-old man, suggesting the possible occurrence of muscle atrophy (Figure 1C). Consistently, the expression levels of atrogin-1 were increased in the vastus lateralis muscles of older people compared with young people, as indicated by RT–qPCR (Figure 1D). Numerous studies have shown that long-term stress accelerates aging. Cortisol is a stress hormone, and the plasma cortisol level increased with aging (Figure 1E). As an enzyme that locally activates glucocorticoids, the mRNA level of 11β-HSD1 in vastus lateralis muscles was positively correlated with age ($r = 0453$, $p = 0.039$) (Figure 1F).

3.2. CR may improve muscle atrophy and muscle function in aged mice by reducing 11β-HSD1

To explore the function of CR in sarcopenia, we analyzed mice under CR initiated at 19 months of age and continued for 3 months to 22 months of age (Figure 2A). Then, we found that body weight was decreased in CR mice compared to normal diet (ND) mice (Figure 2B). Furthermore, we studied muscle strength and function in both the ND and CR groups of mice. We noticed that grip strength/body weight increased in CR mice compared with ND mice (Figure 2C). High-energy muscle contains a large number of mitochondria. Fat deposition will mostly produce toxic lipid intermediates through anaerobic metabolic pathways, which will eventually lead to mitochondrial dysfunction, increased endoplasmic reticulum pressure, and even cell death. Using electron microscopy, we found that CR could reduce lipid droplets in muscle; however, an increased number of droplets could be found in ND aged mice, suggesting that CR reduced mitochondrial damage (Figure 2D). Based on previous observations, the mRNA expression levels of the senescence marker gene $p16^{ink4a}$ and the inflammation gene TNFα were low in aged CR mice (Figures 2E, F).

In response to CR, we noticed that muscle weight/body weight increased in CR mice compared with ND mice (Figure 2G). The size of the muscle fiber in CR mice was larger than that in ND mice (Figure 2H). Moreover, the mRNA levels of the muscle atrophy factors atrogin-1 and MuRF-1 were down-regulated in both TA and GAS (Figures 2I, J). To determine whether CR plays a role in delaying muscle atrophy through 11β-HSD1, we examined 11β-HSD1 expression levels in muscles. The expression level of 11β-HSD1 was markedly decreased in both the TA and GAS of CR mice compared with ND mice (Figure 2K). The western blot results of Atrogin1, MuRF1, and 11β-HSD1 were included in Supplementary Figure 1. These results revealed

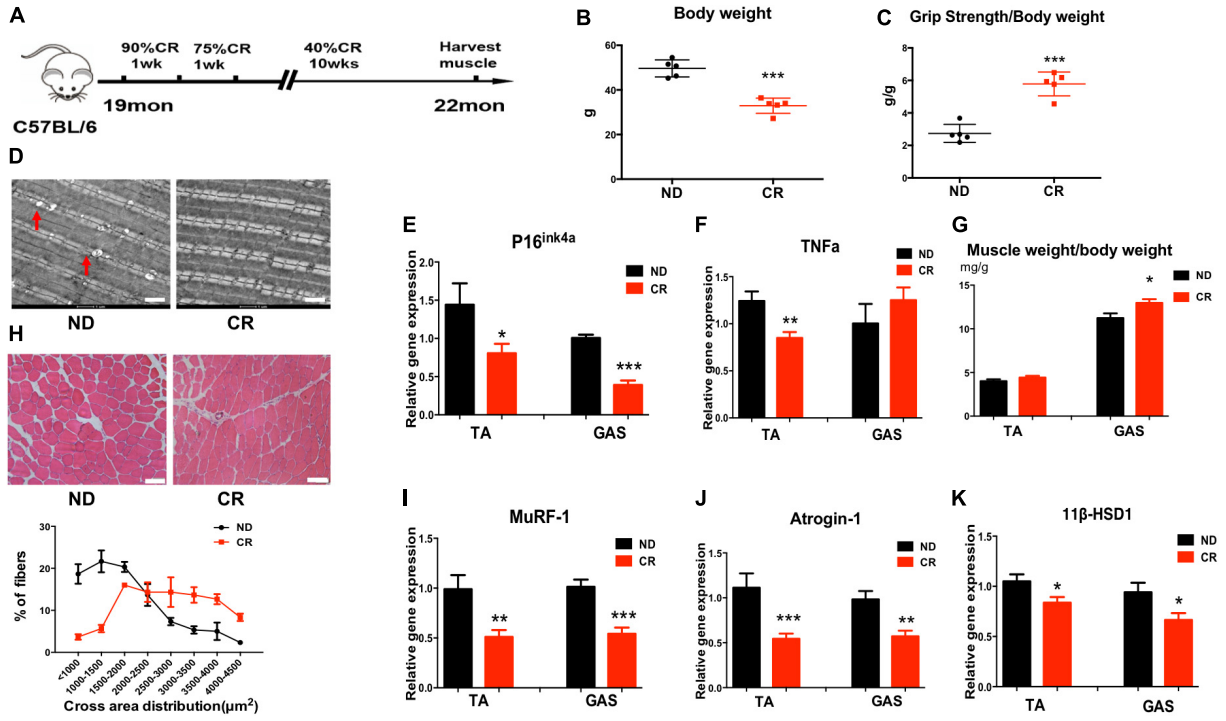

FIGURE 2
Calorie restriction (CR) improves muscle atrophy and muscle function in aged mice by reducing 11β-HSD1. **(A)** CR was initiated in mice at 19 months of age and continued for 3 months to 22 months of age. **(B)** Body weight of CR mice and normal diet (ND) mice (n = 5 mice per group). **(C)** Muscle strength of CR mice and ND mice (n = 5 mice per group). **(D)** Representative images using electron microscopy for vastus lateralis muscles from ND and CR mice. Scale bars, 2 μm. **(E,F)** mRNA quantification of the senescence marker gene $p16^{ink4a}$ and the inflammation gene TNFα. **(G)** Muscle weight/body weight in CR and ND mice (n = 5 mice per group). **(H)** Representative HE staining images of muscle cross sections derived from CR and ND mice. Scale bars, 50 μm. Percentage distribution of muscle fiber cross-sectional area derived from muscles (n = 5 mice per group). **(I,J)** mRNA levels of the muscle atrophy factors atrogin-1 and MuRF-1 in both TA and GAS (n = 5 mice per group). **(K)** mRNA quantification of the 11β-HSD1 genes in both TA and GAS (n = 5 mice per group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

the correlation between the down-regulation of 11β-HSD1 in muscle and CR, suggesting that 11β-HSD1 may be the target of CR.

3.3. Overexpression of 11β-HSD1 leads to muscle atrophy in young mice

Considering that 11β-HSD1 may cause muscle atrophy, we constructed an 11β-HSD1-overexpressing mouse model. The identification of overexpressed mice is included in the Supplementary Figure 2. No differences of body weight, fat mass and lean mass were observed between 11β-HSD1-overexpressing mice and control mice (Figures 3A, C, D); however, grip strength decreased in 11β-HSD1-overexpressing mice compared with control mice (Figure 3B). To further explore whether the elevated 11β-HSD1 level could induce muscle atrophy *in vivo*, we examined lean mass and fiber size and found decreased lean mass and smaller muscle fiber size in 11β-HSD1-overexpressing mice (Figure 3E). Consistent with the morphological changes, the expression levels of atrogin-1 and MuRF1 were significantly increased in overexpression mice, as indicated by RT–qPCR and western blot (Figures 3F, G). These results suggested that 11β-HSD1 can induce atrophy in young mice.

3.4. Muscle-specific 11β-HSD1 knockout rescued age-related muscle atrophy in aged mice

To investigate whether reducing the 11β-HSD1 expression level could rescue muscle atrophy in old mice, we generated muscle-specific 11β-HSD1 knockout mice (MyoD-Cre11β-$HSD1^{-/-}$) by crossing MyoD-cre mice with 11β-HSD1 flox/flox mice (Supplementary Figure 2). 11β-$HSD1^{flox/flox}$ littermates were used as controls. Mice at 22 months of age were healthy. No tumors, obesity, injury, or other diseases were detected. Although the body weight and fat mass of the mice were not different between the control and MyoD-cre 11β-$HSD1^{-/-}$ mice (Figures 4A, B), both the lean mass and muscle strength were increased in muscle-specific 11β-HSD1

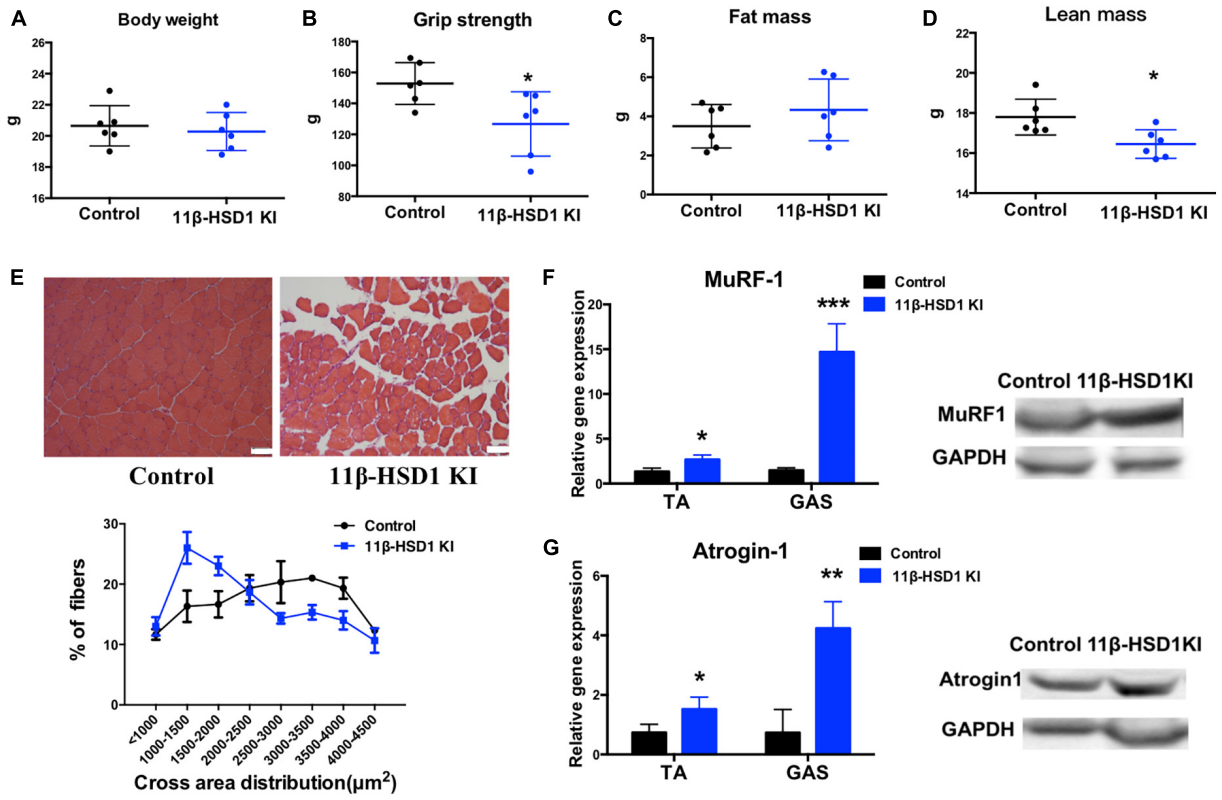

FIGURE 3
Overexpression of 11β-HSD1 leads to muscle atrophy in young mice. **(A)** Body weight of 11β-HSD1 KI mice and control mice (n = 6 mice per group) at 2 months of age. **(B)** Muscle strength of 11β-HSD1 KI mice and control mice (n = 6 mice per group). **(C)** Fat mass of 11β-HSD1 KI mice and control mice (n = 6 mice per group). **(D)** Lean mass of 11β-HSD1 KI mice and control mice (n = 6 mice per group). **(E)** Representative HE staining images of muscle cross sections derived from 11β-HSD1 KI mice and control mice. Scale bars, 50 μm. Percentage distribution of muscle fiber cross-sectional area derived from muscles. **(F,G)** mRNA and protein levels of the muscle atrophy factors atrogin-1 and MuRF-1 in both TA and GAS (n = 6 mice per group). TA, Tibialis anterior muscle; GAS, gastrocnemius. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

knockout mice (Figures 4C, D). Figure 4E shows representative HE staining images of muscle cross sections, and muscle-specific 11β-HSD1 knockout delayed muscle atrophy in 20-month-old mice (Figure 4E). We further found that the gene and protein expression levels of the muscle atrophy factors atrogin-1 and MuRF1 were down-regulated upon muscle-specific 11β-HSD1 knockout (Figures 4F, G). Additionally, the mRNA level of the senescence marker gene $p16^{ink4a}$ in muscles was decreased (Figure 4H). Considering that MyoD is one of the main markers of muscle stem cells (MuSCs), these data demonstrated that 11β-HSD1 may rescue age-related muscle atrophy by maintaining the function of MuSCs.

3.5. The differentiation ability of MuSCs in 11β-HSD1 KO mice and CR mice was improved

To further confirm that 11β-HSD1 plays essential roles in maintaining the function and homeostasis of MuSCs, we first isolated MuSCs from CR and 11β-HSD1$^{-/-}$ mice and checked the effects of 11β-HSD1 on MuSCs (Figure 5A). As expected, 11β-HSD1 expression was significantly decreased in the MuSCs of both 11β-HSD1 knockout and CR mice (Figure 5B). Notably, there was no obvious difference in the proliferation rates of MuSCs sorted from 22-month-old CR and 11β-HSD1$^{-/-}$ mice and their wild-type counterparts detected by MTT assay (Figure 5C). However, we observed that muscle-specific 11β-HSD1 knockout mice and CR mice displayed better differentiation ability, as indicated by larger myofibers (Figure 5A). In addition, both 11β-HSD1 knockout mice and CR mice showed increased expression levels of MyHC and MyOG during the differentiation of MuSCs (Figure 5D). Consistent with the results of gene, both 11β-HSD1 knockout and CR mice showed increased immunofluorescence staining of MyHC (Figure 5E). Taken together, these data demonstrated that inhibition of 11β-HSD1 expression promoted myoblast differentiation. CR may promote MuSC differentiation and delay muscle atrophy by down-regulating 11β-HSD1 in MuSCs.

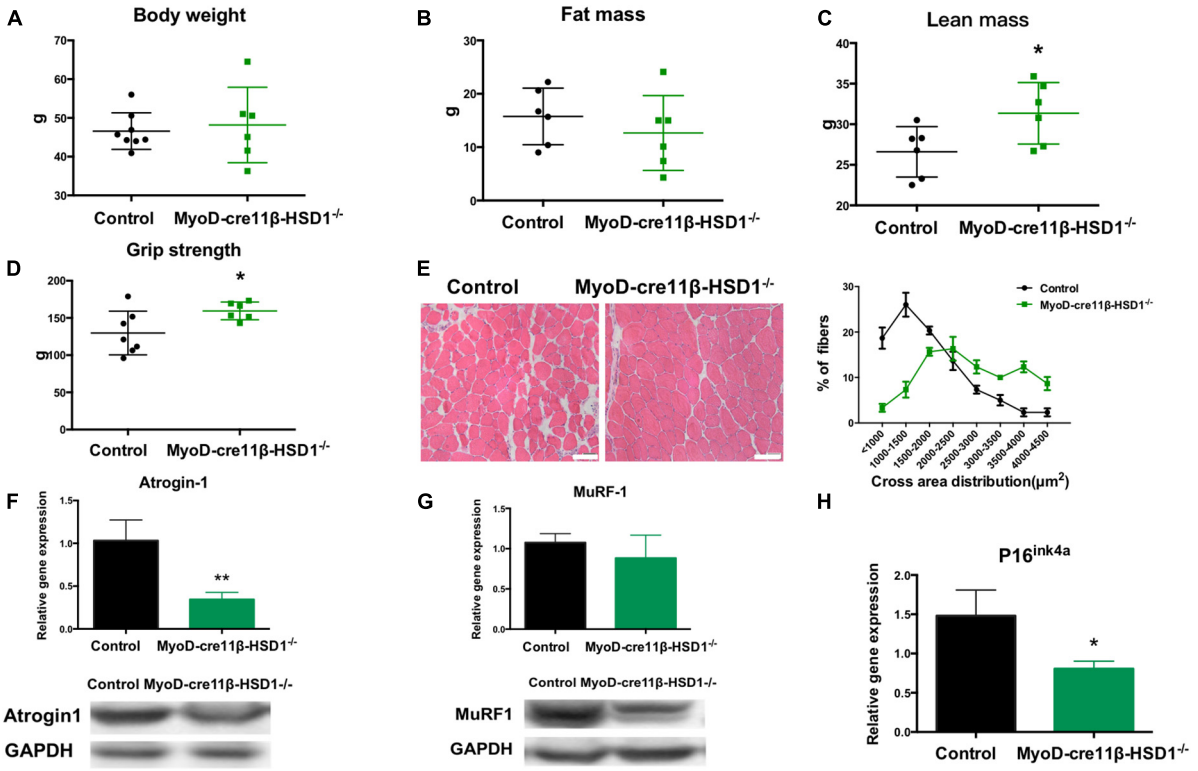

FIGURE 4
Muscle-specific 11β-HSD1 knockout rescued age-related muscle atrophy in aged mice. **(A)** Body weight of MyoD-cre 11β-HSD1 KO mice (n = 6) and control mice (n = 8) at 22 months of age. **(B)** Muscle strength of MyoD-cre 11β-HSD1 KO mice (n = 6) and control mice (n = 6). **(C)** Lean mass of MyoD-cre 11β-HSD1 KO mice (n = 6) and control mice (n = 6). **(D)** Fat mass of MyoD-cre 11β-HSD1 KO mice (n = 6) and control mice (n = 7). **(E)** Representative HE staining images of muscle cross sections derived from muscle-specific 11β-HSD1 knockout mice and control mice. Scale bars, 50 μm. Percentage distribution of muscle fiber cross-sectional area derived from muscles. **(F,G)** mRNA and protein levels of the muscle atrophy factors atrogin-1 and MuRF-1 in muscles (n = 6 mice per group). **(H)** mRNA quantification of the senescence marker gene $p16^{ink4a}$ in muscles (n = 6 mice per group). *$p < 0.05$, **$p < 0.01$.

3.6. 11β- HSD1 inhibitor BVT.2733 improved mitochondrial function in MuSCs

Mitochondria are an important production unit of skeletal muscle and an important production site of oxidative phosphorylation and ATP synthesis, known as the "power house" (17). Consistent with our previous results, 11β-HSD1-overexpressing mice displayed impaired myoblast fusion and myotube formation in MuSCs. As shown in Figure 6A, we labeled MyHC with fluorescent staining to detect myogenic conditions and found 11β-HSD1-overexpressing mice displayed impaired myoblast fusion and myotube formation in MuSCs, suggesting that the myogenic process was inhibited by 11β-HSD1 during differentiation. After treatment with the 11β-HSD1 inhibitor BVT.2733, myotube formation increased. Furthermore, the MuSCs were collected, and the levels of mitochondrial fusion and cleavage genes were detected by RT–PCR. We observed that the fusion gene Opa1 was decreased and the cleavage gene Fis-1 was increased in 11β-HSD1-overexpressing mice, and treatment with the inhibitor BVT.2733 reversed this change (Figures 6B, C). Importantly, overexpression of 11β-HSD1 led to a marked decrease in mitochondrial oxygen consumption under basal conditions and after stimulation with FCCP, as measured by OCR (Figure 6D), which was consistent with the reduction in mitochondrial ATP compatibility levels. Additionally, the 11β-HSD1 inhibitor BVT.2733 increased ATP synthesis in MuSCs (Figure 6E). Collectively, these results suggest that inhibition of 11β-HSD1 in MuSCs may improve mitochondrial function.

4. Discussion

In the present study, we showed that 11β-HSD1 expression levels were increased in age-related muscle atrophy. CR may improve muscle atrophy and muscle function in aged mice by reducing 11β-HSD1 (Figure 7). So far, CR is the only known method considered to extend lifespan and reduce the

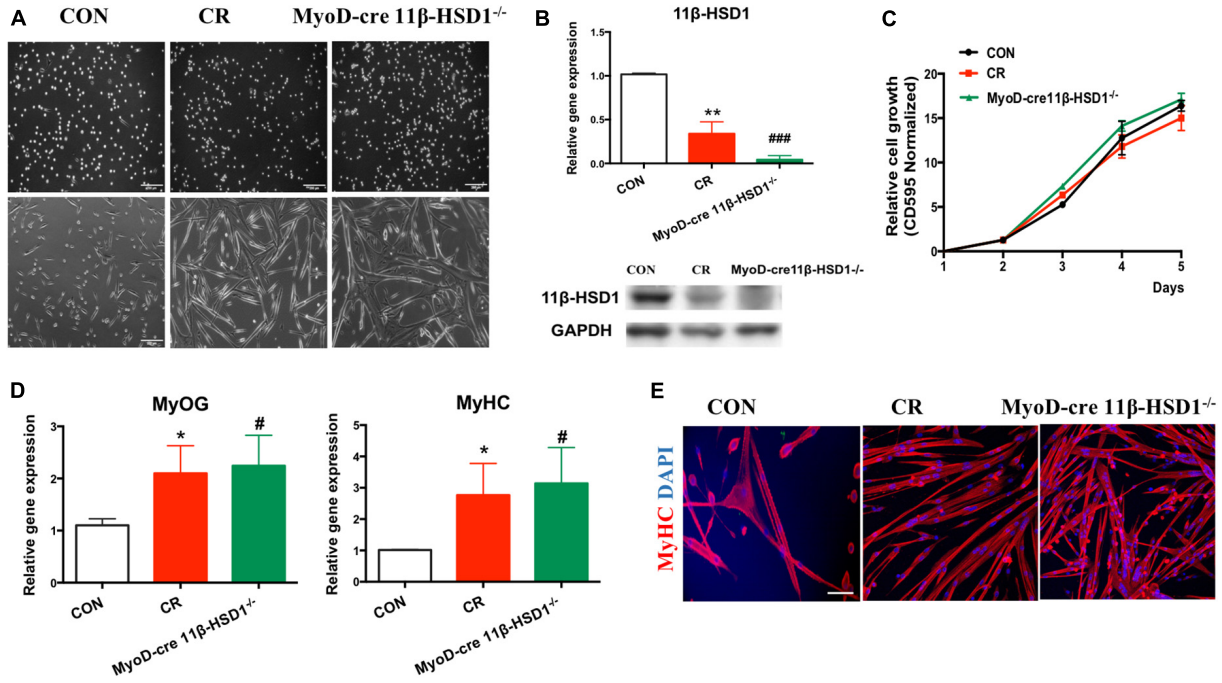

FIGURE 5
The differentiation ability of mouse muscle stem cells (MuSCs) in MyoD-cre 11β-HSD1 KO and CR mice was improved. **(A)** MuSCs were isolated from CR and MyoD-cre 11β-HSD1$^{-/-}$ and control mice at 22 months of age. Muscle-specific 11β-HSD1 knockout mice and CR mice displayed better differentiation ability, as indicated by their larger myofibers. Scale bars, 200 μm. **(B)** Gene and protein expression levels of 11β-HSD1 in MuSCs of CR and MyoD-cre 11β-HSD1$^{-/-}$ and control mice. **(C)** The proliferation rates of MuSCs sorted from 22-month-old CR and 11β-HSD1$^{-/-}$ mice and their wild-type counterparts were detected by MTT assay. **(D)** Gene expression levels of MyHC and MyOG in 11β-HSD1$^{-/-}$ mice and CR mice during the differentiation of MuSCs. *$p < 0.05$, **$p < 0.01$; #$p < 0.01$, ###$p < 0.001$. *CR mice compared with CON; #MyoD-cre 11β-HSD1$^{-/-}$ mice compared with CON. **(E)** Representative immunofluorescent staining images of cultured MuSCs at 3 days of differentiation from CR and MyoD-cre 11β-HSD1$^{-/-}$ and control mice at 22 months of age. Red indicates MyHC staining; DAPI indicates nuclei. Scale bars, 200 μm.

age-related diseases (18). A study in 2010 at the University of Wisconsin Primate Center showed that CR significantly reduced age-related mortality in monkeys compared with control animals (19). In addition, the CR group also had significantly lower rates of sarcopenia, type 2 diabetes, cancer, and cardiovascular disease (20). Currently, there are few studies on CR in sarcopenia and they have conflicting results (21). Although most studies suggested that CR is beneficial to muscle metabolism, it has also been reported that CR may promote age-related muscle loss, lower body mass index, and increase the risk of disability and mortality in the elderly (18). It is worth noting that different levels of nutrition, age at initiation of CR, duration of caloric restriction and changes in dietary macronutrient content are all potentially important factors in CR (22, 23). Thus, we speculated that CR improves sarcopenia in elderly individuals with good nutritional status. In our study, 40% CR did not cause malnutrition in older mice. Additionally, we compared our CR diet with the micronutrient standards set by the National Research Council for Laboratory Mice and found that our young CR mice continued to meet the micronutrient standards. Therefore, our findings demonstrated promising sarcopenia protection with 40% CR in older mice.

The mechanism of CR delaying sarcopenia is unclear. Current studies generally study the regulation of mitochondrial function, inflammation, oxidative stress, apoptosis and autophagy (24). Glucocorticoid (GC) is a stress hormone, and studies have shown that 24-hour urine GC output in elderly individuals is related to DNA damage (25). A follow-up study of nearly 3,000 adults conducted by Dutch researchers showed that GC levels are related to shortening of telomere length in peripheral white blood cells, which can aggravate aging (26). Some studies have found that GCs can directly inhibit the function of muscle stem cells and lead to muscular atrophy by up-regulating myostatin (27). Thus, the reduction in GC secretion may be a way to improve sarcopenia. As the local amplifier of GC, 11β-HSD1 is expressed in muscle, fat, heart, brain, skin, and other tissues (11). Some studies have found that brain 11β-HSD1 can increase the local GC level in brain tissue, thereby promoting brain aging and damaging memory function in mice (12). The expression level and activity of the 11β-HSD1 gene are also significantly increased in elderly skin, promoting

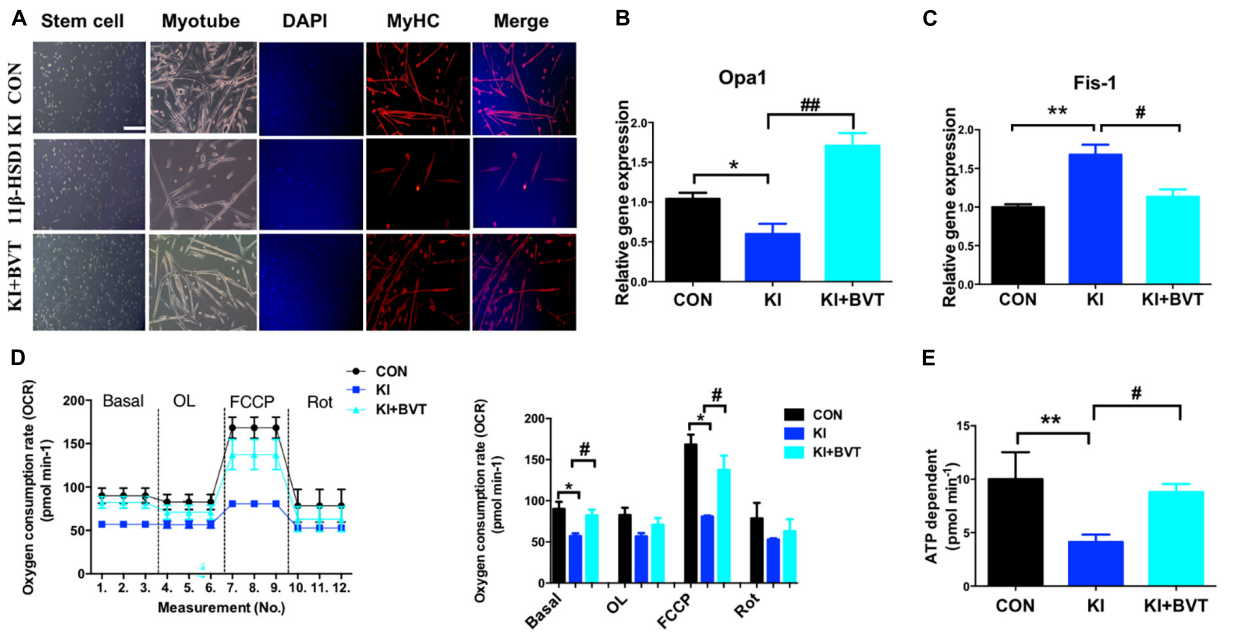

FIGURE 6
11β-HSD1 inhibitor BVT.2733 improved mitochondrial function in mouse muscle stem cells (MuSCs). **(A)** Representative immunofluorescent staining images of cultured MuSCs at 3 days of differentiation from 11β-HSD1 KI mice and 11β-HSD1 inhibitor BVT.2733-treated KI mice and control mice at 2 months of age. Red indicates MyHC staining; DAPI indicates nuclei. Scale bars, 200 μm. **(B)** mRNA levels of the mitochondrial fusion gene Opa1 in MuSCs from 11β-HSD1 KI mice and 11β-HSD1 inhibitor BVT.2733-treated KI mice and control mice. **(C)** mRNA levels of the mitochondrial cleavage gene Fis-1 in MuSCs. **(D)** Oxygen consumption rate measurement during the differentiation of MuSCs from 2-month-old mice. **(E)** ATP synthesis rate measurement during the differentiation of MuSCs from 2-month-old mice. *$p < 0.05$, **$p < 0.01$; #$p < 0.01$, ##$p < 0.01$. *KI mice compared with control; #KI + BVT compared with control.

skin aging (13). Increased expression of 11β-HSD1 in muscle tissue of elderly individuals is significantly correlated with decreased muscle strength (14). In our study, we first found that 11β-HSD1 plays a key role in the relationship between CR and sarcopenia and further found that muscle-specific knockout of 11β-HSD1 could delay muscle atrophy and improve muscle function in aged mice. Thus, confirmation of 11β-HSD1 as a key regulator of aging-related muscle atrophy has important therapeutic significance for sarcopenia.

Another novel finding was that CR can promote the differentiation of MuSCs by down-regulating 11β-HSD1. As the largest organ in the body, skeletal muscle accounts for approximately 40–50% of the adult male's total weight (28). Sarcopenia caused by aging is a multilayered and complex process involving many factors (29, 30). Changing one factor alone cannot prevent or cure sarcopenia. MuSCs are responsible for skeletal muscle regeneration. When the muscle is damaged, resting MuSCs are activated and differentiate and fuse to form muscle tubes, which are arranged in order and fuse to form muscle fibers. Currently, MuSCs are the most ideal seed cells for cell therapy (2). The fusion of MuSCs is the most important contributor to and participant in muscle regeneration (31). Here, we used 11β-HSD1-overexpressing and knockout mice and CR mice for primary MuSC culture. The differentiation ability of MuSCs in 11β-HSD1 KO mice and CR mice was significantly improved. In contrast, the differentiation ability in overexpression mice was significantly reduced. CR can improve the function of MuSCs and delay muscle aging, which is an important direction for the prevention of sarcopenia in elderly individuals.

The life activities of cells depend on mitochondria to provide energy and participate in various activities in the body (32). A previous study found that mitochondria play an important role in maintaining the pluripotency of stem cells and inducing differentiation, and with differentiation, mitochondrial aerobic metabolism gradually becomes dominant (33). In recent years, mitochondrial dysfunction has been proposed as a biomarker of muscle stem cell senescence (34). The cell energy metabolizer is programmed to control the addition of drugs to the culture medium at a specific time to measure the increase in the rate of oxygen consumption under different conditions. FCCP is the uncoupling agent of mitochondrial oxidized phosphoric acid that causes the mitochondrial membrane to depolarize and form proton leakage, increasing the oxygen consumption of mitochondria to reach the maximum respiration rate (35). Here, we assessed mitochondrial function by measuring mitochondrial oxygen consumption and calculated mitochondrial ATP production

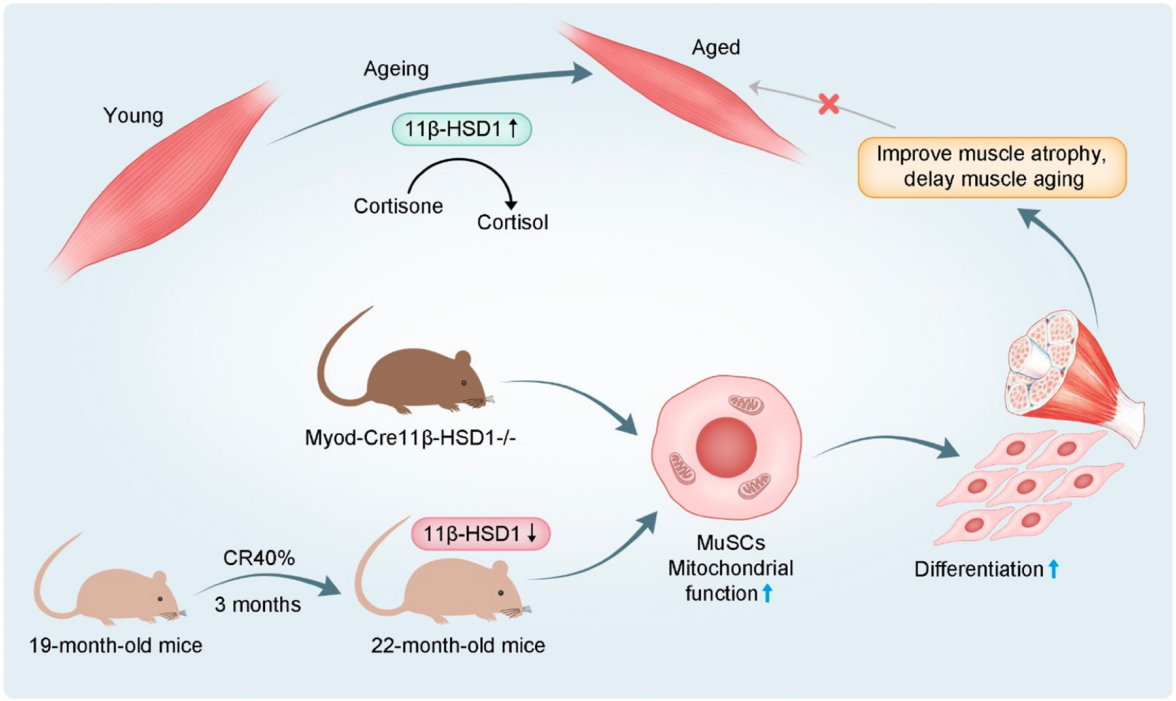

FIGURE 7
Schematic diagram 1. 11β-HSD1 expression level was increased in age-related muscle atrophy. 2. Muscle-specific 11β-HSD1 knockout rescued age-related muscle atrophy. 3. CR promoted the mitochondrial function and differentiation ability of MuSCs by reducing 11β-HSD1, thereby delaying muscle atrophy in aged mice.

capacity. Overexpression of 11β-HSD1 led to a marked decrease in mitochondrial oxygen consumption under basal conditions and after stimulation with FCCP, which was consistent with the reduction in mitochondrial ATP compatibility levels. Additionally, mitochondrial function is closely related to structure. When mitochondrial fusion lysis activity in the cell is impaired, it leads to cellular functional defects (36, 37). We observed that the fusion gene Opa1 was decreased and the cleavage gene Fis-1 was increased in 11β-HSD1-overexpressing mice, and treatment with the inhibitor BVT.2733 reversed this change. It will be interesting to determine the specific mechanisms by which 11β-HSD1 regulates mitochondrial function in skeletal muscle during aging in the future.

There were certain limitations to this study. For example, since nutrition and physical activity are two important factors affecting sarcopenia, we believe that being able to add physical activity of mice is more complete for the whole study. Secondly, western blot or fluorescent immunohistochemistry data should be added for Opa1 and Fis-1 and Inflammatory factors TNFα and aging gene p16. Thirdly, considering the level of GC is closely associated with 11β-HSD1, we should not only focus on the expression level of 11β-HSD, but also the expression level of GC. Moreover, muscle weight, body weight and muscle strength have complicated relationships, and it is meaningful to study the relationship between them. Also, in order to avoid selection bias, we should try to expand the sample size especially MyoD-Cre 11β-HSD1 knockout mice. These shortcomings merit further study.

In conclusion, our findings highlight that increased 11β-HSD1 is a hallmark of aged muscles. Furthermore, CR in aged mice reduced the local effective concentration of GC through 11β-HSD1, thereby promoting mitochondrial function and the differentiation ability of MuSCs (Figure 7). Together, we speculate that targeting an 11β-HSD1-dependent metabolic pathway may represent a novel strategy for developing therapeutics against age-related muscle atrophy.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University

(protocol code 2019-SR-481 and 2020-01-07). Written informed consent to participate in this study was provided by the participants or their legal guardian/next of kin.

Author contributions

SL designed the experiments, conducted the study, and wrote the manuscript. QS performed data analysis and mapping. HL and WX performed animal model and experimental operation. QC obtained tissue specimens. YL, XW, and GD performed project administration and supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1027055/full#supplementary-material

References

1. Hirani V, Blyth F, Naganathan V, Le Couteur D, Seibel M, Waite L, et al. Sarcopenia is associated with incident disability, institutionalization, and mortality in community-dwelling older men: the concord health and ageing in men project. *J Am Med Dir Assoc.* (2015) 16:607–13.

2. Blau H, Cosgrove B, Ho A. The central role of muscle stem cells in regenerative failure with aging. *Nat Med.* (2015) 21:854–62. doi: 10.1038/nm.3918

3. Sousa-Victor P, García-Prat L, Serrano A, Perdiguero E, Muñoz-Cánoves P. Muscle stem cell aging: regulation and rejuvenation. *Trends Endocrinol Metab.* (2015) 26:287–96.

4. Spindler S. Caloric restriction: from soup to nuts. *Ageing Res Rev.* (2010) 9:324–53. doi: 10.1016/j.arr.2009.10.003

5. Sharples A, Hughes D, Deane C, Saini A, Selman C, Stewart C. Longevity and skeletal muscle mass: the role of IGF signalling, the sirtuins, dietary restriction and protein intake. *Aging Cell.* (2015) 14:511–23. doi: 10.1111/acel.12342

6. Yang L, Licastro D, Cava E, Veronese N, Spelta F, Rizza W, et al. Long-term calorie restriction enhances cellular quality-control processes in human skeletal muscle. *Cell Rep.* (2016) 14:422–8. doi: 10.1016/j.celrep.2015.12.042

7. Cerletti M, Jang Y, Finley L, Haigis M, Wagers A. Short-term calorie restriction enhances skeletal muscle stem cell function. *Cell Stem Cell.* (2012) 10:515–9.

8. Ionescu-Tucker A, Cotman C. Emerging roles of oxidative stress in brain aging and Alzheimer's disease. *Neurobiol Aging.* (2021) 107:86–95.

9. Bartsch T, Wulff P. The hippocampus in aging and disease: from plasticity to vulnerability. *Neuroscience.* (2015) 309:1–16.

10. Reynolds R, Dennison E, Walker B, Syddall H, Wood P, Andrew R, et al. Cortisol secretion and rate of bone loss in a population-based cohort of elderly men and women. *Calcif Tissue Int.* (2005) 77:134–8. doi: 10.1007/s00223-004-0270-2

11. Chapman K, Holmes M, Seckl J. 11β-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev.* (2013) 93:1139–206. doi: 10.1152/physrev.00020.2012

12. Sarabdjitsingh R, Zhou M, Yau J, Webster S, Walker B, Seckl J, et al. Inhibiting 11β-hydroxysteroid dehydrogenase type 1 prevents stress effects on hippocampal synaptic plasticity and impairs contextual fear conditioning. *Neuropharmacology.* (2014) 81:231–6. doi: 10.1016/j.neuropharm.2014.01.042

13. Tiganescu A, Tahrani A, Morgan S, Otranto M, Desmoulière A, Abrahams L, et al. 11β-Hydroxysteroid dehydrogenase blockade prevents age-induced skin structure and function defects. *J Clin Invest.* (2013) 123:3051–60. doi: 10.1172/JCI64162

14. Kilgour A, Gallagher I, MacLullich A, Andrew R, Gray C, Hyde P, et al. Increased skeletal muscle 11βHSD1 mRNA is associated with lower muscle strength in ageing. *PLoS One.* (2013) 8:e84057. doi: 10.1371/journal.pone.0084057

15. Loerz C, Staab-Weijnitz C, Huebbe P, Giller K, Metges C, Rimbach G, et al. Regulation of 11β-hydroxysteroid dehydrogenase type 1 following caloric restriction and re-feeding is species dependent. *Chem Biol Interact.* (2017) 276:95–104. doi: 10.1016/j.cbi.2017.02.018

16. Fu X, Xiao J, Wei Y, Li S, Liu Y, Yin J, et al. Combination of inflammation-related cytokines promotes long-term muscle stem cell expansion. *Cell Res.* (2015) 25:1082–3.

17. Bulthuis E, Adjobo-Hermans M, Willems P, Koopman W. Mitochondrial morphofunction in mammalian cells. *Antioxid Redox Signal.* (2019) 30:2066–109.

18. Dirks A, Leeuwenburgh C. Caloric restriction in humans: potential pitfalls and health concerns. *Mech Ageing Dev.* (2006) 127:1–7. doi: 10.1016/j.mad.2005.09.001

19. Colman R, Anderson R, Johnson S, Kastman E, Kosmatka K, Beasley T, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science.* (2009) 325:201–4.

20. Fontana L, Partridge L, Longo V. Extending healthy life span–from yeast to humans. *Science.* (2010) 328:321–6. doi: 10.1126/science.1172539

21. Mattison J, Roth G, Beasley T, Tilmont E, Handy A, Herbert R, et al. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature.* (2012) 489:318–21.

22. Boldrin L, Ross J, Whitmore C, Doreste B, Beaver C, Eddaoudi A, et al. The effect of calorie restriction on mouse skeletal muscle is sex, strain and time-dependent. *Sci Rep.* (2017) 7:5160. doi: 10.1038/s41598-017-04896-y

23. Giller K, Huebbe P, Doering F, Pallauf K, Rimbach G. Major urinary protein 5, a scent communication protein, is regulated by dietary restriction and subsequent re-feeding in mice. *Proc Biol Sci.* (2013) 280:20130101. doi: 10.1098/rspb.2013.0101

24. Masoro E. Overview of caloric restriction and ageing. *Mech Ageing Dev.* (2005) 126:913–22.

25. Revesz D, Verhoeven J, Milaneschi Y, de Geus E, Wolkowitz O, Penninx B. Dysregulated physiological stress systems and accelerated cellular aging. *Neurobiol Aging.* (2014) 35:1422–30.

26. Aschbacher K, O'Donovan A, Wolkowitz O, Dhabhar F, Su Y, Epel E. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology.* (2013) 38:1698–708. doi: 10.1016/j.psyneuen.2013.02.004

27. Dong Y, Pan J, Zhang L. Myostatin suppression of Akirin1 mediates glucocorticoid-induced satellite cell dysfunction. *PLoS One.* (2013) 8:e58554. doi: 10.1371/journal.pone.0058554

28. Frontera W. Physiologic changes of the musculoskeletal system with aging: a brief review. *Phys Med Rehabil Clin N Am.* (2017) 28:705–11.

29. Bouche M, Munoz-Canoves P, Rossi F, Coletti D. Inflammation in muscle repair, aging, and myopathies. *Biomed Res Int.* (2014) 2014:821950.

30. Lardies-Sanchez B, Sanz-Paris A, Perez-Nogueras J, Serrano-Oliver A, Torres-Anoro M, Cruz-Jentoft A. Influence of nutritional status in the diagnosis of sarcopenia in nursing home residents. *Nutrition.* (2017) 41:51–7.

31. Cosgrove B, Gilbert P, Porpiglia E, Mourkioti F, Lee S, Corbel S, et al. Rejuvenation of the muscle stem cell population restores strength to injured aged muscles. *Nat Med.* (2014) 20:255–64. doi: 10.1038/nm.3464

32. Hepple R. Mitochondrial involvement and impact in aging skeletal muscle. *Front Aging Neurosci.* (2014) 6:211. doi: 10.3389/fnagi.2014.00211

33. Seyer P, Grandemange S, Busson M, Carazo A, Gamaleri F, Pessemesse L, et al. Mitochondrial activity regulates myoblast differentiation by control of c-Myc expression. *J Cell Physiol.* (2006) 207:75–86. doi: 10.1002/jcp.20539

34. Zhang H, Ryu D, Wu Y, Gariani K, Wang X, Luan P, et al. NAD^+ repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science.* (2016) 352:1436–43.

35. Leduc-Gaudet J, Picard M, St-Jean Pelletier F, Sgarioto N, Auger M, Vallée J, et al. Mitochondrial morphology is altered in atrophied skeletal muscle of aged mice. *Oncotarget.* (2015) 6:17923–37.

36. Moore A, Wong Y, Simpson C, Holzbaur E. Dynamic actin cycling through mitochondrial subpopulations locally regulates the fission-fusion balance within mitochondrial networks. *Nat Commun.* (2016) 7:12886. doi: 10.1038/ncomms12886

37. Liu R, Jin P, Yu L, Wang Y, Han L, Shi T, et al. Impaired mitochondrial dynamics and bioenergetics in diabetic skeletal muscle. *PLoS One.* (2014) 9:e92810. doi: 10.1371/journal.pone.0092810

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Serum creatinine/cystatin C ratio as a muscle mass evaluating tool and prognostic indicator for hospitalized patients: A meta-analysis

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Objective: Sarcopenia is a syndrome of decreased muscle mass and deficits in muscle strength and physical function. We aimed to investigate the relationship between creatinine/cystatin C ratio (CCR) and sarcopenia and the prognostic value of CCR in hospitalized patients.

Materials and methods: We searched for relevant studies in PubMed, EMBASE, and the Cochrane Database up to August 25, 2022. Meta-analyses were performed to evaluate the relationship between CCR and skeletal muscle [computed tomography-assessed skeletal muscle (CTASM), muscle strength, and physical performance], prognosis and important clinical outcomes in hospitalized adults. The pooled correlation coefficient, the area under the receiver operating characteristic (ROC) curves, and hazard ratio (HR) together with their 95% confidence intervals (CIs) were calculated. We also conducted subgroup analyses to explore the sources of heterogeneity.

Results: A total of 38 studies with 20,362 patients were eligible. These studies were of moderate to high quality. Our results showed that CCR was significant correlations with all CTASM types (Fisher's Z ranged from 0.35 to 0.5; P values ranged from < 0.01 to 0.01), handgrip strength (Fisher's $Z = 0.39$; 95% CI, 0.32–0.45; $P < 0.001$) and gait speed (Fisher's $Z = 0.25$; 95% CI, 0.21–0.30; $P < 0.001$). The ROC curves suggested that CCR had good diagnostic efficacy (0.689; 95% CI, 0.632–0.746; $P < 0.01$) for sarcopenia. CCR can reliably predict mortality in hospitalized patients, which was confirmed by regression analysis of CCR as both continuous (HR 0.78; 95% CI, 0.72–0.84; $P < 0.01$) and categorical variables (HR 2.05; 95% CI, 1.58–2.66; $P < 0.0001$). In addition, less evidence showed that higher CCR was independently associated with a shorter duration of mechanical ventilation, reduced length of stay in the intensive care unit and hospital, less nutritional risk, and decreased complications in hospitalized patients.

Conclusion: CCR could be a simple, economical, and effective screening tool for sarcopenia in hospitalized patients, and it is a helpful prognostic factor for mortality and other important clinical outcomes.

Systematic review registration: https://inplasy.com/inplasy-2022-9-0097/, identifier INPLASY202290097.

KEYWORDS

creatinine/cystatin C ratio, mortality, hospitalized, meta-analysis, sarcopenia

Introduction

Sarcopenia is traditionally been considered a syndrome characterized by reduced muscle mass, deficiencies in muscle strength, and impairments physical function (1). It has been thought that sarcopenia is more prevalent in old patients, especially those over 65 (2, 3). Sarcopenia, however, is common in hospitalized patients of all ages and is associated with various adverse outcomes, including impaired organ functions, infectious complications, prolonged length of stay (LOS) in intensive care unit (ICU) or hospital, and even increased mortality rates (4–7). Therefore, adequate body muscle reserve is crucial for hospitalized patients' recovery and survival.

Previous indicators used to evaluate muscle reserve, such as anthropometrics, lab tests, subjective judgment, and body mass index (BMI), fail to reflect the patient's body composition accurately (8). Clinicians may now directly measure muscle mass thanks to advances in imaging and software technologies (9, 10). In particular, computed tomography (CT), has been recognized as the gold standard for identifying skeletal muscle because it can accurately distinguish skeletal muscle and fat mass using a single cross-sectional slice at multiple body levels (11). However, high cost, radiological damage, and equipment unavailability limit the widespread use of these techniques (10, 11). In addition, these techniques are not conducive to continuous monitoring of muscle mass changes. As a result, there is an urgent need for other simple and inexpensive biomarkers to diagnose and monitor sarcopenia.

Serum creatinine and cystatin C are widely used in clinical practice to assess renal function. And the ratio of the two markers (serum creatinine/serum cystatin C) x100, known as CCR, has recently attracted interest. In 2016, Kashani et al. validated the correlation between muscle mass and CCR, which they defined as the "sarcopenia index" (12). Moreover, the authors found that CCR could predict hospital and 90-day mortality in patients who did not have acute renal damage. Since then, CCR has been increasingly used for critically ill patients (13, 14), the elderly (6, 7), organ transplant recipients (15, 16), and type 2 diabetic patients (17). However, substantial variation in study design, sample size, demographics, and muscle assessment among these studies lead to inconsistent results (6, 7, 12, 13, 18). Furthermore, there are no meta-analyses to examine the values of CCR on muscle mass measurement and prognosis in these patient populations.

Several studies on CCR in hospitalized patients have been published recently (14, 19–24). Therefore, with the power of meta-analysis, we aimed to perform a systematic review and meta-analysis of available published articles about hospitalization patients to investigate (1) whether CCR is a better and more accurate index of muscle mass, muscle strength, and gait speed; (2) the applicability of using CCR as a screening method for sarcopenia; and (3) the association of CCR with clinical outcomes (i.e., mortality, duration of mechanical ventilation, length of stay in ICU or hospital, and complications).

Materials and methods

The systematic review was already registered in the International Platform of Registered Systematic Review and Meta-analysis Protocols database, and it is now available in its entirety on inplasy.com.[1] It was carried out in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (25; **Supplementary file 1**).

Search strategy

We conducted a systematic search of relevant studies in PubMed, EMBASE, and the Cochrane Library from their establishment to August 25, 2022 (The last search date). Using a combination of MeSH and keywords, search terms included "creatinine," "cystatin C," "creatinine/cystatin C ratio,"

Abbreviations: CCR, creatinine/cystatin C ratio; CI, confidence interval; CTASM, computed tomography-assessed skeletal muscle; HGS, handgrip strength; GS, gait speed; ICU, intensive care unit; LOS, length of stay; MD, mean difference; MV, mechanical ventilation; NOS, Newcastle-Ottawa Scale; OR, odds ratio; RCTs, randomized controlled trials; SD, standard deviations.

1 https://inplasy.com/inplasy-2022-9-0097/

"creatinine to cystatin C ratio," "creatinine over cystatin C ratio," "creatinine-to-cystatin C ratio," and "sarcopenia index." We restricted the language to English. Two authors (W-HZ and YY) independently imported the papers into Endnote X7 to exclude duplicate research and screen the literature (titles, abstracts, and full texts). We read meta-analyses, reviews, and comments to find more potential articles. The reference lists of the included full-text papers were also examined. We included the most recent published or reported data for republished studies. Disagreements were resolved through discussions between the two authors.

Inclusion and exclusion criteria

We included articles investigating the correlation between CCR and CT-assessed skeletal muscle and the predictive prognosis value of CCR in hospitalized patients. The particular inclusion criteria were as follows, based on the PICOS (population, intervention, comparison, outcome, design) principle:

(1) adult (> 18 years old) hospitalized patients.
(2) evaluation of skeletal muscle amount (area) or quality (density) as determined by CT using any clear and objective methods.
(3) studies should report the correlation between CCR and CTASM or patient survival information.
(4) eligible studies had a cohort, case-control, or randomized controlled study design.

We excluded the studies that reported data without predefined outcomes and focused on animals or pregnant women. Studies available only in abstract form or meeting reports were also excluded.

Data extraction

Two authors (W-HZ and YY) independently extracted data from included studies on the following items: first author, publication year, geographic location, study design, research period, population, sample size, demographic characteristics, disease severity, details on CT technique (muscle measured, CT-scan level), sarcopenia criteria and prevalence, outcomes of interest and methodological quality.

Quality assessment

Two authors (W-HZ and YY) independently assessed the quality of each included study using the Newcastle-Ottawa Scale (NOS) for cohort studies (26). The NOS is divided into three domains depending on the cohort's selection, the comparability of the groups, and the quality of the results. The included research was granted a maximum of one point for each item in the selection and outcome domains, and a maximum of two points for the comparability domain. The scale ratings ranged from 0 to 9, with 8 or 9 being categorized as good quality, 6 or 7 as moderate quality, and 5 or less as low quality. Disagreements were recognized and addressed through discussion.

Statistical analysis

The primary outcome of the current meta-analysis was to investigate the suitability of CCR as a predictive mortality tool in hospitalized patients. To minimize potential interference factors, we only pooled the regression analysis findings of the included studies to investigate the link between CCR and mortality at the longest follow-up available. The HR in related studies were converted to their natural logarithms, and standard error (SE) values were determined using these logarithms and their respective 95% CI.

Secondary outcomes included associations between CCR and CTASM evaluation, muscle strength, gait speed, nutrition screening tool, or other clinical outcomes (i.e., duration of mechanical ventilation, length of stay in ICU or hospital, and complications). As to these outcomes, we conducted related meta-analyses individually for the various data reporting types as follows among the included studies. (1) For the studies that provided the correlation coefficient between CCR and predefined outcomes (i.e., CTASM, handgrip strength [HGS], gait speed [GS], and nutrition screening tool), we performed a meta-analysis by quantitatively summarizing the correlation coefficient statistic (r) estimates. Fisher's Z and its SE were calculated using r and sample size (N) as follows: $Zr = (1/2)[\log e(1 + r) - \log e(1 - r)]$, $SEzr = 1/\mathrm{sqrt}[N-3]$ (27). After appropriate transformation, we used the inverse variance-weighted approach to determine effect sizes and the associated 95% confidence intervals (CI). (2) For the studies reporting the diagnostic value of CCR for detecting sarcopenia, we pooled Area under the curve (AUC) values using the mean and standard error SE values and weighted them using the inverse-variance method (28). (3) We collected and pooled OR with 95% CI *via* the generalized inverse-variance method for studies that showed an association between CCR and sarcopenia using regression analysis. Unless otherwise noted in the above meta-analyses, we preferred the adjusted analysis results.

We used the I^2 statistic to quantify heterogeneity ($I^2 < 50$ and $> 50\%$ were classified as low and high heterogeneity, respectively) (29). When there was significant heterogeneity, a random-effects model was used; otherwise, a fixed-effects model was utilized (30). We then performed sensitivity analyses, removing one study at a time to demonstrate the impact of that study on the pooled effect estimates. Visually inspecting

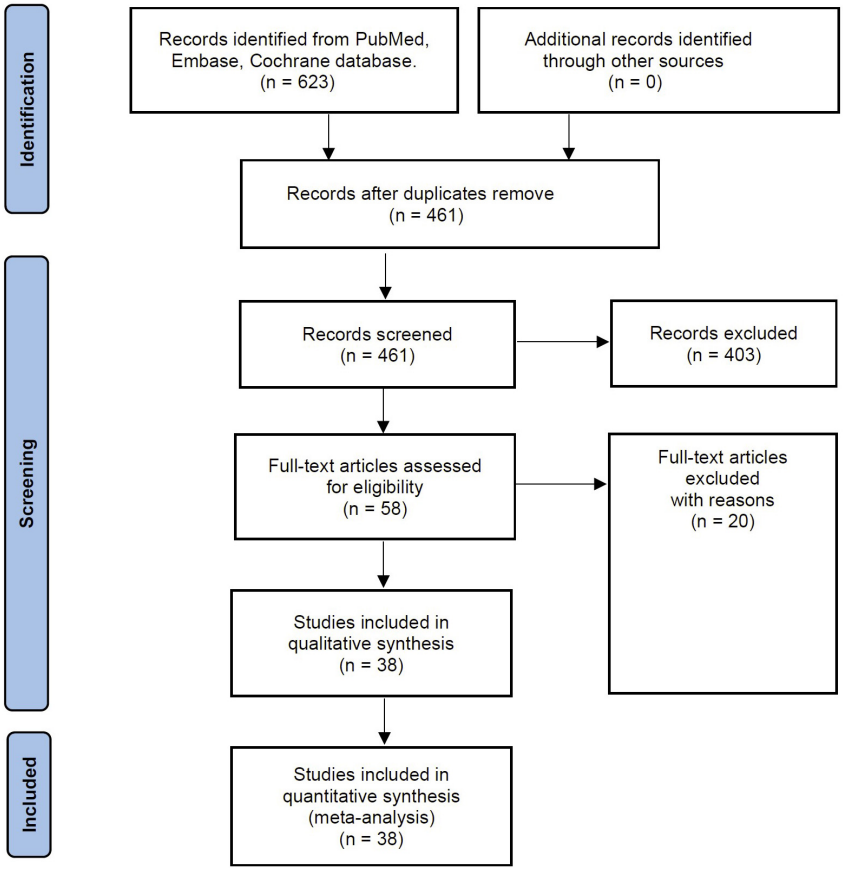

FIGURE 1
Flow chart of literature selection.

funnel plots for asymmetry was used to determine publication bias. Meta-analysis was conducted when data from at least 3 studies were available. *P* values of less than 0.05 were regarded as statistically significant. We utilized R version 3.6.2 for all statistical analyses in the current meta-analysis.

Subgroup analyses were performed to find the potential sources of heterogeneity on the following properties:

(1) Geographic location: Asian and other countries;
(2) Patient population: critical illness, cancer, medical, and surgery patients; and
(3) Gender (i.e., male and female).

Results

Study selection

The comprehensive literature search yielded 213 studies. Following evaluation of the title, abstract, and full text, 38 papers involving 20,362 participants fulfilled the criteria for inclusion in the current systematic review (5–7, 12–24, 31–52). **Figure 1** shows how the search strategy flows and the selection process.

Study characteristics and methodological quality

Table 1 and **Supplementary file 2** present the characteristics of the eligible studies. All these observational studies were published between 2016 and 2022 in six countries (China $n = 16$, Japan $n = 9$, Korea $n = 6$, USA $n = 4$, Argentina $n = 2$, and France $n = 1$). These studies focused on patients with critical illnesses, cancer, medical and surgical departments, and unselected hospitalized patients. The participants' mean age ranged from 47 to 88 years old, and their BMI was from 20.7 to 28 kg/m^2. The included studies have a mean mortality rate of 13% (ranging from 8.7 to 43.2%). The criteria and cut-offs for evaluating the sarcopenia varied among the studies. Twenty studies used regression analyses to investigate the relationship between CCR and mortality (5–7, 12, 14, 15, 18, 19, 23, 24, 31, 32, 37–40, 46, 49, 51). Regarding the relationship between CCR

and muscle evaluation, 21 studies provided Pearson correlation coefficient (r) levels between CCR and CTASM, 12 evaluated the diagnostic value of CCR in sarcopenia, and six used adjusted HR/OR to predict sarcopenia.

The study quality ranged from moderate to high, according to the specifics of the quality evaluation in **Supplementary Table 2** (Scores range from 6 to 9). Overall, 24 studies were judged to be of good quality, while 14 study was considered to be of moderate quality (**Table 1**).

Findings from meta-analysis

Prediction of mortality by CCR

Twenty studies with 13,560 patients investigated the impact of CCR on mortality in hospitalized patients using HR (5–7, 12, 14, 15, 18, 19, 23, 24, 31, 32, 37–40, 46, 49, 51). Among these studies, 13 studies with 11,355 patients reported the CCR treated as a continuous variable, and the pooled results showed a higher CCR was independently associated with a lower risk of mortality (HR 0.78; 95% CI, 0.72–0.84; I^2 = 94%; $P < 0.01$, **Figure 2A**; 5–7, 12, 19, 23, 24, 31, 32, 37, 38, 40, 49). A total of 10 studies including 9,164 patients reported the risk estimation according to CCR categories (6, 7, 13, 14, 18, 19, 37–39, 46). When pooled, there was a significant prognostic role for the CCR category on patients' mortality (HR 2.05; 95% CI, 1.58–2.66; I^2 = 93%; $P < 0.0001$). That is, patients with low values of CCR were less likely to survive than patients with high values (**Figure 2B**). A graph shaped symmetrical inverted funnel indicates there is no publication bias (**Supplementary Figure 1**).

Supplementary Figures 2–7 shows the detailed information of subgroup analyses by CCR categories and CCR treated as a continuous variable. Significant associations between CCR and all-cause mortality were also confirmed in most subgroups (**Supplementary Figures 2–7**).

The relationship between CCR and CTASM

There were 25 studies with 7,868 patients evaluated the correlation between CCR and CTASM from hospitalized patients using the correlation coefficient. As to the CTASM types, skeletal muscle area (SMA) was the most reported (n = 12), followed by the skeletal muscle index (SMI, defined as SMA divided by BSA, n = 11), appendicular skeletal muscle (n = 3), and appendicular skeletal muscle index (n = 3). The pooled results showed positive and significant correlations between CCR and all the four types of CTASM (Fisher's Z ranged from 0.35 to 0.5; P values ranged from < 0.01 to 0.01) with the heterogeneity from 61 to 82% (**Figure 3**).

The diagnostic value of CCR for detecting sarcopenia

Twelve articles presented the AUC value for CCR in the diagnosis of sarcopenia (7, 21, 24, 34, 40–42, 45–47, 49, 50). Among them, two studies only reported AUC values [male/female: 0.813/0.613 (50); 0.752/0.754 (24)], and the other 10 provided the mean AUC and SE values. When pooled, the AUC value of CCR to predict sarcopenia was 0.689 (95% CI, 0.632–0.746; I^2 = 82%; $P < 0.01$) (**Supplementary Figure 8**).

The relationship between CCR and HGS

There were 13 studies with 5,771 patients evaluated the correlation between CCR and HGS (6, 7, 21, 22, 24, 34–36, 41, 42, 46, 47, 49). There were positive and significant correlations between CCR and all the CTASM types (Fisher's Z = 0.39; 95% CI, 0.32–0.45; I^2 = 82%; $P < 0.001$) (**Figure 4**).

The relationship between CCR and GS

There were 5 studies with 1,661 patients assessed the correlation between CCR and GS (7, 21, 24, 42, 46). There were positive and significant correlations between CCR and all the GS (Fisher's Z = 0.25; 95% CI, 0.21–0.30; I^2 = 0%; $P < 0.001$) (**Figure 5**).

The relationship between CCR and nutrition risk

Only four studies described the relationship between CCR and nutrition risk (6, 13, 16, 45). In the study by Abe et al. (45), the authors found that CCR was positively correlated with Mini Nutritional Assessment Short Form scores (r = 0.138, P = 0.03). Barreto et al. reported that CCR was a fair predictor of malnutrition as defined by the modified-NUTRIC score (AUC = 0.61) and with > 90% sensitivity and > 90% specificity (13). After adjusting for potential confounders, the CCR remained independently predictive of malnutrition [OR per 10-unit decrease in CCR, 1.15 (1.05, 1.26); P = 0.002]. Similar results were also seen in the study by Ren and coauthors, which showed that lower CCR was independently associated with nutritional risk/malnutrition whether or not CCR was treated as a continuous or category variable (6). In contrast, one study focused on cancer patients found no differences in the incidence of nutrition risk screening 2002 score ≥ 3 between the low and high CCR groups (P = 0.172) (16).

The relationship between CCR and other clinical outcomes

Three studies (12, 13, 51) provided data on the relationship between the CCR and the duration of MV, of which the study by Wang et al. suggested that CCR at admission significantly correlated with MV (r = 0.138, P = 0.001) (51). As to the other studies that provided specific data on this topic, one reported an increase in the CCR significantly predicted ventilator liberation [aHR 1.07 (0.97, 1.19); P = 0.18] (5), and the other suggested the duration of MV was significantly lower for those with a higher CCR [−1 day for each 10 units of CCR (95% CI, −1.4 to −0.2; P = 0.006)] (12).

Intensive care unit (ICU) and hospital LOS were available in three studies (5, 37, 51), of which the study by Wang

TABLE 1 Characteristics of included studies in the current meta-analysis.

Study	Country	Design	Population	Sample	Age, year	Male, %	BMI	Follow-up	Mean CCR	Study quality
Abe et al. (45)	Japan	R, SC	Patients with CHF	248	77	49	22.4	In-hospital	NA	7
Barreto et al. (5)	USA	P, MC	ICU patients	171	63	61	26	90 days	84	9
Barreto et al. (13)	USA	R, SC	ICU patients	398	65	58	28	90 days	69	8
Chen et al. (31)	China	R, SC	Cancer	664	65	70	NA	28 months	76	8
Fujita et al. (21)	Japan	CS, SC	Patients with IPF	49	73	90	22.3	N/A	86	6
Fu et al. (34)	China	CS, SC	Cancer	182	55	63	21.6	N/A	79	8
Huang et al. (22)	China	CS, SC	AECOPD	104	67	100	20.6	N/A	96	6
Huang et al. (23)	China	R, SC	CAP	769	79	62	21.5	In-hospital	N/A	8
Lchikawa et al. (35)	Japan	R, SC	Chronic liver disease	303	66	50	23.8	NA	70	7
Jung et al. (14)	Korea	R, SC	ICU patients with RRT	1,588	65	60	25.4	90 days	92	8
Jung et al. (37)	Korea	R, SC	Cancer	3,060	61	54	23.6	1 year	82	8
Kashani et al. (15)	USA	R, SC	Lung transplant patients	28	58	54	25.9	1 year	106	6
Kashani et al. (12)	USA	R, SC	ICU patients	226	68	46	28	90 days	50	8
Kim et al. (19)	Korea	R, SC	Patients with CAD	1,928	65	71	24.9	3 years	110	8
Kim et al. (38)	Korea	R, SC	Patients with CABG	605	72	72	23.9	30 days PO	87	8
Lee et al. (39)	Korea	R, MC	Patients with CAD	1,086	72	63	24.6	3 years	105	8
Lchikawa et al. (36)	Japan	R, SC	Liver disease	313	65	48	23.6	NA	70	6
Lin et al. (41)	China	CS, SC	Non-d-CKD	272	66	57	26	N/A	100	8
Lin et al. (40)	China	R, SC	Non-d-CKD	1,141	71	58	25.5	Until death	98	8
Lin et al. (42)	China	CS, SC	CKD	297	69	57	26.3	N/A	51	8
Liu et al. (32)	China	R, SC	AIS	217	68	64	NA	3 months	71	8
Mauro et al. (43)	Argentina	R, SC	ALT	215	55	55	28.4	11.7 months	56	7
Nishiki et al. (20)	Japan	R, SC	COPD	201	72	95	22.3	NA	86	6
Osaka et al. (17)	Japan	P, MC	D2M	285	67	56	25.3	NA	NA	9
Okubo et al. (44)	Japan	R, SC	Patients with hip fracture	130	88	22	20.7	In-hospital	66	6
Ren et al. (6)	China	P, SC	Old patients	758	86	78	23	212 days	72	9
Romeo et al. (46)	Argentina	R, SC	Patients undergoing TAVR	100	84	36	27.2	1 year	69	7
Shin (47)	Korea	R, SC	D2M	1,577	63	58	25.2	NA	84	8
Sun et al. (18)	China	R, SC	Cancer	327	62	72	21.9	3 years	75	8
Tamai et al. (48)	Japan	R, SC	Cancer	50	65	60	24.1	36.5 months	79	6
Tang et al. (49)	China	P, SC	Cancer	579	59	65	23.2	Until death	73	9
Tang et al. (7)	China	R, SC	Hospitalized older patients	248	81	81	22.5	3 years	74	8
Ulmann et al. (50)	France	R, SC	Cancer	44	65	68	24.4	NA	79	6
Wang et al. (51)	China	R, SC	Neurocritically ill patients	538	57	63	22.9	In-NCU	70	8
Yang et al. (16)	China	R, SC	Postoperative cancer patients	417	58	60	23.3	30 days PO or in-hospital	61	6

(Continued)

TABLE 1 (Continued)

Study	Country	Design	Population	Sample	Age, year	Male, %	BMI	Follow-up	Mean CCR	Study quality
Yang et al. (33)	China	R, SC	D2M	193	55	59	26.1	In-hospital	73	8
Yanishi et al. (52)	Japan	P, SC	Kidney transplant recipients	62	47	67	22.4	NA	104	7
Zheng et al. (24)	China	R, DB	Cancer	989	67	80	22.7	7 months	65	8

AECOPD, acute exacerbation of chronic obstructive pulmonary disease; AIS, acute ischemic stroke; R, retrospective; ALT, awaiting liver transplantation; BMI, Body Mass Index; CABG, coronary artery bypass grafting; CAD, coronary artery disease; CAP, community-acquired pneumonia; CCR, creatinine/cystatin C ratio; CHF, chronic heart failure; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; CS, cross-sectional; DB, data base; D2M, type 2 diabetes; ICU, intensive care unit; IPF, idiopathic pulmonary fibrosis; MC, multi-centers; NA, not available; N/A, not applicable; NCU, neurocritical care unit; P, prospective; PO, postoperative; RRT, renal replacement therapy; SC, single-center; TAVR, transcatheter aortic valve replacement.

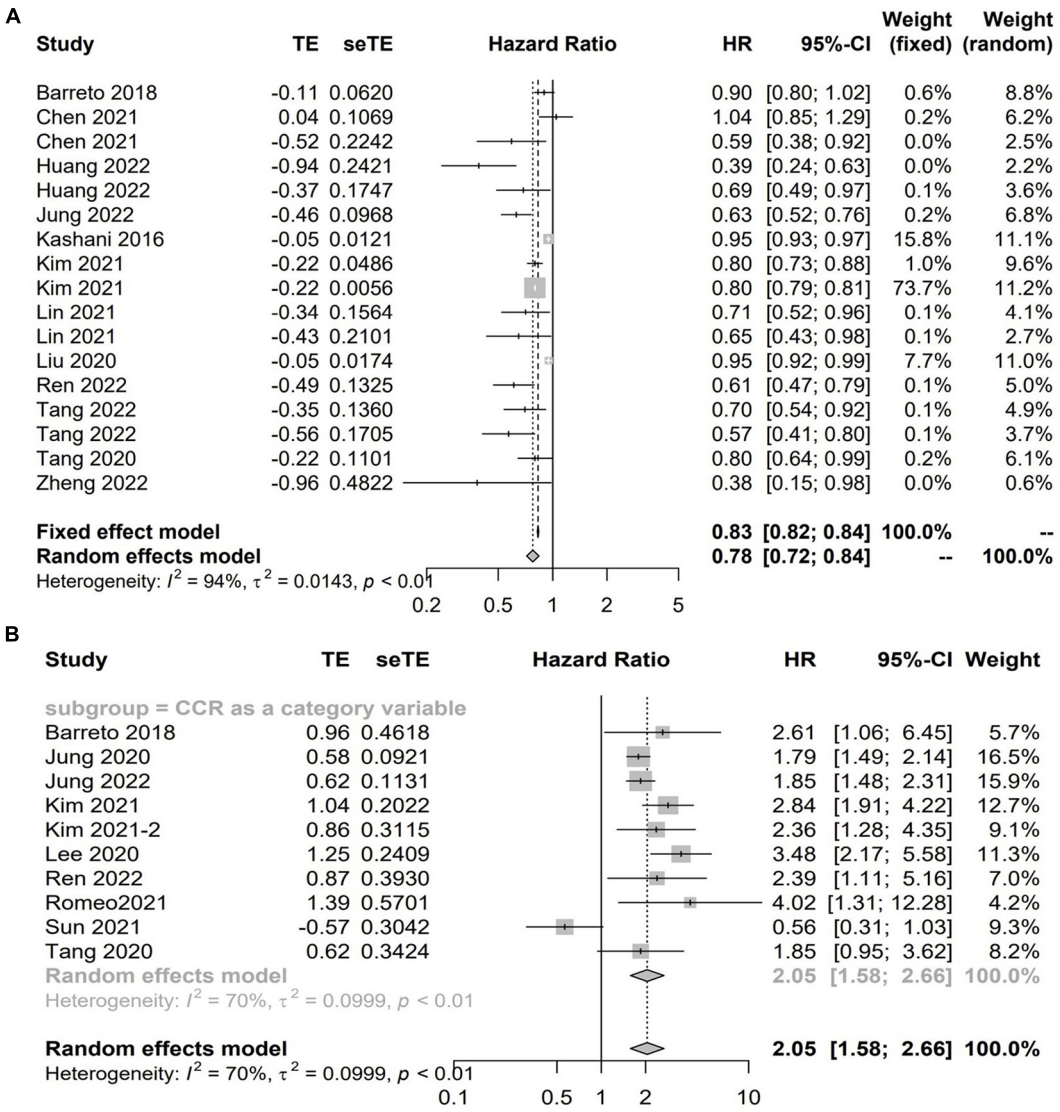

FIGURE 2
The forest plot in assessing the impact of creatinine/cystatin C ratio (CCR) on mortality in hospitalized patients by hazard ratio (HR) using regression analysis as continuous **(A)** and categorical variables **(B)**.

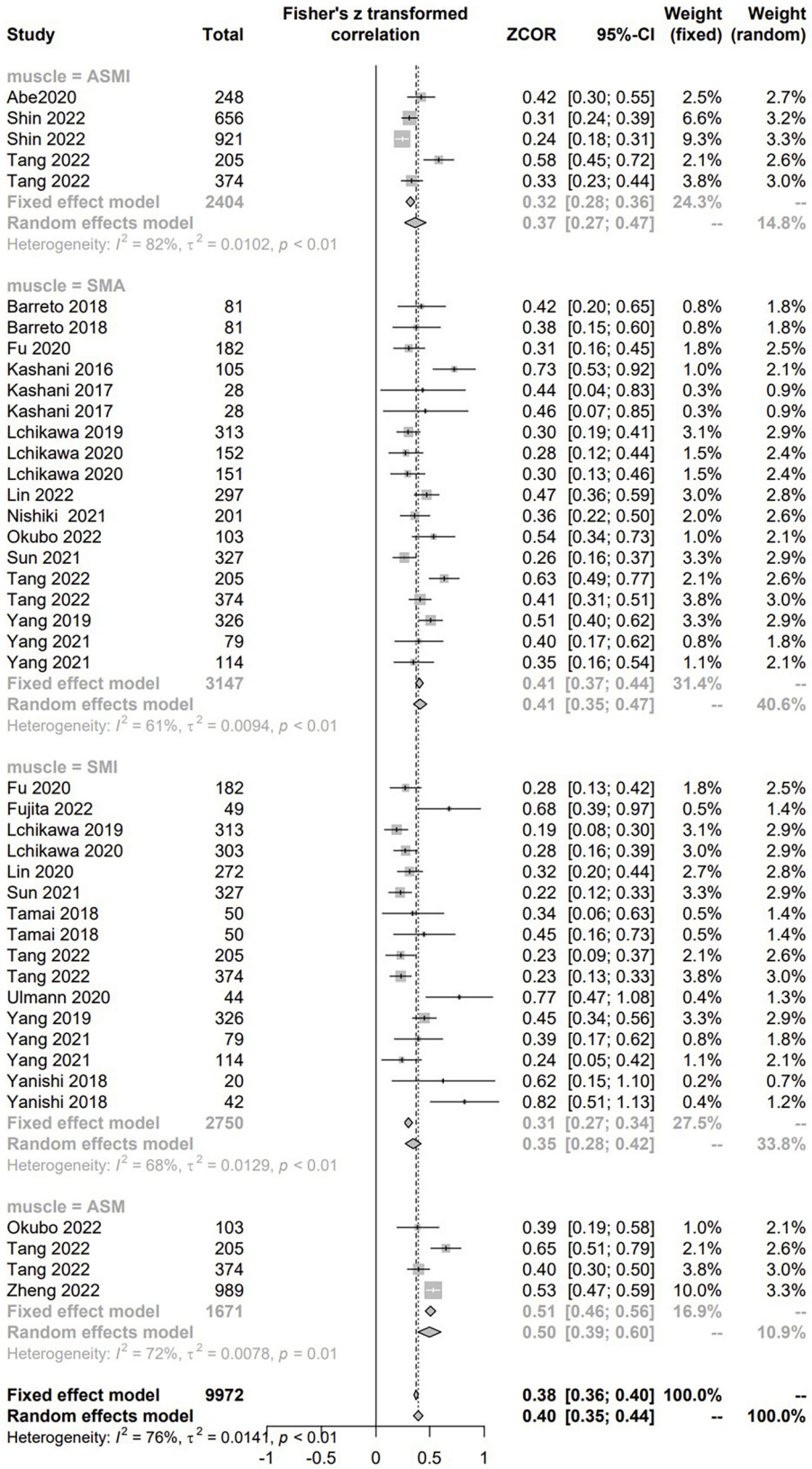

FIGURE 3
The pooled estimate of the relationship between serum creatinine/cystatin C ratio and computed tomography-assessed skeletal muscle.

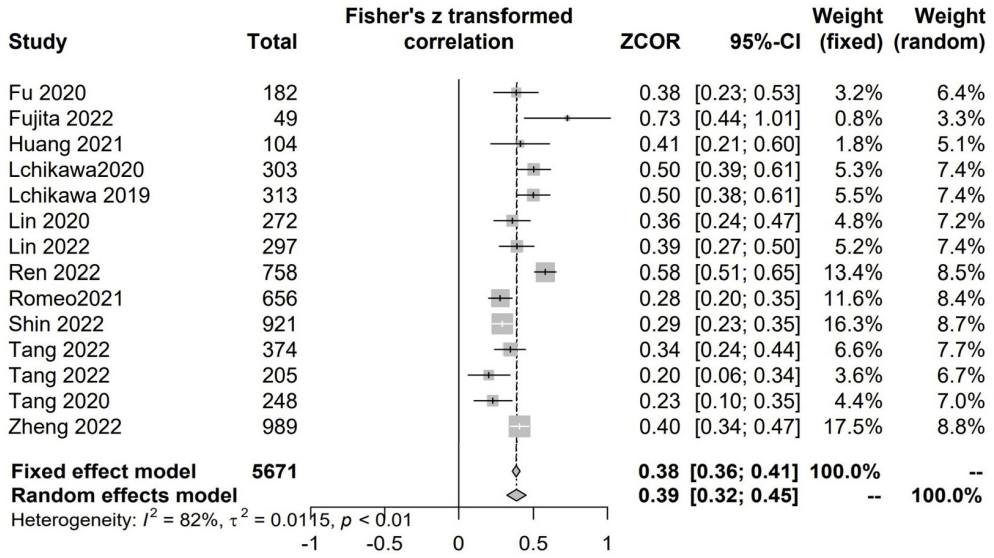

FIGURE 4
The pooled estimate of the relationship between serum creatinine/cystatin C ratio and handgrip strength.

et al. suggested that CCR at admission significantly correlated with ICU LOS (r = 0.161, P < 0.001) (51). Jung et al. found significant trends toward shorter ICU (P = 0.002) and hospital LOS (P < 0.001) in the higher CCR quartiles (37). Similarly, Barreto et al. suggested that an increase in the CCR significantly predicted more rapid discharge from the ICU [aHR, 1.06 (0.99, 1.14); P = 0.003] and hospital [aHR, 1.10 (1.03, 1.18) P = 0.007] (5).

Seven studies focused on the predictive value of CCR on the overall complications. The pooled findings from five studies that provided specific data on this topic showed that a decreased CCR was independently associated with a higher mortality risk (HR 1.66; 95% CI, 1.17–2.36; I^2 = 83%; P < 0.01) (7, 16, 24, 38, 39; **Supplementary Figure 9**). In the remaining two studies, one reported that CCR was significantly lower in patients with cardiovascular disease (P = 0.008) and lower extremity arterial disease (P = 0.004) (33), and the other found no associations between CCR and adverse reactions (31).

Discussion

We conducted the current meta-analysis of 38 studies to assess the value of CCR in hospitalized patients. Our results showed that CCR had a significant correlation with CTASM, GS and HGS, and the ROC curves suggested that CCR had good diagnostic efficacy for sarcopenia, indicating that CCR is an ideal alternative biomarker to screen sarcopenia. CCR, on the other hand, can reliably predict mortality in hospitalized patients, which was confirmed by regression analysis of CCR as both continuous and categorical variables. In addition, less evidence showed that higher CCR was independently associated with a shorten MV duration, reduced ICU and hospital LOS, less nutritional risk, and decreased complications in patients.

CCR as a muscle mass evaluating tool in hospitalized patients

Recent findings suggest CCR is associated with disease-related catabolism because it reflects altered muscle mass (12). Serum creatinine is mainly produced by creatine phosphate during skeletal muscle metabolism (53). Therefore, patients with reduced muscle mass have lower creatinine levels. Also, cystatin C is a low molecular weight protein produced continuously by all nucleated cells and is readily filtered, absorbed, and broken down in the proximal renal tubules. It is not influenced by muscle metabolism (54). As a result, among patients with stable kidney function, one of the key factors of the difference between these two measures, skeletal muscle mass, is one of the primary determinants of the difference between these two markers. The effect of muscle mass on creatinine can be estimated by the ratio to cystatin C, which provides an easily accessible and reproducible assessment of muscle in patients at high risk for catabolism (12, 13).

Our results suggest that the CCR is an inexpensive, valid, objective, and reproducible tool for muscle mass assessment. We validated that CCR can screen for sarcopenia in three domains. CCR was significantly correlated not only with SAM/SMI, but also with GS and HGS. This supports the current sarcopenia definition in guidelines, that is, sarcopenia is assessed by skeletal

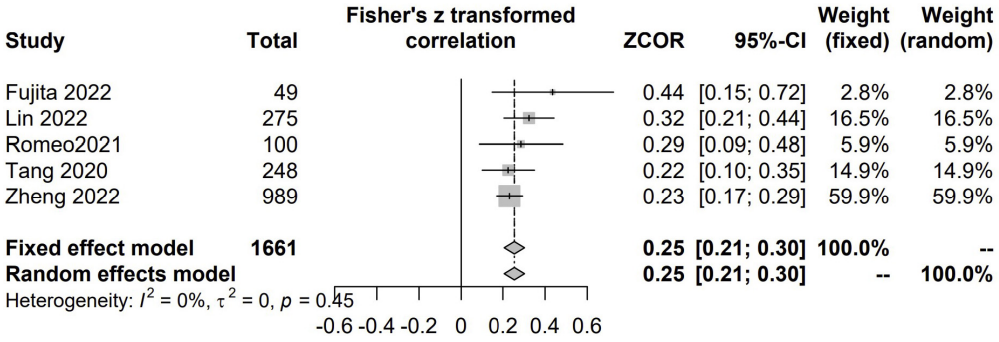

FIGURE 5
The pooled estimate of the relationship between serum creatinine/cystatin C ratio and gait speed.

muscle mass, muscle strength, and physical performance (55). Also, subgroup analyses suggested that medical, surgical, cancer, trauma, and ICU patients maintained consistently high correlations, meaning that CCR can be applied in various conditions (**Figure 3**).

In addition, the included studies used different definitions of sarcopenia. For example, most authors used diagnostic thresholds reported in previous studies to define sarcopenia or based on arbitrary thresholds from diverse populations (e.g., lowest 25th quartile, 33rd quartile, or median). These definitions were another source of heterogeneity in our results. Based on different sarcopenia definitions, we found that sarcopenia remained robustly correlated with CCR for all. On the other hand, different definitions have prevented obtaining a uniform CCR cut-off value for the diagnosis of sarcopenia in the current manuscript. Therefore, given the differences in disease, body size, dietary structure, and nutritional interventions among the included inpatients, there is still a need to establish appropriate CCR thresholds based on the local sarcopenic population in the future clinical application of CCR.

CCR as a nutritional screening tool in hospitalized patients

Several studies included in our meta-analysis demonstrate that CCR can be utilized as a surrogate indication for a variety of nutritional screening techniques (6, 13, 16, 45). These studies found that CCR remained an independent predictor of nutrition risk/malnutrition in patients after adjusting for age, gender, Acute Physiology and Chronic Health Evaluation (APACHE) III score, Sequential Organ Failure Assessment (SOFA) score, and BMI (6, 13, 16, 45). Low CCR values generally indicate reduced muscle tissue, which is associated with malnutrition. In contrast, high CCR values indicate a complete muscle tissue mass and functional status and can identify malnutrition at the initial stage.

Furthermore, some studies suggest that CCR is superior to some traditional nutritional risk indicators (e.g., NUTRIC score), which are established and validated only for nutritional status and not as a surrogate for muscle mass (53). Thus, the independence from the subjective provision of information, weight data, and complex calculations, as well as the simplicity and repeatability, make CCR a favorable choice for clinical decision support of busy bedside clinicians compared to previous, more sophisticated tools. Although the studies we included involved a wide range of inpatient populations such as ICU, geriatric patients, and medical patients, given the number of studies, further confirmation in a larger sample is needed in the future.

CCR as a prognostic indicator in hospitalized patients

We provide evidence for the association of sarcopenia with inpatient prognosis by evaluating CCR. A possible explanation for the association of CCR with mortality is that CCR may reflect muscle mass (12), the latter has been demonstrated to influence outcomes in various patient populations. Current literature suggests that sarcopenia is related to reduced protein synthesis and enhanced degradation induced by wasting, physical restraints, infection, prolonged mechanical ventilation and sedation, and muscle relaxants in various hospitalized settings (56). Especially in ICU patients with multiple organ dysfunction syndromes, the cross-sectional area of muscle fibers decreases at a rate of 3 to 4% per day, resulting in skeletal muscle atrophy, which affects vital physiological functions (4). As a result, muscle is also considered one of the failing organs of multiple organ dysfunction syndrome and has received widespread attention.

Although the pathophysiological relationship between muscle loss and patients' prognosis is not fully understood, several studies have suggested it may be associated with a high catabolic state, cytokine impairment, and insulin signaling,

leading to glucose intolerance (57). Sarcopenia also decreases the body's ability to respond adequately to inflammatory stimuli and delays the implementation of rehabilitation (4, 57). Under these conditions, patient immune function may be reduced, leading to a high CCR, i.e., high risk of sarcopenia, associated with various complications, prolonged ICU or hospital stay, prolonged mechanical ventilation, and ultimately increased risk of patient death, as shown by our findings.

Strengths and limitations of the study

To the best of our knowledge, this is the first meta-analysis to assess the value of CCR on muscle evaluation and prognosis in hospitalized patients. We included 38 studies with more than 20,000 patients with sufficient statistical power to conduct subgroup analysis based on potential influencing factors. The vast majority were based on multiple regression analyses of the included studies. Most of these included studies adjusted for a variety of possible confounders, including demographic variables (e.g., age and sex), anthropometric measures (e.g., BMI), nutritional status (e.g., subjective global assessment, NUTRIC score, disease severity (e.g., SOFA, APACHE-II score), disease-specific (e.g., tumor type, stage, and type of treatment), and physical fitness status as a prognostic factor. Thus, our findings somewhat control these confounding factors when evaluating muscle, prognosis, and other clinical outcome-related CCR measures.

Our meta-analysis has several limitations. (1) The observational design of all included studies excluded any causal inference. Also, patients included only in CCR tests in retrospective studies were prone to selection bias. (2) The small sample may be subject to false-positive bias; the small number of included studies in some subgroup analyses interpreted the results with caution. (3) The mean age of the included patients varied widely (47–88 years), but there was insufficient data to further explore the effect of age on mortality. (4) The uneven distribution of underlying disease in the study population may also have a different prognostic value. (5) CCR is unlikely to be at a steady state under AKI conditions, making the application of muscle mass ratios less desirable than in other clinical situations. Of note, cystatin C is a cathepsins inhibitor, and its levels increased in hyperthyroidism, obesity, metabolic syndrome, diabetes mellitus type 2, and different types of inflammation, albeit in different degrees (58, 59). However, most included studies did not exclude these confounding factors. (6) Patients' medications or other interventions may affect the results of our study. Therefore, to reduce the effect of the intervention, we used the longest follow-up time of each included study. (7) The vast majority of the included studies were from Asian countries, so more data from other ethnicities are needed for confirmation.

Conclusion

The results of our study indicated significant correlations between CCR and skeletal muscle evaluation and a prognostic role of CCR that higher circulating CCR levels were positively associated with the less risk of all-cause mortality in hospitalized patients. Thus, we recommended using CCR as a new prognostic biomarker to provide better information not only in decision correlations for muscle mass assessment but also in the prediction of survival and other associated clinical outcome.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Author contributions

W-HZ and YY contributed to analysis and drafting of the article. Y-BZ contributed to data collection and analysis. H-BH contributed to the conception of the study, design, revisions of this manuscript, and was responsible for the integrity of the work as a whole, from inception to publication of the article. All authors approved the final version submitted for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1058464/full#supplementary-material

References

1. Cruz-Jentoft AJ, Landi F, Topinková E, Michel JP. Understanding sarcopenia as a geriatric syndrome. *Curr Opin Clin Nutrit Metab Care.* (2010) 13:1–7. doi: 10.1097/MCO.0b013e328333c1c1

2. Shafiee G, Keshtkar A, Soltani A, Ahadi Z, Larijani B, Heshmat R. Prevalence of sarcopenia in the world: a systematic review and meta- analysis of general population studies. *J Diab Metab Disord.* (2017) 16:21. doi: 10.1186/s40200-017-0302-x

3. Almohaisen N, Gittins M, Todd C, Sremanakova J, Sowerbutts AM, Aldossari A, et al. Prevalence of undernutrition, frailty and sarcopenia in community-dwelling people aged 50 years and above: systematic review and meta-analysis. *Nutrients.* (2022) 14:1537. doi: 10.3390/nu14081537

4. Puthucheary ZA, Rawal J, McPhail M, Connolly B, Ratnayake G, Chan P, et al. Acute skeletal muscle wasting in critical illness. *JAMA.* (2013) 310:1591–600. doi: 10.1001/jama.2013.278481

5. Barreto EF, Poyant JO, Coville HH, Dierkhising RA, Kennedy CC, Gajic O, et al. Validation of the sarcopenia index to assess muscle mass in the critically ill: a novel application of kidney function markers. *Clin Nutr.* (2019) 38:1362–7. doi: 10.1016/j.clnu.2018.05.031

6. Ren C, Su H, Tao J, Xie Y, Zhang X, Guo Q. Sarcopenia index based on serum creatinine and cystatin C is associated with mortality, nutritional risk/malnutrition and sarcopenia in older patients. *Clin Interv Aging.* (2022) 17:211–21. doi: 10.2147/CIA.S351068

7. Tang T, Zhuo Y, Xie L, Wang H, Yang M. Sarcopenia index based on serum creatinine and cystatin C is associated with 3-year mortality in hospitalized older patients. *Sci Rep.* (2020) 10:1260. doi: 10.1038/s41598-020-58304-z

8. Earthman CP. Body composition tools for assessment of adult malnutrition at the bedside: a tutorial on research considerations and clinical applications. *JPEN J Parenter Enteral Nutr.* (2015) 39:787–822. doi: 10.1177/0148607115595227

9. Koo BK. Assessment of muscle quantity, quality and function. *J Obes Metab Syndrome.* (2022) 31:9–16. doi: 10.7570/jomes22025

10. Connolly B, MacBean V, Crowley C, Lunt A, Moxham J, Rafferty GF, et al. Ultrasound for the assessment of peripheral skeletal muscle architecture in critical illness: a systematic review. *Crit Care Med.* (2015) 43:897–905. doi: 10.1097/CCM.0000000000000821

11. Tolonen A, Pakarinen T, Sassi A, Kyttä J, Cancino W, Rinta-Kiikka I, et al. Methodology, clinical applications, and future directions of body composition analysis using computed tomography (CT) images: a review. *Eur J Radiol.* (2021) 145:109943. doi: 10.1016/j.ejrad.2021.109943

12. Kashani KB, Frazee EN, Kukralova L, Sarvottam K, Herasevich V, Young PM, et al. Evaluating muscle mass by using markers of kidney function: development of the sarcopenia index. *Crit Care Med.* (2017) 45:e23–9. doi: 10.1097/CCM.0000000000002013

13. Barreto EF, Kanderi T, DiCecco SR, Lopez-Ruiz A, Poyant JO, Mara KC, et al. Sarcopenia index is a simple objective screening tool for malnutrition in the critically Ill. *JPEN J Parenter Enteral Nutr.* (2019) 43:780–8. doi: 10.1002/jpen.1492

14. Jung CY, Joo YS, Kim HW, Han SH, Yoo TH, Kang SW, et al. Creatinine-Cystatin C ratio and mortality in patients receiving intensive care and continuous kidney replacement therapy: a retrospective cohort study. *Am J Kidney Dis.* (2021) 77:509-516.e1. doi: 10.1053/j.ajkd.2020.08.014

15. Kashani K, Sarvottam K, Pereira NL, Barreto EF, Kennedy CC. The sarcopenia index: a novel measure of muscle mass in lung transplant candidates. *Clin Trans.* (2018) 32:e13182. doi: 10.1111/ctr.13182

16. Yang J, Zhang T, Feng D, Dai X, Lv T, Wang X, et al. A new diagnostic index for sarcopenia and its association with short-term postoperative complications in patients undergoing surgery for colorectal cancer. *Colorectal Dis.* (2019) 21:538–47. doi: 10.1111/codi.14558

17. Osaka T, Hamaguchi M, Hashimoto Y, Ushigome E, Tanaka M, Yamazaki M, et al. Decreased the creatinine to cystatin C ratio is a surrogate marker of sarcopenia in patients with type 2 diabetes. *Diabetes Res Clin Pract.* (2018) 139:52–8. doi: 10.1016/j.diabres.2018.02.025

18. Sun J, Yang H, Cai W, Zheng J, Shen N, Yang X, et al. Serum creatinine/cystatin C ratio as a surrogate marker for sarcopenia in patients with gastric cancer. *BMC Gastroenterol.* (2022) 22:26. doi: 10.1186/s12876-022-02093-4

19. Kim CH, Rhee TM, Woo Park K, Soon Park C, Kang J, Han JK, et al. Association between low muscle mass and prognosis of patients with coronary artery disease undergoing percutaneous coronary intervention. *J Am Heart Assoc.* (2021) 10:e018554. doi: 10.1161/JAHA.120.018554

20. Nishiki K, Nojiri M, Kato R, Shinomiya S, Oikawa T, Ishizaki T, et al. Serum creatinine/cystatin c ratio associated with cross-sectional area of erector spinae muscles and pulmonary function in patients with chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis.* (2021) 16:3513–24. doi: 10.2147/COPD.S339243

21. Fujita K, Ohkubo H, Nakano A, Takeda N, Fukumitsu K, Fukuda S, et al. Serum creatinine/cystatin C ratio is a surrogate marker for sarcopenia in patients with idiopathic pulmonary fibrosis. *BMC Pulm Med.* (2022) 22:203. doi: 10.1186/s12890-022-02000-3

22. Huang D, Xie C, Sun C, Chen M, Li L, Yi H, et al. Serum creatinine to cystatin C ratio is an effective indicator for muscle strength decline in men with acute exacerbation of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis.* (2022) 17:781–9. doi: 10.2147/COPD.S356314

23. Huang S, Zhao L, Liu Z, Li Y, Wang X, Li J, et al. The effectiveness of the sarcopenia index in predicting septic shock and death in elderly patients with community-acquired pneumonia. *BMC Geriatr.* (2022) 22:341. doi: 10.1186/s12877-022-03029-z

24. Zheng C, Wang E, Li JS, Xie K, Luo C, Ge QY, et al. Serum creatinine/cystatin C ratio as a screening tool for sarcopenia and prognostic indicator for patients with esophageal cancer. *BMC Geriatr.* (2022) 22:207. doi: 10.1186/s12877-022-02925-8

25. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg.* (2010) 8:336–41. doi: 10.1016/j.ijsu.2010.02.007

26. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol.* (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z

27. Lenhard W, Lenhard A. *Hypothesis Tests for Comparing Correlations*. Germany: Psychometrica (2014).

28. Zhou XH, Obuchowski NA, McClish DK. *Statistical Methods in Diagnostic Medicine*. New York, NY: Wiley (2002). doi: 10.1002/9780470317082

29. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

30. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Controlled Clin Trials.* (1986) 7:177–88. doi: 10.1016/0197-2456(86)90046-2

31. Chen XHL, Shen Y, Wu X, Dong B, Hao Q. The role of baseline sarcopenia index in predicting chemotherapy-induced undesirable effects and mortality in older people with stage III or IV NonSmall cell Lung cancer. *J Nutr Health Aging.* (2021) 25:878–82. doi: 10.1007/s12603-021-1633-3

32. Liu WZX, Tan X, Yang L, Wang Y, Diao S, Huang S, et al. Predictive value of serum creatinine/cystatin C in acute ischemic stroke patients under nutritional intervention. *J Nutr Health Aging.* (2021) 25:335–9. doi: 10.1007/s12603-020-1495-0

33. Yang Q, Zhang M, Sun P, Li Y, Xu H, Wang K, et al. Cre/CysC ratio may predict muscle composition and is associated with glucose disposal ability and macrovascular disease in patients with type 2 diabetes. *BMJ Open Diab Res Care.* (2021) 9:e002430. doi: 10.1136/bmjdrc-2021-002430

34. Fu X, Tian Z, Wen S, Sun H, Thapa S, Xiong H, et al. A new index based on serum creatinine and cystatin C is useful for assessing sarcopenia in patients with advanced cancer. *Nutrition.* (2021) 82:111032. doi: 10.1016/j.nut.2020.111032

35. Ichikawa T, Miyaaki H, Miuma S, Motoyoshi Y, Yamashima M, Yamamichi S, et al. Calculated body muscle mass as a useful screening marker for low skeletal muscle mass and sarcopenia in chronic liver disease. *Hepatol Res.* (2020) 50:704–14. doi: 10.1111/hepr.13492

36. Ichikawa T, Miyaaki H, Miuma S, Motoyoshi Y, Yamashima M, Yamamichi S, et al. Indices calculated by serum creatinine and cystatin C as predictors of liver damage, muscle strength and sarcopenia in liver disease. *Biomed Rep.* (2020) 12:89–98. doi: 10.3892/br.2020.1273

37. Jung CY, Kim HW, Han SH, Yoo TH, Kang SW, Park JT. Creatinine-cystatin C ratio and mortality in cancer patients: a retrospective cohort study. *J Cachexia Sarcopenia Muscle.* (2022) 13:2064–72. doi: 10.1002/jcsm.13006

38. Kim HJ, Kim HB, Kim HY, Shim JK, Lee C, Kwak YL. Associations of creatinine/cystatin C ratio and postoperative pulmonary complications in elderly patients undergoing off-pump coronary artery bypass surgery: a retrospective study. *Sci Rep.* (2021) 11:16881. doi: 10.1038/s41598-021-96442-0

39. Lee HS, Park KW, Kang J, Ki YJ, Chang M, Han JK, et al. Sarcopenia index as a predictor of clinical outcomes in older patients with coronary artery disease. *J Clin Med.* (2020) 9:3121. doi: 10.3390/jcm9103121

40. Lin YL, Chang IC, Liou HH, Wang CH, Lai YH, Kuo CH, et al. Serum indices based on creatinine and cystatin C predict mortality in patients with non-dialysis chronic kidney disease. *Sci Rep.* (2021) 11:16863. doi: 10.1038/s41598-021-96447-9

41. Lin YL, Chen SY, Lai YH, Wang CH, Kuo CH, Liou HH, et al. Serum creatinine to cystatin C ratio predicts skeletal muscle mass and strength in patients with non-dialysis chronic kidney disease. *Clin Nutr.* (2020) 39:2435–41. doi: 10.1016/j.clnu.2019.10.027

42. Lin YL, Wang CH, Chang IC, Hsu BG. A novel application of serum creatinine and cystatin c to predict sarcopenia in advanced CKD. *Front Nutr.* (2022) 9:828880. doi: 10.3389/fnut.2022.828880

43. Mauro E, Diaz JM, Garcia-Olveira L, Spina JC, Savluk L, Zalazar F, et al. Sarcopenia HIBA score predicts sarcopenia and mortality in patients on the liver transplant waiting list. *Hepatol Commun.* (2022) 6:1699–710. doi: 10.1002/hep4.1919

44. Okubo N, Yoshida T, Tanaka K, Okada N, Hosoi K, Ohara M, et al. Serum creatinine to cystatin C ratio reflects preoperative and early postoperative walking ability in older patients with hip fracture. *J Cachexia Sarcopenia Muscle.* (2022) 13:945–54. doi: 10.1002/jcsm.12940

45. Abe K, Yano T, Katano S, Ohori K, Ishigo T, Moniwa N, et al. Utility of the sarcopenia index for assessment of muscle mass and nutritional status in patients with chronic heart failure: comparison with anthropometric parameters. *Geriatr Gerontol Int.* (2020) 20:388–9. doi: 10.1111/ggi.13876

46. Romeo FJ, Chiabrando JG, Seropian IM, Raleigh JV, de Chazal HM, Garmendia CM, et al. Sarcopenia index as a predictor of clinical outcomes in older patients undergoing transcatheter aortic valve replacement. *Catheter Cardiovasc Interv.* (2021) 98:E889–96. doi: 10.1002/ccd.29799

47. Shin JY. Low serum creatinine to cystatin C ratio is independently associated with sarcopenia and high carotid plaque score in patients with type 2 diabetes. *Nutr Metab Cardiovasc Dis.* (2022) 32:1454–62. doi: 10.1016/j.numecd.2022.02.005

48. Tamai Y, Iwasa M, Kawasaki Y, Yoshizawa N, Ogura S, Sugimoto R, et al. Ratio between estimated glomerular filtration rates of creatinine and cystatin C predicts overall survival in patients with hepatocellular carcinoma. *Hepatol Res.* (2019) 49:153–63. doi: 10.1111/hepr.13230

49. Tang T, Xie L, Hu S, Tan L, Lei X, Luo X, et al. Serum creatinine and cystatin C-based diagnostic indices for sarcopenia in advanced non-small cell lung cancer. *J Cachexia Sarcopenia Muscle.* (2022) 13:1800–10. doi: 10.1002/jcsm.12977

50. Ulmann G, Kai J, Durand JP, Neveux N, Jouinot A, De Bandt JP, et al. Creatinine-to-cystatin C ratio and bioelectrical impedance analysis for the assessement of low lean body mass in cancer patients: comparison to L3-computed tomography scan. *Nutrition.* (2021) 81:110895. doi: 10.1016/j.nut.2020.110895

51. Wang S, Xie L, Xu J, Hu Y, Wu Y, Lin Z, et al. Predictive value of serum creatinine/cystatin C in neurocritically ill patients. *Brain Behav.* (2019) 9:e01462. doi: 10.1002/brb3.1462

52. Yanishi M, Kinoshita H, Tsukaguchi H, Kimura Y, Koito Y, Sugi M, et al. The creatinine/cystatin C ratio provides effective evaluation of muscle mass in kidney transplant recipients. *Int Urol Nephrol.* (2019) 51:79–83. doi: 10.1007/s11255-018-2015-6

53. Kashani K, Rosner MH, Ostermann M. Creatinine: from physiology to clinical application. *Eur J Int Med.* (2020) 72:9–14. doi: 10.1016/j.ejim.2019.10.025

54. Björk J, Nyman U, Berg U, Delanaye P, Dubourg L, Goffin K, et al. Validation of standardized creatinine and cystatin C GFR estimating equations in a large multicentre European cohort of children. *Pediatric Nephrol.* (2019) 34:1087–98. doi: 10.1007/s00467-018-4185-y

55. Dent E, Morley JE, Cruz-Jentoft AJ, Arai H, Kritchevsky SB, Guralnik J, et al. International Clinical Practice Guidelines for Sarcopenia (ICFSR): screening, diagnosis and management. *J Nutr Health Aging.* (2018) 22:1148–61. doi: 10.1007/s12603-018-1139-9

56. Moisey LL, Mourtzakis M, Cotton BA, Premji T, Heyland DK, Wade CE, et al. Skeletal muscle predicts ventilator-free days, ICU-free days, and mortality in elderly ICU patients. *Crit Care.* (2013) 17:R206. doi: 10.1186/cc12901

57. Batt J, Herridge MS, Dos Santos CC. From skeletal muscle weakness to functional outcomes following critical illness: a translational biology perspective. *Thorax.* (2019) 74:1091–8. doi: 10.1136/thoraxjnl-2016-208312

58. Zi M, Xu Y. Involvement of cystatin C in immunity and apoptosis. *Immunol Lett.* (2018) 196:80–90. doi: 10.1016/j.imlet.2018.01.006

59. Xu Y, Ding Y, Li X, Wu X. Cystatin C is a disease-associated protein subject to multiple regulation. *Immunol Cell Biol.* (2015) 93:442–51. doi: 10.1038/icb.2014.121

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Serum transthyretin and aminotransferases are associated with lean mass in people with coronary heart disease: Further insights from the CARE-CR study

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Background: Low muscle mass disproportionately affects people with coronary heart disease compared to healthy controls but is under-researched and insufficiently treated. Inflammation, poor nutrition, and neural decline might contribute to low muscle mass. This study aimed to assess circulatory biomarkers related to these mechanisms [albumin, transthyretin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and C-terminal agrin fragment] and their relationship with muscle mass in people with coronary heart disease. Our findings could be beneficial to indicate mechanisms of sarcopenia, detect sarcopenia, and evaluate treatment.

Methods: Serum blood samples from people with coronary heart disease were analysed for biomarker concentrations using enzyme-linked immunosorbent assays. Skeletal muscle mass was estimated using dual X-ray absorptiometry derived appendicular lean mass and reported as skeletal muscle index (SMI; kgm^{-2}), and as a proportion of total body mass [appendicular skeletal mass (ASM%)]. Low muscle mass was defined as a SMI <7.0 and <6.0kgm^{-2}, or ASM% <25.72 and <19.43% for men and women, respectively. Associations between biomarkers and lean mass were adjusted for age and inflammation.

Results: Sixty-four people were assessed; 14 (21.9%) had low muscle mass. People with low muscle mass had lower transthyretin (effect size 0.34, p=0.007), ALT (effect size 0.34, p=0.008), and AST (effect size 0.26, p=0.037) concentrations, compared to those with normal muscle mass. SMI was associated with inflammation-corrected ALT (r=0.261, p=0.039) and with inflammation- and age-adjusted AST/ALT ratio (r=−0.257, p=0.044). Albumin and C-terminal agrin fragment were not associated with muscle mass indices.

Conclusion: Circulatory transthyretin, ALT and AST were associated with low muscle mass in people with coronary heart disease. Low concentrations of these biomarkers might indicate that low muscle mass is partially explained by poor nutrition and high inflammation in this cohort. Targeted treatments to address these factors could be considered for people with coronary heart disease.

KEYWORDS

agrin, albumin, aminotransferases, biomarkers, coronary heart disease, muscle, sarcopenia, transthyretin

1. Introduction

Between 1990 and 2019, coronary heart disease (CHD)-related mortality declined at a greater rate (61%) than CHD incidence (37%) (1). In the era of modern medical management, people with a CHD diagnosis live for longer and many will require increased support to manage their long-term health. An important component of healthy ageing is maintaining skeletal muscle mass (SMM) (2, 3). This is particularly relevant in people with CHD where there is a higher incidence of low SMM in people with CHD compared to age- and sex -matched adults (4). Emerging research in people with CHD shows that low SMM increases the risk of all-cause mortality, fatal or non-fatal major adverse cardiovascular events, lower fitness (peak oxygen uptake; VO_{2peak}) and poorer quality of life (4–8). However, factors that influence loss of SMM in CHD are poorly defined. The delivery of successful interventions to improve SMM, and subsequently long-term health, in these people requires that we have: (1) the ability to identify those at risk of low SMM early, and (2) a thorough understanding of the factors influencing low SMM. For this purpose, circulatory biomarkers might be useful to complement traditional measures of SMM and strength.

Maladaptive processes and behaviours that contribute to loss of SMM and/ or function are complex. There is compelling evidence that these include neural maladaptation (9, 10), inflammation (11, 12), and sub-optimal nutrition (13, 14). Biomarkers which appear to have a central role in these systems need investigating. C-terminal agrin fragment (CAF) is a circulatory by-product of agrin cleavage by synaptic protease neurotrypsin (15), a process which can lead to neuromuscular junction breakdown (16). In healthy older adults (17, 18) and people with heart failure (19), CAF levels are elevated in those with low, compared to with normal, SMM. Thus, declining neural function might contribute to low SMM. However, it is unclear whether these findings exist in older people with CHD. Albumin and transthyretin are acute-phase response proteins which might indicate inflammation-related nutrition risk (20). In hospitalised people with CHD, albumin and transthyretin levels are lower in the presence of sarcopenia (as defined by the Asian Working Group for Sarcopenia) compared to those defined as non-sarcopenic (21). Whether albumin and transthyretin are associated with low SMM using European cut-off points (22), in people with CHD, requires clarification. Finally, alanine (ALT) and aspartate (AST) aminotransferases are liver/skeletal muscle enzymes (23). Circulatory levels of ALT are elevated in people with type 2 diabetes (24) and metabolic syndrome (25), but lower in the presence of age-related syndromes often characterised by under-nutrition, including sarcopenia (26). The AST/ALT ratio is proposed to be higher in those with sarcopenia compared to those without, although few studies have investigated this to date (27, 28).

Associations between SMM and serum CAF (17–19), albumin, transthyretin (21), ALT and AST (26–28) were reported in healthy older adults and people with chronic health conditions. The present study aimed to investigate the association between estimated SMM, and serum CAF, albumin, transthyretin, ALT and AST, in people with recently diagnosed stable CHD. We hypothesised that people with CHD and low SMM will have higher CAF levels and AST/ALT ratio and lower albumin and transthyretin levels, compared to people with CHD and preserved SMM.

2. Materials and methods

2.1. Study design and participants

Baseline serum blood samples and demographic characteristics used in this cross-sectional study were collected as part of the Cardiovascular and cardiorespiratory Adaptations to Routine Exercise-based Cardiac Rehabilitation (CARE CR) study (29). The CARE CR study protocol was published in detail elsewhere (29). Briefly, clinically stable people with a primary diagnosis of CHD (aged 30–85 years) were referred to the research team by nursing staff, within 2 weeks of a cardiac event or procedure. Participants provided their written informed consent to participate in the study. The CARE CR study was granted ethical approval by the Humber Bridge NHS Research Ethics Committee-Yorkshire and the Humber (12/YH/0278). Ethical approval for assay analysis of serum samples for biomarkers related to sarcopenia was provided by the Northumbria University Health and Life Sciences Ethics Committee (20933). The main findings from the CARE CR study on patient rehabilitation and cardiorespiratory fitness are published elsewhere (4, 30).

2.2. Body composition

Body mass index (BMI; $kg\,m^{-2}$) was calculated using mass (kg) and stature (m). Waist and hip circumferences (cm) were measured at 1 cm above the iliac crest and at the widest aspect of the hips, respectively. Appendicular lean mass (ALM), defined as total lean mass in both arms and legs (kg), was measured using dual X-ray absorptiometry (DXA; Lunar iDXA GE Healthcare Buckinghamshire, United Kingdom), as a proxy for SMM assessment. ALM is expressed as skeletal muscle index (SMI; $kg\,m^{-2}$) and as a percentage of total body mass (appendicular skeletal mass; ASM%). Age-adjusted SMI and ASM% were moderately correlated ($r = 0.507$, $p < 0.001$). We defined low SMI as <7.0 and <6.0 $kg\,m^{-2}$ (22) and low ASM% as <25.72 and <19.43% (31) for men and women, respectively.

Abbreviations: ALT, Alanine aminotransferase; ASM%, Appendicular skeletal muscle; AST, Aspartate aminotransferase; CAF, C-terminal agrin fragment; CHD, Coronary heart disease; DXA, Dual X-ray absorptiometry; Hs-CRP, High sensitivity C-reactive protein; SMI, Skeletal muscle index; SMM, Skeletal muscle mass.

2.3. Maximal cardiopulmonary exercise test

Cardiopulmonary exercise testing was performed using the modified Bruce treadmill protocol (32), as previously described (4, 29). A 12-lead Electrocardiogram (ECG), ECG-gated automated blood pressure, heart rate, and rate of perceived exertion were monitored throughout. Breath-by-breath metabolic gas exchange data were collected using an Oxycon Pro metabolic cart (Jaeger, Hoechburg, Germany). We report VO_{2peak} (ml), defined as the mean VO_2 over the last 30 s of the test; VO_{2peak} was adjusted for body mass (ml kg^{-1} min^{-1}) (4).

2.4. Blood sampling and analysis

Participants abstained from strenuous exercise 24-h prior to attending their baseline study visit. Resting blood samples were drawn by venepuncture and placed in a refrigerated (4°C) centrifuge at 3,000 revolutions per minute, for 15 min. Albumin, aminotransferases and N-terminal pro-brain natriuretic peptide (NT-proBNP) were analysed at the Hull Royal Infirmary in an accredited biochemistry laboratory, as a single measurement on the day of each blood draw. Calibration and quality controls were conducted in accordance with manufacturer's guidelines. The ABX Pentra 400 biochemistry auto analyser (Horiba, Montpellier, France) was used to analyse high sensitivity C-reactive protein (hs-CRP) in duplicate, in accordance with the manufacturer's quality control guidance (4). Remaining plasma and serum samples were stored at −80°C until analysis.

We analysed serum samples in duplicate using commercial enzyme-linked immunosorbent assay (ELISA) for CAF (Abcam #ab216945) and transthyretin (Abcam #ab108895) and followed their standard instructions for serum analysis. Concentrations of transthyretin and CAF were assessed in duplicate and the average of the two measures reported. We re-analysed samples with a coefficient of variation (CV) >40% and when biomarker concentrations were not within the limits of the standard curve. The CV for the assay analyses of transthyretin and CAF were 7.9 and 5.1%, respectively. Routine health-related serum biomarkers evaluated as part of the CARE CR study are reported elsewhere, including NT-proBNP, hs-CRP, glucose, white cell count, total cholesterol, low-density and high-density lipoprotein cholesterol, estimated glomerular filtration rate and triglycerides (4, 30).

Normal adult reference values for circulatory markers of interest are:

- Albumin: 35–50 g/L (33).
- Transthyretin: 30–33 and 25–27 mg/dl in males and females, respectively (34).
- ALT: 9.0–59.0 and 7.8–41.0 U/L in males and females, respectively (35).
- AST: 11.0–34.0 U/L (35)
- CAF: 0.86–4.66 ng/ml (17).

2.5. Statistical analysis

Statistical analyses were performed by a single researcher using commercially available software (SPSS version 28, IBM, New York, NY, United States). Distribution of the data was assessed using visual inspection of histograms, QQ-plots and using the Kolmogorov Smirnov test. Categorical variables are reported as frequency with percentage. Continuous normally distributed variables are reported as mean ± standard deviation. Continuous non-normally distributed variables are reported as median with interquartile range, or median with range where the sample size is ≤3 people. Demographic characteristics are reported for the whole cohort and separately for people with normal or low SMM (defined as low SMI or low ASM%). Differences in demographic characteristics between the two groups were assessed using the Fisher's exact test (categorical variables), a Student's *t*-test (continuous normally distributed), or Mann–Whitney *U* test (continuous non-normally distributed). Two-group comparison of blood biomarkers between people with normal or low SMM were evaluated using Mann–Whitney *U* tests and reported as U statistics, *p*-values, and effect sizes, calculated using the following equation (36):

$$r = \frac{Z}{\sqrt{n}}$$

Absolute r values of 0.2, 0.5, and 0.8 are considered small, moderate and large effect sizes, respectively (37). The relationship between serum biomarker concentrations, SMI and ASM% were calculated using Spearman's rank correlations. It is well-established that age and inflammation influence SMM and some serum biomarkers; people with CHD and low SMM are significantly older than those with normal SMM (38), whilst albumin and transthyretin concentrations decrease in the presence of inflammation (39). Accordingly, we also report non-parametric partial correlations adjusted for age and circulatory hs-CRP concentrations, both separately and together. An r value of <0.3, 0.3–0.5, 0.6–0.8, and >0.8 indicated a poor, fair, moderately strong and very strong associations, respectively (40). Scatterplots of associations between SMI and circulatory markers were plotted with linear regression lines. Where a marker was associated with SMI or ASM% or had a significant effect size for low and normal SMM groups, receiver operating characteristic (ROC) curves were used to investigate the sensitivity and specificity of predicting low SMM as the dichotomous 'state variable'. We report the area under the curve (AUC) with 95% confidence interval (CI) and *p*-values. The AUC value was interpreted as follows: perfect (1.0), excellent (0.9–0.99), good (0.8–0.89), fair (0.7–0.79), poor (0.51–0.69), and no value (0.5) (41). The biomarker concentration cut-off points for prediction of low SMM were selected based on the highest combination of sensitivity and specificity values. We plotted ROC curves and determined biomarker cut-off points for the whole cohort and then separately for men only. Due to a small sample, ROC curves could not be plotted for women only. Statistical significance was set at $p < 0.05$.

3. Results

Sixty-four people were included (63.4 ± 9.8 years; 12.5% female). Participant characteristics, presenting diagnosis, comorbidities, and

TABLE 1 Patient baseline characteristics.

Variable	Mean±standard deviation or frequency (%)		
	All people (n=64)	Low SMM (n=14)	Normal SMM (n=50)
Age (years)	63.4 ± 9.8	67.6 ± 10.6	62.2 ± 9.4
Female	8 (12.5)	5 (35.7)*	3 (6.0)
Body mass index (kg m^{-2})	28.9 ± 3.9	28.9 ± 5.5	28.9 ± 3.3
Body fat content (%)	36.1 ± 6.9	41.9 ± 8.6**	34.5 ± 5.4
Waist/ Hip circumferences ratio[a,b]	0.97 (0.93, 1.02)	0.96 (0.86, 1.0)	0.97 (0.93, 1.0)
Appendicular lean mass (kg)	23.8 ± 4.6	18.9 ± 3.2**	25.2 ± 3.9
Skeletal muscle index (kg m^{-2})	8.7 ± 1.7	6.9 ± 1.2**	9.2 ± 1.4
Appendicular skeletal mass (%)[a]	28.7 (26.2, 30.8)	24.4 (22.0, 25.3)**	29.1 (27.9, 31.0)
$\dot{V}O_{2peak}$ (ml kg^{-1} min^{-1})	23.9 ± 6.0	20.1 ± 5.6*	24.6 ± 5.8
Left ventricular ejection fraction (%)	55.1 ± 7.0	53.9 ± 8.7	55.5 ± 6.5
N-terminal pro-brain natriuretic peptide (NT-proBNP; pg L^{-1})[a,b]	172.0 (64.7, 344.0)	357.0 (112.8, 998.0)*	138.0 (55.8, 273.5)
Presenting diagnosis			
ST-elevation MI (STEMI)	14 (21.9)	3 (21.4)	11 (22.0)
Non-ST-elevation MI (non-STEMI)	21 (32.8)	4 (28.6)	17 (34.0)
Elective percutaneous coronary intervention (PCI)	17 (26.6)	3 (21.4)	14 (28.0)
Coronary artery bypass graft (CABG)	6 (9.4)	2 (14.3)	4 (8.0)
Angina	6 (9.4)	2 (14.3)	4 (8.0)
Comorbidities			
Hypertension	30 (46.9)	8 (57.1)	22 (44.0)
Type 2 diabetes	12 (18.8)	3 (21.4)	9 (18.0)
Chronic obstructive pulmonary disease (COPD)	3 (4.7)	2 (14.3)	1 (2.0)
Hyperlipidaemia	43 (67.2)	9 (64.3)	34 (68.0)
Previous PCI	13 (20.3)	4 (28.5)	9 (18.0)
Previous MI	13 (20.3)	2 (14.2)	11 (22.0)
Previous CABG	5 (7.8)	2 (14.3)	3 (6.0)
Previous cardiac valve surgery	1 (1.6)	0	1 (2.0)
Previous transient ischemic attack	6 (9.4)	3 (21.4)	3 (6.0)
Cancer	10 (15.6)	5 (35.7)*	5 (10.0)
Medications			
Aspirin	62 (96.9)	13 (92.9)	49 (98.0)
Clopidogrel	19 (29.7)	6 (42.9)	13 (26.0)
Ticagrelor	32 (50.0)	4 (28.6)	28 (56.0)
Beta-blockers	57 (89.1)	12 (85.7)	45 (90.0)
Angiotensin converting enzyme (ACE)-inhibitors	38 (59.4)	10 (71.4)	28 (56.0)
Statins	61 (95.3)	14 (100.0)	47 (94.0)
Diuretics	7 (10.9)	3 (21.4)	4 (8.0)
Nitrates (non-GTN)	15 (23.4)	2 (14.3)	13 (26.0)
GTN spray	58 (90.6)	12 (85.7)	46 (92.0)

CABG, coronnary artery bypass graft; GTN, glyceryl trinitrate; MI, myocardial infarction. *p < 0.05 or **p < 0.01 compared to normal SMM group. [a]Values are median (interquartile range). [b]n = 63.

medications, are reported in Table 1. Low ASM% and low SMI were identified in 14.1% (n = 9) and 12.5% (n = 8) of people, respectively. Three people had both low ASM% and low SMI (4.7%) and 14 had either low ASM% or low SMI (21.9%).

Circulatory biomarker concentrations are reported in Table 2. The distribution of biomarker concentrations compared to normal reference values (section 2.4) were as follows: albumin, 92.2% (n = 59) within, 6.3% (n = 4) lower than and 1.6% (n = 1) higher than

TABLE 2 Circulatory biomarker concentrations in people with coronary heart disease with low or normal skeletal muscle mass (SMM).

Biomarker	All					Men					Women				
	Low SMM (n=14)	Normal SMM (n=50)	U	ES	p-Value	Low SMM (n=9)	Normal SMM (n=47)	U	ES	p-Value	Low SMM (n=5)	Normal SMM (n=3)	U	ES	p-Value
Albumin (g/L)	37.50 (36.00, 39.25)	38.50 (37.00, 41.00)	283.00	0.14	0.274	38.00 (36.00, 39.50)	39.00 (37.00, 41.00)	170.00	0.12	0.352	37.00 (36.00, 40.00)	37.00 (36.00, 38.00)	7.00	0.05	0.877
Transthyretin (mg/dl)	29.66 (18.36, 34.08)	37.87 (28.83, 53.63)	183.00	0.34	**0.007****	28.64 (17.51, 34.96)	37.88 (28.87, 54.55)	96.00	0.34	**0.010***	32.07 (22.23, 35.28)	24.34 (24.08, 50.34)	7.00	0.05	0.881
Alanine aminotransferase (U/L)	20.00 (17.00, 24.00)	31.00 (21.75, 41.25)	188.00	0.34	0.**008****	20.00 (19.50, 24.00)	32.00 (22.00, 42.00)	127.50	0.25	0.061	17.00 (14.50, 28.00)	21.00 (19.00, 24.00)	3.00	0.47	0.180
Aspartate aminotransferase (U/L)	22.25 (18.00, 29.13)	27.00 (23.00, 34.75)	221.50	0.26	**0.037***	24.00 (22.25, 31.50)	27.50 (23.00, 35.50)	186.00	0.08	0.569	18.00 (17.25, 20.25)	25.50 (22.50, 27.00)	0.00	0.79	**0.025***
AST/ALT	1.17 (0.93, 1.25)	0.91 (0.69, 1.23)	255.00	0.19	0.123	1.20 (0.92, 1.31)	0.86 (0.68, 1.23)	130.00	0.25	0.069	1.13 (0.76, 1.24)	1.07 (1.06, 1.42)	6.00	0.16	0.655
C-terminal agrin fragment (ng/ml)	3.89 (3.10, 4.24)	3.67 (3.11, 4.48)	335.00	0.03	0.808	4.15 (2.55, 4.53)	3.63 (3.05, 4.36)	206.00	0.02	0.902	3.74 (3.37, 4.02)	4.83 (4.02, 5.25)	1.00	0.69	0.053
Hs C-reactive protein (mg/L)[a]	2.51 (0.42, 4.33)	1.19 (0.50, 3.41)	303.00	0.10	0.445	2.62 (0.58, 4.63)	1.18 (0.48, 3.41)	164.00	0.14	0.289	1.87 (0.32, 4.25)	2.96 (2.17, 8.86)	4.00	0.37	0.297

Bold values indicate statistical significance ($p < 0.05$). *$p < 0.05$ or **$p < 0.01$ compared to normal SMM group. AST/ALT, alanine aminotransferase/aspartate aminotransferase ratio; ES, effect size; U, U statistic from Mann–Whitney U test. Low SMM was skeletal muscle index <7.0 and <6.0 kg m^{-2}, or appendicular skeletal muscle <25.72 and <19.43%, for men and women, respectively. Values are median (range) where $n = 3$. All other values are median (interquartile range).[a]Values from a subset of people included in the present study were been reported elsewhere (4).

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the normal range; transthyretin, 6.3% ($n = 4$) within, 34.4% ($n = 22$) lower than and 59.4% ($n = 38$) higher than the normal range; ALT, 90.6% ($n = 58$) within and 9.4% ($n = 6$) higher than the normal range; AST, 78.1% ($n = 50$) within and 21.9% ($n = 14$) higher than the normal range; and CAF, 78.1% ($n = 50$) within and 21.9% ($n = 14$) higher than the normal range. There were small to moderate effect sizes for lower serum transthyretin (effect size 0.34; 29.66 mg/dl versus 37.87 mg/dl, $p = 0.007$), ALT (effect size 0.34; 20.00 U/L versus 31.00 U/L, $p = 0.008$) and AST (effect size 0.26; 22.25 U/L versus 27.00 U/L, $p = 0.037$) levels in people with low SMM compared to those with normal SMM.

Correlations between circulatory biomarkers, SMI and ASM% are reported in Table 3. Figure 1 shows correlations between SMI and circulatory biomarkers. SMI was associated with hs-CRP -corrected serum ALT levels ($r = 0.261$, $p = 0.039$) and with hs-CRP and age -corrected AST/ALT ratio ($r = -0.257$, $p = 0.044$). In men, after correction for hs-CRP levels and age, SMI was associated with AST ($r = -0.279$, $p = 0.041$) and the AST/ALT ratio ($r = -0.281$, $p = 0.040$). In women, after correction for hs-CRP levels and age, transthyretin was negatively associated with ASM% ($r = -0.889$, $p = 0.018$).

3.1. ROC curve analysis

The prognostic value of transthyretin, ALT, AST, and the AST/ALT ratio for identification of low SMM was assessed using ROC curve analysis. Including all participants, transthyretin (AUC 0.739, 95% CI 0.601, 0.876, $p = 0.007$) and ALT (AUC 0.731, 95% CI 0.576, 0.887, $p = 0.009$) had the greatest predictive capacity to identify low SMM. The AUC for AST level was 0.684 (95% CI 0.516, 0.851, $p = 0.037$) and non-significant for the AST/ALT ratio (AUC 0.636, 95% CI 0.482, 0.790, $p = 0.123$). The optimal cut-off points to indicate risk of low SMM were: a transthyretin value of ≤37.7654 mg/dl (sensitivity 0.857, specificity 0.520), an ALT value of ≤25.00 U/L (sensitivity 0.857, specificity 0.620), and an AST value of ≤24.50 U/L (sensitivity 0.714, specificity 0.620).

Including men only, ROC curve analyses showed the predictive capacity of transthyretin (AUC 0.773, 95% CI 0.603, 0.943, $p = 0.002$), ALT (AUC 0.699, 95% CI 0.509, 0.888, $p = 0.040$) and the AST/ALT ratio (AUC 0.693, 95% CI 0.538, 0.847, $p = 0.014$). The AUC for AST level was non-significant (AUC 0.560, 95% CI 0.357, 0.764, $p = 0.562$). In men, the optimal cut-off points to indicate risk of low SMM where: a transthyretin value of ≤30.3284 mg/dl (sensitivity 0.778, specificity 0.723), an ALT value of ≤25.00 U/L (sensitivity 0.889, specificity 0.660), and an AST/ALT ratio of ≥0.9347 (sensitivity 0.778, specificity 0.553).

4. Discussion

This study aimed to report the association between DXA-estimated SMM and serum albumin, transthyretin, ALT, AST, and CAF in people with CHD. People with low SMM had lower serum transthyretin, AST and ALT levels compared to those with normal SMM, with small to moderate effect sizes. SMI was positively associated with ALT level and negatively associated with the AST/ALT ratio. We found no associations between albumin or CAF levels with any SMM index.

More than one-fifth of people had low SMM. Similarly, others report a prevalence of 25–30% for low SMM in people with CHD (5, 7, 42). Comparatively fewer (12%) apparently healthy, community-dwelling, older adults have low SMM (43). In the current study, presence of comorbidities associated with SMM loss, such as cancer (44) and COPD (45), likely contributed to the higher prevalence of low SMM. Importantly, in a previous CARE CR publication, ASM% was inversely associated with estimated all-cause mortality risk ($r = -0.365$, $p = 0.006$) in people with CHD (4). Thus, interventions to prevent or reverse low SMM should be offered to these people. To support the design and implementation of successful interventions, accurate and readily available methods to assess or monitor changes in SMM are needed.

4.1. Albumin

Albumin is a marker of inflammation-related nutritional risk (20). In agreement with previous studies involving people with liver cirrhosis (46), end-stage renal disease (47) and heart failure (48), we found no association between albumin levels and SMM indices in people with CHD. Interestingly, others report both lower (49–51) and or higher (52) albumin concentrations in older adults with low SMM, compared to those with preserved SMM. The use of albumin levels to infer protein energy malnutrition was previously commonplace in clinical practise (53). Given that lean mass reflects the somatic protein store, the assumption followed that albumin might be useful as a marker of lean mass. However, the use of albumin as a biomarker of malnutrition or body composition has not been without criticism (20, 54). The literature lacks consensus on the existence and/ or direction of the association between albumin and SMM-related variables (46–49, 51, 52), likely due to the role of albumin as an acute-phase response protein.

The inflammation-induced reduction in albumin concentration is underpinned by: decreased albumin synthesis during stress response to prioritise synthesis of essential proteins, increased capillary permeability prompting a shift of albumin from the intravascular to the interstitial space, and a shortened albumin half-life resulting from tissue catabolism (20). In older adults, serum albumin is inversely associated with common inflammatory cytokine, CRP (55). We found no difference in hs-CRP between people with normal or low SMM (Table 2). This could explain the similar albumin levels between groups. Additionally, Chen et al. (56) speculated that sex-specific hormones levels might also impact the association between SMM and albumin levels, after finding these variables to be positively associated in men and negatively associated in women. However, our study included a small sample of women, and we were unable to investigate this hypothesis.

4.2. Transthyretin

Transthyretin levels were significantly lower in people with low versus normal SMM. Similar to albumin, transthyretin is a marker of inflammation-related nutritional risk (20), a key component of malnutrition related to acute or chronic disease (57). Amino acid availability, from dietary protein intake, was proposed to mediate the relationship between transthyretin and lean mass (34). This is because amino acid ingestion promotes lean tissue accretion (58) and also modulates transthyretin synthesis in the liver (59). A

TABLE 3 Correlations between SMI, ASM%, and serum biomarkers.

	All (n=64)		Men (n=56)		Women (n=8)	
	SMI	ASM%	SMI	ASM%	SMI	ASM%
Albumin						
Spearman's corr. (r)	0.229	0.179	0.147	0.104	0.593	0.272
p-value	0.069	0.157	0.279	0.447	0.121	0.515
Partial corr. (r)[a]	0.217	0.141	0.143	0.071	0.738	0.114
p-value	0.087	0.271	0.297	0.607	0.058	0.807
Partial corr. (r)[b]	0.082	0.151	−0.023	0.081	0.765*	0.198
p-value	0.524	0.236	0.868	0.557	**0.045**	0.671
Partial corr. (r)[c]	0.072	0.116	−0.034	0.036	0.763	0.248
p-value	0.579	0.369	0.809	0.795	0.078	0.636
Transthyretin						
Spearman's corr. (r)	0.246	0.132	0.208	0.048	0.048	−0.857**
p-value	**0.050**	0.297	0.124	0.725	0.911	**0.007**
Partial corr. (r)[a]	0.237	0.101	0.204	0.002	0.030	−0.839*
p-value	0.061	0.433	0.135	0.987	0.949	**0.018**
Partial corr. (r)[b]	0.213	0.120	0.142	0.033	0.075	−0.874
p-value	0.094	0.349	0.302	0.808	0.872	**0.010***
Partial corr. (r)[c]	0.206	0.090	0.134	−0.017	0.054	−0.889*
p-value	0.109	0.484	0.335	0.901	0.919	**0.018**
Alanine aminotransferase (ALT)						
Spearman's corr. (r)	0.271*	0.209	0.144	0.048	0.190	0.095
p-value	**0.030**	0.098	0.289	0.726	0.651	0.823
Partial corr. (r)[a]	0.261*	0.175	0.141	0.023	0.200	0.051
p-value	**0.039**	0.170	0.304	0.868	0.668	0.913
Partial corr. (r)[b]	0.158	0.186	−0.032	0.016	0.193	0.106
p-value	0.216	0.144	0.819	0.917	0.678	0.820
Partial corr. (r)[c]	0.150	0.156	−0.040	−0.020	0.277	−0.016
p-value	0.245	0.226	0.776	0.886	0.596	0.977
Aspartate aminotransferase (AST)						
Spearman's corr. (r)	0.038	0.181	−0.169	−0.001	0.238	0.190
p-value	0.766	0.152	0.213	0.993	0.570	0.651
Partial corr. (r)[a]	0.034	0.176	−0.171	−0.013	0.236	0.339
p-value	0.791	0.168	0.212	0.927	0.610	0.457
Partial corr. (r)[b]	−0.017	0.168	−0.275*	−0.018	0.277	0.290
p-value	0.897	0.188	**0.042**	0.895	0.547	0.528
Partial corr. (r)[c]	−0.019	0.165	−0.279*	−0.033	0.279	0.313
p-value	0.825	0.199	**0.041**	0.812	0.592	0.546
AST/ALT ratio						
Spearman's corr. (r)	−0.360**	−0.089	−0.386**	−0.031	−0.048	0.000
p-value	**0.003**	0.484	**0.003**	0.823	0.911	1.00
Partial corr. (r)[a]	−0.351**	−0.043	−0.384**	−0.001	−0.117	0.361
p-value	**0.005**	0.736	**0.004**	0.997	0.803	0.427
Partial corr. (r)[b]	−0.264*	−0.054	−0.285*	−0.002	−0.025	0.135
p-value	**0.036**	0.675	**0.035**	0.986	0.958	0.773

(Continued)

TABLE 3 (Continued)

	All (n=64)		Men (n=56)		Women (n=8)	
	SMI	ASM%	SMI	ASM%	SMI	ASM%
Partial corr. (r)[c]	−0.257*	−0.011	−0.281*	0.037	−0.139	0.393
p-value	**0.044**	0.932	**0.040**	0.792	0.793	0.441
C-terminal agrin fragment						
Spearman's corr. (r)	−0.042	−0.180	−0.008	−0.161	0.429	0.286
p-value	0.741	0.154	0.956	0.235	0.289	0.493
Partial corr. (r)[a]	−0.029	−0.146	−0.002	−0.133	0.429	0.303
p-value	0.823	0.253	0.988	0.334	0.337	0.509
Partial corr. (r)[b]	−0.048	−0.182	−0.015	−0.163	0.436	0.311
p-value	0.706	0.152	0.912	0.234	0.329	0.497
Partial corr. (r)[c]	−0.038	−0.148	−0.008	−0.134	0.476	0.277
p-value	0.770	0.250	0.955	0.333	0.340	0.595

Bold values indicate statistical significance ($p < 0.05$). corr., correlation; ASM%, appendicular skeletal muscle; SMI, skeletal muscle index (kg m^{-2}). Non-parametric partial correlations are corrected for (a) high-sensitivity C-reactive protein, (b) age, and (c) high-sensitivity C-reactive protein and age. *$p < 0.05$; **$p < 0.01$.

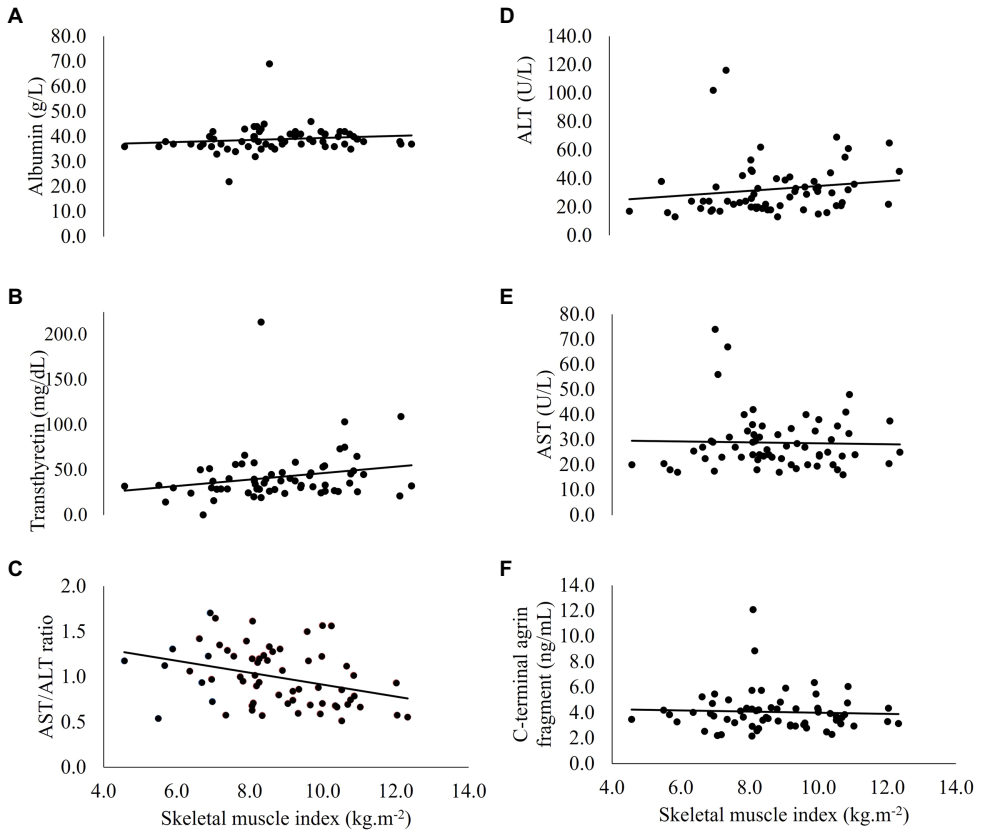

FIGURE 1
Correlations between skeletal muscle index and circulatory **(A)** albumin, **(B)** transthyretin, **(C)** AST/ALT ratio, **(D)** ALT, **(E)** AST, and **(F)** C-terminal agrin fragment, in people with coronary heart disease (n=64). ALT, alanine aminotransferase; AST, aspartate aminotransferase.

strong, positive association (r = 0.58) between transthyretin levels and SMI was previously reported in people at a geriatric outpatient hospital (51). Around 40% of people in the study by Sergi et al. (51) were underweight (BMI <20 kg m^{-2}). Poorer nutritional status likely contributed to a more pronounced inflammatory environment and lean mass loss in this study (51), potentially explaining the strong association between transthyretin and SMI, compared to a non-significant association in the present study (r = 0.246, p = 0.05).

Nevertheless, our detection of significantly lower transthyretin levels in people with low compared to normal SMM is a promising finding, as it become increasingly apparent that transthyretin assessment might have clinical utility as part of a comprehensive medical evaluation (60).

4.3. Aminotransferases

Assessment of liver enzymes ALT and AST is routine in clinical practise (61). As a catalyst in the alanine-glucose cycle, ALT converts pyruvate to amino acid alanine in skeletal muscle and converts alanine back to pyruvate (for glucose production) in the liver (62, 63). A similar cycle is catalysed by AST, where the amino acid and product are aspartate and oxaloacetate, respectively (63). Circulatory levels of ALT and AST are elevated in Type 2 diabetes (24) and metabolic syndrome (25), conditions characterised by insulin resistance and hepatic steatosis. We, and others, demonstrate that ALT levels appear to be lower in the presence of low SMM (26, 50). Contrastingly, in a cross-section of >12,000 adults without liver-related disorders, ALT levels were elevated in those with low SMM compared to normal SMM (64). The direction of the relationship between AST and SMM is similarly contested. We found lower AST concentrations in people with low SMM compared to normal SMM. Others report that low SMM coincided with higher AST concentrations in people with (65, 66) and without (64) liver disease.

Multiple factors likely influence the inconsistency in these findings. First, damaged liver cells release ALT and AST into circulation, explaining their higher serum concentrations in people with liver disorders (67). Secondly, participants with low SMM in the study by Yoo et al. (64) were more often obese with higher fasting blood glucose and insulin levels compared to the normal SMM group, consistent with the theory that aminotransferase levels are elevated in the presence of higher metabolic risk. In the present study, people with reduced SMM had higher average body fat and comparable BMI to people with normal SMM. It could be speculated that differences in intra-abdominal and intra-hepatic steatosis, together with diet quality/alcohol consumption might have influenced aminotransferase concentrations.

Additionally, both ALT and AST require vitamin B_6 as a cofactor, meaning that vitamin B_6 deficiency might contribute to low circulatory ALT and AST (68). Furthermore, vitamin B_6 is mostly stored in striated muscle (69); thus, where lean mass is reduced a smaller pool of vitamin B_6 is available to act as a cofactor for AST and ALT. An estimated 31 and 24% of community-dwelling men and women (≥65 years) are at risk of inadequate vitamin B_6 dietary intake (70). Although not assessed in this study, addressing any dietary deficiencies in people with CHD and low SMM should be prioritised.

4.4. C-terminal agrin fragment

Studies involving older adults (17, 71, 72), people with lung disease (72, 73) and with heart failure (19, 73) have reported an association between high circulatory CAF levels and low SMM. This association is proposed to originate from degeneration of the neuromuscular junction with ageing. Agrin is cleaved by neurotrypsin during normal neural development (15). Excessive agrin cleavage from over-expression of neurotrypsin causes agrin to become deactivated and the neuromuscular junction to break down (16). The product of this breakdown, CAF, is released into the circulation (74). However, the effect of degeneration and remodelling of the neuromuscular junction on SMM loss is debated, with polarising studies arguing that this process contributes to (75) or is protective against (76) muscle atrophy.

We found no association between CAF levels and SMM indices in people with CHD. Others have reported similar non-significant findings when assessing possible associations between CAF and presence of frailty in people with CHD, although an assessment of SMM was not included in their definition of frailty (77). Sánchez-Castellano et al. (78) found no difference in CAF levels between low and normal SMM groups with hip fracture and suggested that elevated CAF levels in both groups indicated neuromuscular degeneration was present in both. In contrast, median CAF values in the low and normal SMM groups were within the normal limits in the present study (0.86–4.66 ng/ml; 17), suggesting that circulatory CAF has limited utility as biomarker for low SMM in this cohort.

4.5. Strengths and limitations

We assessed, in a secondary analysis, multiple proposed biomarkers for low SMM in people with CHD, contributing to our understanding of the factors influencing this complex and under-researched pathology. We included assessment of four biomarkers which are already commonly assessed in clinical practise (albumin, transthyretin, ALT, and AST), aiding the potential transition of our findings into practise.

This study is potentially limited by our use of DXA-derived lean mass to estimate SMM. DXA assessment is the current reference standard, but is limited by the production of variability related to different devices and software versions (79) and the absence of a universally agreed cut-off point for low SMM (80). Furthermore, DXA derived lean mass can be interpreted in several ways (i.e., corrected for stature, body mass or body fat percentage), which often produce conflicting findings when analysed in relation to circulatory biomarkers. This might limit the comparability of our findings with other, similar research. Finally, we included a small sample of women and there was no assessment of muscle strength or function.

4.6. Future research

Future research should evaluate the association between albumin, transthyretin, aminotransferases, CAF and measures of muscular strength alongside SMM. Whether these markers change with targeted lifestyle interventions also requires investigation. Additionally, there appears to be sex differences in median biomarker concentrations and their correlations with SMM indices, although our small sample of women limits the certainty of this finding. Future research might further investigate sex differences in SMM biomarkers in people with CHD.

5. Conclusion

This study aimed to identify associations between SMM indices and circulatory biomarkers in people with CHD. Lower levels of serum transthyretin, AST and ALT were present in people with CHD and low SMM, compared to those with normal SMM. To assist with practical application, we also identified the cut-off points below which transthyretin, ALT and AST indicate high likelihood of low SMM. We found no association between albumin, CAF and SMM indices, suggesting that these markers have limited utility as markers for low SMM in this cohort.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Humber Bridge NHS Research Ethics Committee-Yorkshire and the Humber and Northumbria University Health and Life Sciences Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AO'D, EJ, SN, LI, SG, and SC: conceptualization. SN, AO'D, EJ, and KW: data collection. EJ: analysis and writing original draft. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Vancheri, F, Tate, AR, Henein, M, Backlund, L, Donfrancesco, C, Palmieri, L, et al. Time trends in ischaemic heart disease incidence and mortality over three decades (1990-2019) in 20 Western European countries: systematic analysis of the Global Burden of Disease Study 2019. *Eur J Prev Cardiol*. (2022) 29:396–403. doi: 10.1093/eurjpc/zwab134

2. Wang, DX, Yao, J, Zirek, Y, Reijnierse, EM, and Maier, AB. Muscle mass, strength, and physical performance predicting activities of daily living: a meta-analysis. *J Cachexia Sarcopenia Muscle*. (2020) 11:3–25. doi: 10.1002/jcsm.12502

3. Landi, F, Calvani, R, Picca, A, Tosato, M, Bernabei, R, and Marzetti, E. Emerging research on importance of muscle mass and function. *J Gerontol Geriatr*. (2019) 67:26–31.

4. Nichols, S, O'Doherty, AF, Taylor, C, Clark, AL, Carroll, S, and Ingle, L. Low skeletal muscle mass is associated with low aerobic capacity and increased mortality risk in patients with coronary heart disease–a CARE CR study. *Clin Physiol Funct Imaging*. (2019) 39:93–102. doi: 10.1111/cpf.12539

5. Sato, R, Akiyama, E, Konishi, M, Matsuzawa, Y, Suzuki, H, Kawashima, C, et al. Decreased appendicular skeletal muscle mass is associated with poor outcomes after ST-segment elevation myocardial infarction. *J Atheroscler Thromb*. (2020) 27:1278–87. doi: 10.5551/jat.52282

6. Xue, Q, Wu, J, Ren, Y, Hu, J, Yang, K, and Cao, J. Sarcopenia predicts adverse outcomes in an elderly population with coronary artery disease: a systematic review and meta-analysis. *BMC Geriatr*. (2021) 21:1–10. doi: 10.1186/s12877-021-02438-w

7. Kang, DO, Park, SY, Choi, BG, Na, JO, Choi, CU, Kim, EJ, et al. Prognostic impact of low skeletal muscle mass on major adverse cardiovascular events in coronary artery disease: a propensity score-matched analysis of a single center all-comer cohort. *J Clin Med*. (2019) 8:712. doi: 10.3390/jcm8050712

8. van Venrooij, LMW, Verberne, HJ, de Vos, R, Borgmeijer-Hoelen, MMMJ, van Leeuwen, PAM, and de Mol, BAJM. Postoperative loss of skeletal muscle mass, complications and quality of life in patients undergoing cardiac surgery. *Nutrition*. (2012) 28:40–5. doi: 10.1016/j.nut.2011.02.007

9. Aagaard, P, Suetta, C, Caserotti, P, Magnusson, SP, and Kjær, M. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports*. (2010) 20:49–64. doi: 10.1111/j.1600-0838.2009.01084.x

10. Kwon, YN, and Yoon, SS. Sarcopenia: neurological point of view. *J Bone Metab*. (2017) 24:83–9. doi: 10.11005/jbm.2017.24.2.83

11. Jimenez-Gutierrez, GE, Martínez-Gómez, LE, Martínez-Armenta, C, Pineda, C, Martínez-Nava, GA, and Lopez-Reyes, A. Molecular mechanisms of inflammation in sarcopenia: diagnosis and therapeutic update. *Cells*. (2022) 11:2359. doi: 10.3390/cells11152359

12. Dalle, S, Rossmeislova, L, and Koppo, K. The role of inflammation in age-related sarcopenia. *Front Physiol*. (2017) 8:1045. doi: 10.3389/fphys.2017.01045

13. Abiri, B, and Vafa, M. The role of nutrition in attenuating age-related skeletal muscle atrophy. *Rev New Drug Targets Age-Related Disord*. (2020) 1260:297–318. doi: 10.1007/978-3-030-42667-5_12

14. Zhang, J, Yu, Y, and Wang, J. Protein nutritional support: the classical and potential new mechanisms in the prevention and therapy of sarcopenia. *J Agric Food Chem*. (2020) 68:4098–108. doi: 10.1021/acs.jafc.0c00688

15. Reif, R, Sales, S, Hettwer, S, Dreier, B, Gisler, C, Wölfel, J, et al. Specific cleavage of agrin by neurotrypsin, a synaptic protease linked to mental retardation. *FASEB J*. (2007) 21:3468–78. doi: 10.1096/fj.07-8800com

16. Bolliger, MF, Zurlinden, A, Lüscher, D, Bütikofer, L, Shakhova, O, Francolini, M, et al. Specific proteolytic cleavage of agrin regulates maturation of the neuromuscular junction. *J Cell Sci*. (2010) 123:3944–55. doi: 10.1242/jcs.072090

17. Hettwer, S, Dahinden, P, Kucsera, S, Farina, C, Ahmed, S, Fariello, R, et al. Elevated levels of a C-terminal agrin fragment identifies a new subset of sarcopenia patients. *Exp Gerontol*. (2013) 48:69–75. doi: 10.1016/j.exger.2012.03.002

18. Landi, F, Calvani, R, Lorenzi, M, Martone, AM, Tosato, M, Drey, M, et al. Serum levels of C-terminal agrin fragment (CAF) are associated with sarcopenia in older

multimorbid community-dwellers: results from the ilSIRENTE study. *Exp Gerontol*. (2016) 79:31–6. doi: 10.1016/j.exger.2016.03.012

19. Steinbeck, L, Ebner, N, Valentova, M, Bekfani, T, Elsner, S, Dahinden, P, et al. Detection of muscle wasting in patients with chronic heart failure using C-terminal agrin fragment: results from the Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF). *Eur J Heart Fail*. (2015) 17:1283–93. doi: 10.1002/ejhf.400

20. Evans, DC, Corkins, MR, Malone, A, Miller, S, Mogensen, KM, Guenter, P, et al. The use of visceral proteins as nutrition markers: an ASPEN position paper. *Nutr Clin Pract*. (2021) 36:22–8. doi: 10.1002/ncp.10588

21. Zhang, N, Zhu, WL, Liu, XH, Chen, W, Zhu, ML, Sun, XH, et al. Related factors of sarcopenia in hospitalized elderly patients with coronary heart disease. *Zhonghua Xin Xue Guan Bing Za Zhi*. (2019) 47:979–84. doi: 10.3760/cma.j.issn.0253-3758.2019.12.007

22. Cruz-Jentoft, AJ, Bahat, G, Bauer, J, Boirie, Y, Bruyère, O, Cederholm, T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. (2019) 48:16–31. doi: 10.1093/ageing/afy169

23. Kalas, MA, Chavez, L, Leon, M, Taweesedt, PT, and Surani, S. Abnormal liver enzymes: a review for clinicians. *World J Hepatol*. (2021) 13:1688–98. doi: 10.4254/wjh.v13.i11.1688

24. Ko, S-H, Baeg, MK, Han, K-D, Ko, S-H, and Ahn, Y-B. Increased liver markers are associated with higher risk of type 2 diabetes. *World J Gastroenterol*. (2015) 21:7478–87. doi: 10.3748/wjg.v21.i24.7478

25. Hanley, AJG, Williams, K, Festa, A, Wagenknecht, LE, D'Agostino, RB Jr, and Haffner, SM. Liver markers and development of the metabolic syndrome: the insulin resistance atherosclerosis study. *Diabetes*. (2005) 54:3140–7. doi: 10.2337/diabetes.54.11.3140

26. Vespasiani-Gentilucci, U, De Vincentis, A, Ferrucci, L, Bandinelli, S, Antonelli Incalzi, R, and Picardi, A. Low alanine aminotransferase levels in the elderly population: frailty, disability, sarcopenia, and reduced survival. *J Gerontol A Biol Sci Med Sci*. (2018) 73:925–30. doi: 10.1093/gerona/glx126

27. He, Y, Ding, F, Yin, M, Zhang, H, Hou, L, Cui, T, et al. High serum AST/ALT ratio and low serum INS*PA product are risk factors and can diagnose sarcopenia in middle-aged and older adults. *Front Endocrinol*. (2022) 13:843610. doi: 10.3389/fendo.2022.843610

28. Yin, M, Zhang, H, Liu, Q, Ding, F, Deng, Y, Hou, L, et al. Diagnostic performance of clinical laboratory indicators with sarcopenia: results from the west China health and aging trend study. *Front Endocrinol*. (2021) 12:785045. doi: 10.3389/fendo.2021.785045

29. Nichols, S, Nation, F, Goodman, T, Clark, AL, Carroll, S, and Ingle, L. CARE CR-Cardiovascular and cardiorespiratory Adaptations to Routine Exercise-based Cardiac Rehabilitation: a study protocol for a community-based controlled study with criterion methods. *BMJ Open*. (2018) 8:e019216. doi: 10.1136/bmjopen-2017-019216

30. Nichols, S, Taylor, C, Goodman, T, Page, R, Kallvikbacka-Bennett, A, Nation, F, et al. Routine exercise-based cardiac rehabilitation does not increase aerobic fitness: a CARE CR study. *Int J Cardiol*. (2020) 305:25–34. doi: 10.1016/j.ijcard.2020.01.044

31. Levine, ME, and Crimmins, EM. The impact of insulin resistance and inflammation on the association between sarcopenic obesity and physical functioning. *Obesity*. (2012) 20:2101–6. doi: 10.1038/oby.2012.20

32. Bruce, RA, Kusumi, F, and Hosmer, D. Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease. *Am Heart J*. (1973) 85:546–62. doi: 10.1016/0002-8703(73)90502-4

33. Busher, J. Serum albumin and globulin In: HK Walker, WD Hall and JW Hurst, editors. *Clinical Methods: The History, Physical, and Laboratory Examinations*. *3rd* ed. Boston: Butterworths (1990)

34. Ingenbleek, Y, and Bernstein, LH. Plasma transthyretin as a biomarker of lean body mass and catabolic states. *Adv Nutr*. (2015) 6:572–80. doi: 10.3945/an.115.008508

35. Ceriotti, F, Henny, J, Queraltó, J, Ziyu, S, Özarda, Y, Chen, B, et al. Common reference intervals for aspartate aminotransferase (AST), alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) in serum: results from an IFCC multicenter study. *Clin Chem Lab Med*. (2010) 48:1593–601. doi: 10.1515/CCLM.2010.315

36. Tomczak, M, and Tomczak, E. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. *Trends Sport Sci*. (2014) 21:19–25.

37. Coolican, H. *Research Methods and Statistics in Psychology*. London, UK: Psychology Press (2017).

38. Nichols, S, O'Doherty, A, Carroll, S, and Ingle, L. Influence of appendicular skeletal muscle mass on resting metabolic equivalents in patients with cardiovascular disease: Implications for exercise training and prescription. *Eur J Prev Cardiol*. (2020) 27:1001–3. doi: 10.1177/2047487319856432

39. Epstein, FH. Acute-phase proteins and other systemic responses to inflammation. *Complement*. (1999) 3:C9.

40. Chan, Y. Biostatistics 104: correlational analysis. *Singap Med J*. (2003) 44:614–9.

41. Carter, JV, Pan, J, Rai, SN, and Galandiuk, S. ROC-ing along: Evaluation and interpretation of receiver operating characteristic curves. *Surgery*. (2016) 159:1638–45. doi: 10.1016/j.surg.2015.12.029

42. Harada, H, Kai, H, Niiyama, H, Nishiyama, Y, Katoh, A, Yoshida, N, et al. Effectiveness of cardiac rehabilitation for prevention and treatment of sarcopenia in patients with cardiovascular disease-a retrospective cross-sectional analysis. *J Nutr Health Aging*. (2017) 21:449–56. doi: 10.1007/s12603-016-0743-9

43. Peng, LN, Lee, WJ, Liu, LK, Lin, MH, and Chen, LK. Healthy community-living older men differ from women in associations between myostatin levels and skeletal muscle mass. *J Cachexia Sarcopenia Muscle*. (2018) 9:635–42. doi: 10.1002/jcsm.12302

44. Rausch, V, Sala, V, Penna, F, Porporato, PE, and Ghigo, A. Understanding the common mechanisms of heart and skeletal muscle wasting in cancer cachexia. *Oncogenesis*. (2021) 10:1. doi: 10.1038/s41389-020-00288-6

45. Barreiro, E, and Jaitovich, A. Muscle atrophy in chronic obstructive pulmonary disease: molecular basis and potential therapeutic targets. *J Thorac Dis*. (2018) 10:S1415–24. doi: 10.21037/jtd.2018.04.168

46. Zhao, M, Zhou, X, Yuan, C, Li, R, Ma, Y, and Tang, X. Association between serum irisin concentrations and sarcopenia in patients with liver cirrhosis: a cross-sectional study. *Sci Rep*. (2020) 10:16093. doi: 10.1038/s41598-020-73176-z

47. Dong, J, Li Yj, LX, Hp, G, Zuo, L, and Hy, W. Correlations of lean body mass with nutritional indicators and mortality in patients on peritoneal dialysis. *Kidney Int*. (2008) 73:334–40. doi: 10.1038/sj.ki.5002644

48. Prenner, SB, Pillutla, R, Yenigalla, S, Gaddam, S, Lee, J, Obeid, MJ, et al. Serum albumin is a marker of myocardial fibrosis, adverse pulsatile aortic hemodynamics, and prognosis in heart failure with preserved ejection fraction. *J Am Heart Assoc*. (2020) 9:e014716. doi: 10.1161/JAHA.119.014716

49. Baumgartner, RN, Koehler, KM, Romero, L, and Garry, PJ. Serum albumin is associated with skeletal muscle in elderly men and women. *Am J Clin Nutr*. (1996) 64:552–8. doi: 10.1093/ajcn/64.4.552

50. Portal, D, Hofstetter, L, Eshed, I, Dan-Lantsman, C, Sella, T, Urban, D, et al. L3 skeletal muscle index (L3SMI) is a surrogate marker of sarcopenia and frailty in non-small cell lung cancer patients. *Cancer Manag Res*. (2019) 11:2579–88. doi: 10.2147/CMAR.S195869

51. Sergi, G, Coin, A, Enzi, G, Volpato, S, Inelmen, EM, Buttarello, M, et al. Role of visceral proteins in detecting malnutrition in the elderly. *Eur J Clin Nutr*. (2006) 60:203–9. doi: 10.1038/sj.ejcn.1602289

52. Tachi, Y, Kozuka, A, Hirai, T, Ishizu, Y, Honda, T, Kuzuya, T, et al. Impact of myosteatosis on skeletal muscle volume loss in patients with chronic liver disease. *J Gastroenterol Hepatol*. (2018) 33:1659–66. doi: 10.1111/jgh.14133

53. Spiekeman, AM. Nutritional assessment (protein nutriture). *Anal Chem*. (1995) 67:429–36. doi: 10.1021/ac00108a026

54. Bouillanne, O, Hay, P, Liabaud, B, Duché, C, Cynober, L, and Aussel, C. Evidence that albumin is not a suitable marker of body composition-related nutritional status in elderly patients. *Nutrition*. (2011) 27:165–9. doi: 10.1016/j.nut.2009.12.007

55. Sullivan, DH, Johnson, LE, Dennis, RA, Roberson, PK, Heif, M, Garner, KK, et al. The Interrelationships among albumin, nutrient intake, and inflammation in elderly recuperative care patients. *J Nutr Health Aging*. (2011) 15:311–5. doi: 10.1007/s12603-010-0297-1

56. Chen, Z, Song, C, Yao, Z, et al. Associations between albumin, globulin, albumin to globulin ratio and muscle mass in adults: results from the national health and nutrition examination survey 2011–2014. *BMC Geriatr*. (2022) 22:383. doi: 10.1186/s12877-022-03094-4

57. White, JV, Guenter, P, Jensen, G, Malone, A, and Schofield, M. Consensus Statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: characteristics recommended for the identification and documentation of adult malnutrition (undernutrition). *J Acad Nutr Diet*. (2012) 112:730–8. doi: 10.1016/j.jand.2012.03.012

58. Pennings, B, Groen, B, de Lange, A, Gijsen, AP, Zorenc, AH, Senden, JMG, et al. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. *Am J Physiol Endocrinol Metab*. (2012) 302:E992–9. doi: 10.1152/ajpendo.00517.2011

59. de Jong, FA, and Schreiber, G. Messenger RNA levels of plasma proteins in rat liver during protein depletion and refeeding. *J Nutr*. (1987) 117:1795–800. doi: 10.1093/jn/117.10.1795

60. Ranasinghe, RN, Biswas, M, and Vincent, RP. Prealbumin: the clinical utility and analytical methodologies. *Ann Clin Biochem*. (2022) 59:7–14. doi: 10.1177/0004563220931885

61. Kwo, PY, Cohen, SM, and Lim, JK. ACG clinical guideline: evaluation of abnormal liver chemistries. *Am J Gastroenterol*. (2017) 112:18–35. doi: 10.1038/ajg.2016.517

62. Felig, P. The glucose-alanine cycle. *Metabolism*. (1973) 22:179–207. doi: 10.1016/0026-0495(73)90269-2

63. McGill, MR. The past and present of serum aminotransferases and the future of liver injury biomarkers. *EXCLI J*. (2016) 15:817–28. doi: 10.17179/excli2016-800

64. Yoo, KD, Jun, DW, Lee, KN, Lee, HL, Lee, OY, Yoon, BC, et al. Sarcopenia is a risk factor for elevated aminotransferase in men independently of body mass index, dietary habits, and physical activity. *Dig Liver Dis*. (2015) 47:303–8. doi: 10.1016/j.dld.2014.12.014

65. Mizuno, N, Seko, Y, Kataoka, S, Okuda, K, Furuta, M, Takemura, M, et al. Increase in the skeletal muscle mass to body fat mass ratio predicts the decline in transaminase

in patients with nonalcoholic fatty liver disease. *J Gastroenterol*. (2019) 54:160–70. doi: 10.1007/s00535-018-1485-8

66. Takata, R, Nishikawa, H, Enomoto, H, Iwata, Y, Ishii, A, Miyamoto, Y, et al. Relationship between skeletal muscle mass and liver fibrosis markers for patients with hepatitis C virus related liver disease. *Medicine*. (2017) 96:e8761. doi: 10.1097/MD.0000000000008761

67. Ramaiah, SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food Chem Toxicol*. (2007) 45:1551–7. doi: 10.1016/j.fct.2007.06.007

68. Vanderlinde, RE. Review of pyridoxal phosphate and the transaminases in liver disease. *Ann Clin Lab Sci*. (1986) 16:79–93.

69. van den Berg, H. Vitamin B6 status and requirements in older adults. *Br J Nutr*. (1999) 81:175–6. doi: 10.1017/S0007114599000355

70. ter Borg, S, Verlaan, S, Hemsworth, J, Mijnarends, DM, Schols, JM, Luiking, YC, et al. Micronutrient intakes and potential inadequacies of community-dwelling older adults: a systematic review. *Br J Nutr*. (2015) 113:1195–206. doi: 10.1017/S0007114515000203

71. Drey, M, Sieber, C, Bauer, J, Uter, W, Dahinden, P, Fariello, R, et al. C-terminal agrin fragment as a potential marker for sarcopenia caused by degeneration of the neuromuscular junction. *Exp Gerontol*. (2013) 48:76–80. doi: 10.1016/j.exger.2012.05.021

72. Qaisar, R, Karim, A, Muhammad, T, and Shah, I. Circulating biomarkers of accelerated sarcopenia in respiratory diseases. *Biology*. (2020) 9:322. doi: 10.3390/biology9100322

73. Qaisar, R, Karim, A, Muhammad, T, Shah, I, and Khan, J. Prediction of sarcopenia using a battery of circulating biomarkers. *Sci Rep*. (2021) 11:8632. doi: 10.1038/s41598-021-87974-6

74. Stephan, A, Mateos, JM, Kozlov, SV, Cinelli, P, Kistler, AD, Hettwer, S, et al. Neurotrypsin cleaves agrin locally at the synapse. *FASEB J*. (2008) 22:1861–73. doi: 10.1096/fj.07-100008

75. Rowan, SL, Rygiel, K, Purves-Smith, FM, Solbak, NM, Turnbull, DM, and Hepple, RT. Denervation causes fiber atrophy and myosin heavy chain co-expression in senescent skeletal muscle. *PLoS One*. (2012) 7:e29082. doi: 10.1371/journal.pone.0029082

76. Willadt, S, Nash, M, and Slater, CR. Age-related fragmentation of the motor endplate is not associated with impaired neuromuscular transmission in the mouse diaphragm. *Sci Rep*. (2016) 6:1–8. doi: 10.1038/srep24849

77. de Souza Ramos, JTG, Ferrari, FS, Andrade, MF, de Melo, CS, Boas, PJFV, Costa, NA, et al. Association between frailty and C-terminal agrin fragment with 3-month mortality following ST-elevation myocardial infarction. *Exp Gerontol*. (2022) 158:111658. doi: 10.1016/j.exger.2021.111658

78. Sánchez-Castellano, C, Martín-Aragón, S, Bermejo-Bescós, P, Vaquero-Pinto, N, Miret-Corchado, C, Merello de Miguel, A, et al. Biomarkers of sarcopenia in very old patients with hip fracture. *J Cachexia Sarcopenia Muscle*. (2020) 11:478–86. doi: 10.1002/jcsm.12508

79. Buckinx, F, Landi, F, Cesari, M, Fielding, RA, Visser, M, Engelke, K, et al. Pitfalls in the measurement of muscle mass: a need for a reference standard. *J Cachexia Sarcopenia Muscle*. (2018) 9:269–78. doi: 10.1002/jcsm.12268

80. Lee, K, Shin, Y, Huh, J, Sung, YS, Lee, I-S, Yoon, K-H, et al. Recent issues on body composition imaging for sarcopenia evaluation. *Korean J Radiol*. (2018) 20:205–17. doi: 10.3348/kjr.2018.0479

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Identification of novel pathways and immune profiles related to sarcopenia

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Introduction: Sarcopenia is a progressive deterioration of skeletal muscle mass strength and function.

Methods: To uncover the underlying cellular and biological mechanisms, we studied the association between sarcopenia's three stages and the patient's ethnicity, identified a gene regulatory network based on motif enrichment in the upregulated gene set of sarcopenia, and compared the immunological landscape among sarcopenia stages.

Results: We found that sarcopenia (S) was associated with GnRH, neurotrophin, Rap1, Ras, and p53 signaling pathways. Low muscle mass (LMM) patients showed activated pathways of VEGF signaling, B-cell receptor signaling, ErbB signaling, and T-cell receptor signaling. Low muscle mass and physical performance (LMM_LP) patients showed lower enrichment scores in B-cell receptor signaling, apoptosis, HIF-1 signaling, and the adaptive immune response pathways. Five common genes among DEGs and the elastic net regression model, *TTC39DP*, *SLURP1*, *LCE1C*, *PTCD2P1*, and *OR7E109P*, were expressed between S patients and healthy controls. *SLURP1* and *LCE1C* showed the highest expression levels among sarcopenic Chinese descent than Caucasians and Afro-Caribbeans. Gene regulatory analysis of top upregulated genes in S patients yielded a top-scoring regulon containing GATA1, GATA2, and GATA3 as master regulators and nine predicted direct target genes. Two genes were associated with locomotion: *POSTN* and *SLURP1*. *TTC39DP* upregulation was associated with a better prognosis and stronger immune profile in S patients. The upregulation of *SLURP1* and *LCE1C* was associated with a worse prognosis and weaker immune profile.

Conclusion: This study provides new insight into sarcopenia's cellular and immunological prospects and evaluates the age and sarcopenia-related modifications of skeletal muscle.

KEYWORDS

sarcopenia, low muscle mass, low physical performance, immunology, pathway analysis, bioinformatics, machine learning

1. Introduction

Sarcopenia is a syndrome characterized by a progressive deterioration of the skeletal muscle mass strength and function with a risk of developing adverse outcomes, such as motor disability and death (1). The study of sarcopenia and the development of preventive or therapeutic approaches represent public health priorities. The European Working Group on Sarcopenia in Older People (EWGSOP) recommends categorizing sarcopenia into three groups, namely pre-sarcopenia, sarcopenia, and severe sarcopenia (1). They proposed that the pre-sarcopenia stage is characterized by low muscle mass with no impact on muscle strength or physical performance, whereas the sarcopenia stage is characterized by low muscle mass with low muscle strength or low physical performance, and severe sarcopenia is characterized by the presence of all three criteria (1). Low physical performance is defined as a gait speed of <0.8 m/s, while low muscle strength is defined by handgrip strength of <30 kg for men and <20 kg for women (1). Low muscle mass is measured by a skeletal muscle mass index from 7.23 to 8.87 kg/m^2 in men and from 5.45 to 6.42 kg/m^2 in women (2). The pathophysiology of sarcopenia stages is still complex and partially characterized. There is an inadequate understanding of the underlying cellular and biological mechanisms driving the development of this disease. Till today, the current understanding of the regulation of muscle mass is mainly derived from animal studies data, with limited knowledge of the key regulatory processes in human muscle or blood. Animal studies suggested that the PI3k-Akt pathway is strongly linked to muscle synthesis (3). Most studies on sarcopenia in humans have suggested that a reduced synthetic response primarily drives the loss of muscle mass termed as anabolic resistance (4, 5).

The decline in immune function with age, known as immunosenescence, has been well-documented (6, 7). Immunosenescence includes inflammaging, which is the presence of a chronic proinflammatory state with age (8). Inflammaging is characterized by increased levels of proinflammatory cytokines, such as interleukin 1β (IL1β), interleukin 6 (IL6), tissue necrosis factor-alpha (TNFα), C-reactive protein (CRP), and a reduced level of anti-inflammatory cytokines, such as interleukin 10 (IL10) (8). Investigation of the immune system's role in different sarcopenia stages shows that the immune system's dysregulation may play a role in disease progression (8).

The current knowledge on the available sarcopenia biomarkers, such as functional, biological, or imaging-related biomarkers, that could be utilized in clinical trials remains inadequate. This study aimed to uncover the underlying cellular and biological mechanisms driving the development of sarcopenia and identify novel pathways and biomarkers that differentiate between different sarcopenia stages using unsupervised and supervised machine learning methods. Then, we studied the association of sarcopenia's three stages with the patient's ethnicity. In addition, we identified gene regulatory network mapping based on motif enrichment in the upregulated gene set of sarcopenia and identified the gene modules of coexpressed genes among the three sarcopenia stages. Finally, we compared the immunological landscape among sarcopenia stages based on their gene expression profiles. This study aimed to provide new insight into sarcopenia's cellular and immunological prospects, which is considered reliable and promising to evaluate the age and sarcopenia-related modifications of skeletal muscle.

2. Methods

2.1. Datasets

We downloaded the RNA-Seq gene expression in human muscle biopsies of 119 older adults (GSE111017) from the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/). This dataset includes three individual datasets: Hertfordshire Sarcopenia, Jamaica Sarcopenia, and Singapore Sarcopenia. Hertfordshire's Caucasian dataset includes four sarcopenia patients with low muscle strength and physical performance, five with low muscle mass strength, three with low physical performance, and 28 healthy individuals (aged 68–77 years). The inclusion criteria of the Hertfordshire Caucasian dataset were the availability of birth records detailing birth weight, and men were excluded if they had heart disease, myositis, neuromuscular conditions, or diabetes. The diagnosis of sarcopenia among Caucasians was based on the EWGSOP algorithm (1, 9). Jamaica's Afro-Caribbean dataset includes nine sarcopenia patients with low muscle strength and physical performance, five with low muscle mass strength, 11 with low physical performance, and 14 healthy individuals, and all participants were of at least three grandparents of African origin (aged 63–89 years) (9). The diagnosis of sarcopenia among Afro-Caribbeans was also based on the EWGSOP definition (1). Singapore's Chinese dataset includes 20 sarcopenia patients and 20 healthy individuals without information on low muscle mass strength and the physical performance of older adults (aged 65–79 years). Each Chinese participant gave written self-reported ethnicity, and the diagnosis of sarcopenia was based on the AWGSOP definition (10). Lean mass was measured using DXA scanning (9). We merged the three datasets using the "merge" function in the R package "base", performed batch effects adjustment, and normalized the combined data for further analyses.

2.2. Quantification of the enrichment levels of immune signatures

We used the single-sample gene-set enrichment analysis (ssGSEA) score (11) to evaluate the enrichment level of an immune signature in older adults based on the expression profiles of its marker genes. The ssGSEA score represents the enrichment score of a gene set in a sample based on the degree to which the gene set is coordinately upregulated/downregulated in the sample. We analyzed 30 immune signatures, including anti-inflammatory cytokines, B cells, CD4$^+$ Regulatory T cells, CD8$^+$ T cells, central memory T cells, cytolytic activity, cytolytic protein perforin, dendritic cells, effector memory T cell, effector T cell, effector Treg T cells, exhausted T cell, granulocyte, IGF1, immune-modulatory molecules, macrophages, MHC Class I, myokines, naive T cell, NK antimicrobial protein granulysin, NK encoded activating receptors, NK encoded inhibitory receptors, proinflammatory cytokines, resident memory T cell, resting Treg T cells, senescence-associated

secretory phenotype (SASP), *Th1* cells, *Th2* cells, Type I IFN response, and Type II IFN response. The gene sets representing these immune signatures are listed in Supplementary material S1.

2.3. Pathway analysis

We identified differentially expressed genes (DEGs) between sarcopenia (S) and non-sarcopenia, low muscle mass (LMM)/non, and low physical performance (LMM_LP)/non using Student's *t*-test with a threshold of adjusted *P*-value [false discovery rate (FDR)] of <0.05 and fold change (FC) of mean expression levels of >1.3. Based on the DEGs, we identified KEGG (12) and GO (13) pathways differentially enriched between the identified groups using the "pathfindR" R package (14), with a threshold of FDR $<$ 0.05. The FDR was calculated by using the Bonferroni method (15).

2.4. Class prediction

We predicted sarcopenia (S) patients vs. non-sarcopenia control individuals based on the combined gene expression profiles. We performed 3-fold cross-validation (CV) in 33 sarcopenia patients and 86 control individuals. We trained the elastic net regression model classifier and predicted sarcopenia patients vs. non-sarcopenia control individuals. We used the CV to select the best λ with a fixed α parameter of 0.5. We reported the prediction performance (accuracy, specificity, balanced accuracy, and the Kappa statistic) in the 3-fold CV. We carried out the class prediction algorithm in the "caret" R package (16).

2.5. Gene regulatory and weighted correlation networks

We used iRegulon software (17) to directly enable gene regulatory network mapping based on motif enrichment in the upregulated gene set of S patients. The library of motifs used was 10K [9,713 position weight matrices (PWMs)], track collection of 1,120 ChiP-seq tracks, the putative regulatory region of 20 kb centered around transcription start site (TSS), motif ranking database 20 kb centered around TSS (seven species), and track ranking database of 20 kb centered around TSS (ChiP-seq-derived). In addition, we used an enrichment score threshold of 3.0, a ROC threshold for AUC calculation of 0.03, and a rank threshold of 5,000. For transcription factor (TF) prediction, the maximum FDR on the motif similarity threshold was 0.001. The weighted gene coexpression network analysis (WGCNA) was used to identify the gene modules of coexpressed genes among the three sarcopenia stages (LMM, LMM_LP, and S). Based on the expression correlations between the hub genes in gene modules, we identified the enriched GO pathways that have significant correlations with specific traits. We analyzed the WGCNA using the R package "WGCNA" (18).

2.6. Statistical analysis

We used Student's *t*-tests (two-tailed) to compare two classes of samples. In comparisons among three classes of samples, we used ANOVA tests (two-tailed). In evaluating correlations between gene expression levels and immune signatures' enrichment levels and between expression correlations between two genes, we used Pearson's correlation coefficients (*R*). We employed FDR to adjust *P*-values in multiple tests. The FDR was calculated using the Benjamini–Hochberg methods. We used abbreviations of S, LMM, and LMM_LP to represent sarcopenia, low muscle mass, and low muscle mass with low physical performance but without sarcopenia, respectively.

3. Results

3.1. Comparison of blood transcriptomes between sarcopenia, low muscle strength, and low physical performance patients with healthy individuals

We first identified the transcriptional signature of S patients compared to healthy individuals. This analysis revealed significant expression differences in sarcopenia patients in 459 unique protein-coding transcripts (419 upregulated and 40 downregulated genes, Supplementary material S1). In LMM patients, we found 74 unique protein-coding transcripts (60 upregulated and 14 downregulated genes, Supplementary material S1). In LMM_LP patients, we found 27 unique protein-coding transcripts (20 upregulated and seven downregulated genes, Supplementary material S1). There were five common genes among the three sets of DEGs, including *AC092184.1* (uncategorized gene); *FAM239B* (family with sequence similarity 239 member B pseudogene); *HSFX2* (heat shock transcription factor family, X-linked 2); *PTCD2P1* (pentatricopeptide repeat domain 2 pseudogene 1); and *SLURP1* (secreted LY6/PLAUR domain containing 1).

We analyzed pathway enrichment on significant genes to identify activated or repressed pathways in a given sample or group. We calculated the agglomerated z-score of each enriched pathway per sample, followed by unsupervised hierarchical clustering of the most significant KEGG and GO pathways between the pre-defined groups. We found that sarcopenia was associated with increased expression of genes related to the GnRH, neurotrophin, Rap1, Ras, and p53 signaling pathways (Figure 1A). In addition, we found that sarcopenia patients showed significant enrichment in RNA polymerase II-specific DNA-binding transcription factor binding and negative regulation of cyclin-dependent protein serine/threonine kinase activity pathways (Figure 1B). Furthermore, the significantly activated pathways in LMM patients included the VEGF, B-cell receptor, ErbB, and T-cell receptor signaling pathways (Figures 1C, D). Interestingly, LMM_LP patients showed lower enrichment scores in the B-cell receptor signaling, apoptosis, HIF-1 signaling, and the adaptive immune response pathways than LMM patients (Figures 1E, F). These results indicate that immune responses and biological and metabolic dysregulation occurred simultaneously with the patient's muscle mass and function deterioration.

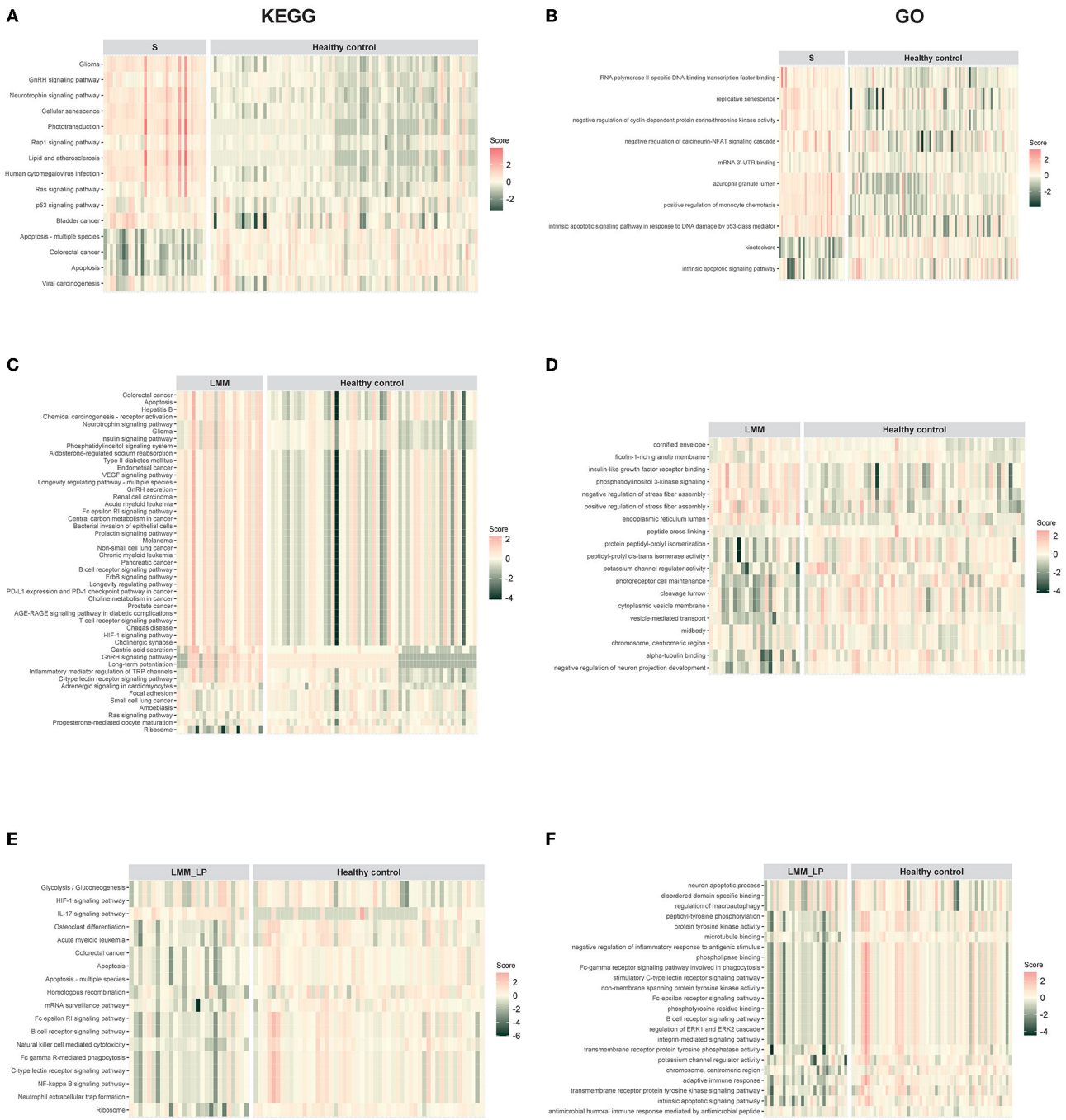

FIGURE 1
Pathway enrichment analysis for sarcopenia (S), low muscle mass (LMM), and low muscle mass with physical performance (LMM_LP) by unsupervised hierarchical clustering. **(A)** KEGG and **(B)** GO pathways enrichment analysis for sarcopenia (S) vs. healthy controls. **(C)** KEGG and **(D)** GO pathways enrichment analysis for low muscle mass (LMM) vs. healthy controls. **(E)** KEGG and **(F)** GO pathways enrichment analysis for low muscle mass with physical performance (LMM_LP) vs. healthy controls. The enrichment agglomerated z-score was calculated for each enriched term per sample.

3.2. Application of elastic net regression model with feature selection to discriminate sarcopenia from healthy states

To identify the fewest transcripts as diagnostic biomarkers for S patients, we used the elastic net regression to derive a discriminating model in the transcriptomic data from S patients and healthy controls. We trained this model on multiple random samples of the transcriptomic data using CV and ranked all the genes based on their regression coefficients (Figure 2A). Among deferentially expressed genes (DEGs) and the elastic net regression model, we found five common genes (*TTC39DP*, *SLURP1*, *LCE1C*, *PTCD2P1*, and *OR7E109P*) that discriminated between sarcopenia patients and healthy controls with a different range (Figure 2A).

The relative expressions of these genes were generally upregulated or downregulated in sarcopenia patients than in healthy controls (Figure 2B). To alleviate sampling error, we performed 3-fold CV train/test sequences to obtain an average accuracy of 0.97, 95% CI of 0.8871 and 0.9995, kappa of 0.95, a sensitivity of 0.97, a specificity of 1.00, and a balanced accuracy of 0.98. Among these top-ranked genes, we found that *SLURP1* and *LCE1C* were upregulated in S patients, while *TTC39DP*, *OR7E109P*, and *PTCD2P1* were downregulated in them (Figure 2B). *TTC39DP* (tetratricopeptide repeat domain 39D, pseudogene) was the highest ranked among the five genes and discriminated between S patients and healthy controls with a regression coefficient of 95.99. *SLURP1* and *LCE1C* discriminated between S patients and healthy controls with regression coefficients of 62.45 and 34.54, respectively (Figure 2A).

We further analyzed the expression differences of *TTC39DP*, *SLURP1*, and *LCE1C* in S, LMM, and LMM_LP among Caucasian, Afro-Caribbean, and Chinese descents (Figure 2C). *SLURP1* and *LCE1C* showed the highest expression levels in sarcopenic Chinese descent. In addition, *SLURP1* expression levels were higher in the S group of Caucasian descent than in the LMM and LMM_LP groups. *SLURP1* expression levels were higher in the LMM_LP group of Afro-Caribbean descent than in the S and LMM groups. *TTC39DP* showed the maximum expression levels among the LMM_LP group of Caucasian and Afro-Caribbean descent. These results indicate that different expression levels of *TTC39DP*, *SLURP1*, and *LCE1C* among the pre-defined groups depend on the patient's ethnicity.

3.3. Gene regulatory and weighted correlation networks of sarcopenia, low muscle mass, and low physical performance patients

We analyzed 96 significantly upregulated genes under S status using iRegulon software. The analysis yields a top-scoring regulon containing GATA1, GATA2, and GATA3 as master regulators and nine predicted direct target genes, namely *EHF*, *SLURP1*, *MYEOV*, *SERPINB2*, *SERPINA12*, *ARHGAP40*, *PCDH15*, *SULT2B1*, and *POSTN* (Figure 3A). The predicted GATA1, GATA2, and GATA3 targets are likely functional targets associated with the different pathways, including signaling, response to stimulus, regulation of the biological process, multicellular organismal process, metabolic process, locomotion, localization, growth, developmental process, cellular process, biological regulation, and biological adhesion behavior (Figure 3B). Interestingly, two genes were associated with locomotion: *POSTN* and *SLURP1*. *POSTN* has been associated with metastatic outgrowth in melanoma (19), while the association of *SLURP1* with locomotion remains limited.

Furthermore, we conducted further analysis to investigate biomarkers of different sarcopenia stages, according to different pathophysiologic mechanisms. These mechanisms include the neuromuscular junction, endocrine system, growth factor, muscle protein turnover, behavior-mediated pathways, inflammation-mediated pathways, and redox-related factors (20). However, due to the small sample size, significant results were found only in muscle protein turnover and neuromuscular junction pathways (Figure 3C). As expected, muscle protein turnover and neuromuscular junction pathways were higher and lower with severe sarcopenic patients than with LMM and LMM_LP, respectively. These results indicate the association of sarcopenia with the impairment of neurophysiological functions, alteration in the transduction of the action potentials of muscle, and structural alterations of the muscles.

In addition, WGCNA identified 34 significant gene modules differentially enriched between the S, LMM, and LMM_LP groups (Figure 3D). The 34 representative colors of these modules are indicated in salmon2, maroon, orangered1, sienna4, lightpink4, yellowgreen, paleturquoise, steelblue, lavenderblush2, lightslateblue, skyblue4, thistle1, mediumorchid, red, violet, coral3, mediumpurple3, yellow4, indianred4, plum, antiquewhite2, mediumpurple4, salmon4, mediumpurple2, lightcoral, brown2, saddle brown, lightgreen, darkmagenta, lightcyan, darkgreen, grey60, greenyellow, and lightsteelblue. The representative GO terms for these gene modules were olfactory receptor activity, response to ketamine, galanin receptor activity, galanin-activated signaling pathway, intrinsic component of nuclear outer membrane, cardiac left ventricular formation, chemokine (C-C motif) ligand 4 production, keratin filament, dihydrofolate reductase activity, regulation of endothelial cell activation, myoblast development, spermatogenesis exchange of chromosomal protein, negative regulation of high-density lipoprotein particle clearance, RISC complex, ATP:ADP antiporter activity, positive regulation of interleukin-17 secretion, CCR3 chemokine receptor binding, extrinsic component of synaptic vesicle membrane, intermediate filament, phytanate-CoA ligase activity, oxidative phosphorylation uncoupler activity, negative regulation of heart induction by canonical Wnt signaling, otic vesicle formation, regulation of transcription from RNA polymerase II promoter involved in forebrain neuron fate commitment, cornification, epidermal cell differentiation, neuron fate commitment, sensory perception, photoreceptor outer segment membrane, detection of chemical stimulus involved in sensory perception, keratinocyte differentiation, formation of quadruple SL/U4/U5/U6 snRNP, dynein complex, and positive regulation of cell growth, respectively.

The S group was associated with enriched pathways of intrinsic component of the nuclear outer membrane, cardiac left ventricle formation, chemokine (C-C motif) ligand 4 production, keratin filament, dihydrofolate reductase activity, regulation of endothelial cell activation, intermediate filament, phytanate-CoA ligase activity, oxidative phosphorylation uncoupler activity, negative regulation of heart induction by canonical Wnt signaling, otic vesicle formation, regulation of transcription from RNA polymerase II promoter involved in forebrain neuron fate commitment, cornification, epidermal cell differentiation, sensory perception, photoreceptor outer segment membrane, detection of chemical stimulus involved in sensory perception, formation of quadruple SL/U4/U5/U6 snRNP, dynein complex, and positive regulation of cell growth (Figure 3D). In contrast, the red RISC complex module was negatively associated with the S group. Moreover, the LMM and LMM_LP groups shared two common pathways with the S group, namely epidermal cell differentiation and the RISC

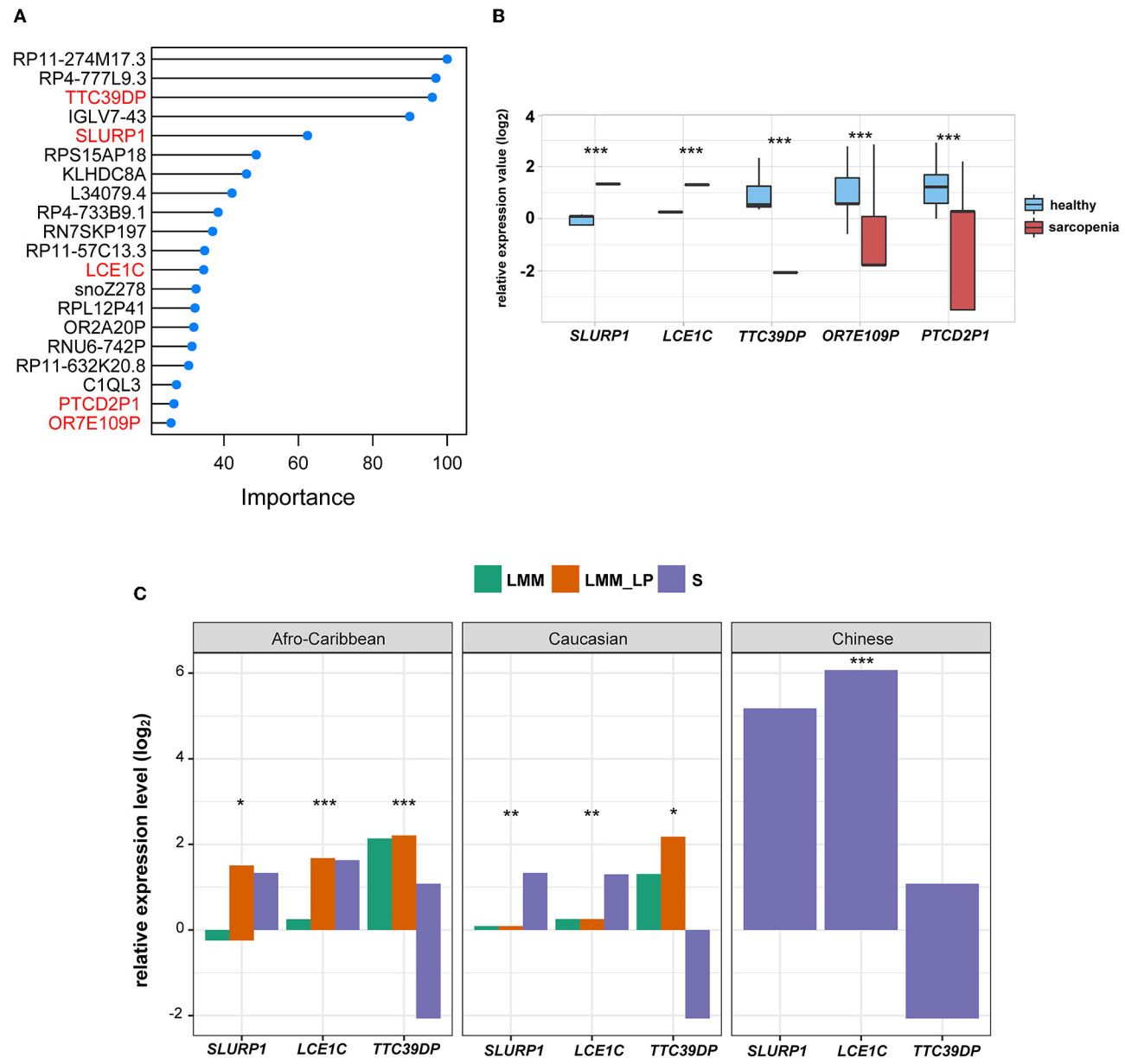

FIGURE 2
Discrimination of sarcopenia from healthy states using elastic net regression model and the association of the novel biomarkers with patient's ethnicity. **(A)** The elastic regression model discriminates sarcopenia from a healthy control based on gene expression profile. Model importance was identified as regression coefficients. **(B)** Comparison of the expression levels of the top-ranked genes between sarcopenia and the healthy controls between differentially expressed gene analysis and the elastic net regression model (*t*-test, *p*-values are shown. *$p < 0.5$, **$p < 0.01$, and ***$p < 0.001$). **(C)** Comparison of the expression levels of *SLURP1*, *LCE1C*, and *TTC39DP* among the S, LMM, and LMM_LP groups (ANOVA test, p-values are shown. *$p < 0.5$, **$p < 0.01$, and ***$p < 0.00$).

complex. The RISC complex pathway was highly enriched in the LMM and LMM_LP groups, while the S group observed the opposite. Interfering RNAs (siRNAs) and microRNAs (miRNAs) act *via* RNA-induced silencing complexes (RISC) and can regulate gene transcripts negatively (21). RISC involves many biological processes, such as brain aging (21). It has been proven that the dysregulation of miRNA expression has been associated with reduced muscle plasticity (22), as observed in our results, and, as a consequence, will impair skeletal muscle adaptations to exercise (22).

3.4. The immune profile of sarcopenia, low muscle mass, and low physical performance patients

We analyzed the association of different immune signatures with patients' muscle mass and function deterioration. First, we conducted a *t*-test analysis to identify the immune signatures (30 immune signatures) differentiating sarcopenia from healthy individuals. A total of 14 immune signatures significantly differentiated sarcopenia from healthy individuals (Figure 4A).

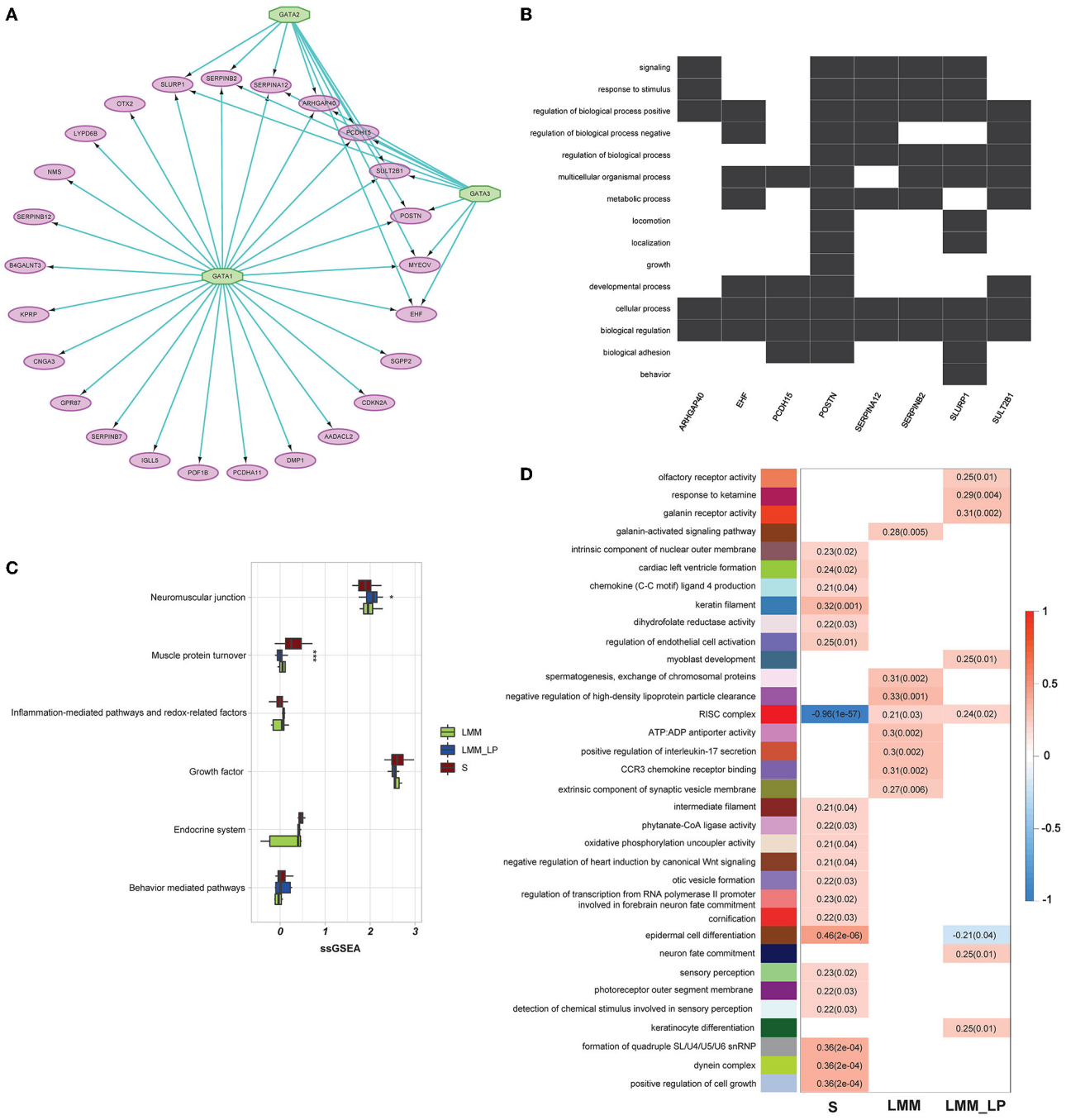

FIGURE 3
Gene regulatory, weighted correlation networks, and pathophysiological mechanisms of different sarcopenia stages, low muscle mass, and low physical performance patients. **(A)** Gene regulatory network of the top upregulated genes under sarcopenia using iRegulon software. Results show GATA1, 2, and 3 as top regulators and their relationship with the nine predicted direct targets. **(B)** Heatmap-like functional classification depicts the linkages of predicted genes and biological concepts (e.g., GO terms or KEGG pathways). **(C)** Comparison of the pathophysiological mechanisms between the S, LMM, and LMM_LP groups (ANOVA test, *p*-values are shown. $*p < 0.5$, $**p < 0.01$, and $***p < 0.00$). **(D)** Significant gene modules and their representative gene ontology differentially enriched between sarcopenia (S), low muscle mass (LMM), and low muscle mass with physical performance (LMM_LP), which were identified by WGCNA. The *P*-values are shown in parentheses.

Only proinflammatory cytokines were significantly upregulated in sarcopenia than in healthy controls. In contrast, Th1&Th2 cells, resident memory T cells, NK activating receptor, NK antimicrobial protein granulysin, granulocytes, exhausted T cells, effector memory T cells, cytolytic protein perforin, cytolytic activity, $CD4^+$ T cells, B cells, and anti-inflammatory cytokines were less enriched in sarcopenia patients than in healthy controls. Second, we used the ANOVA test to compare immune signatures' expression levels among the S, LMM, and LMM_LP groups. In total, nine immune signatures significantly differentiate the three groups (Figure 4B).

Type I IFN response and dendritic cells were higher in sarcopenia patients than in the other two groups. However, signatures of Th1&Th2 cells, resident memory T cells, granulocytes, $CD4^+$ T cells, B cells, and anti-inflammatory cytokines declined with worsening muscle function and disease progression. These results strongly correlate immune system dysregulation with disease progression and muscle function deterioration.

Furthermore, we used the elastic net regression to derive a discriminating model of the 30 immune signatures based on patients with and without sarcopenia after training this model on multiple random samples of the immune signatures using CV. We ranked all the immune signatures based on their regression coefficients. We found that Th2 cells and granulocytes could discriminate between sarcopenia and healthy individuals with a regression coefficient of 100 and 77.9, respectively (Figure 4C). Figures 4A, B show that the Th2 cells were significantly downregulated in sarcopenia patients relative to healthy controls, LMM, and LMM_LP. These results indicate that sarcopenia patients have weaker immune responses than other pre-defined groups.

3.5. Association of *TTC39DP, SLURP1*, and *LCE1C* expression levels with immune signatures

In addition, we analyzed the association of various immune signatures with the pre-defined top-ranked genes (*TTC39DP*, *SLURP1*, and *LCE1C*) that discriminated sarcopenia from healthy individuals. *TTC39DP* has shown a significant positive correlation with 11 immune signatures and one negative correlation with proinflammatory cytokines (Figure 5A). The highest positive correlations were observed in granulocytes and B cells. In contrast, *SLURP1* and *LCE1C* significantly negatively correlated with immunomodulatory molecules and exhausted T cells (Figures 5B–E). Interestingly, *TTC39DP* expression levels were much lower in sarcopenia patients than in healthy controls, and its upregulation correlated with a better prognosis and stronger immune profile in older people. In contrast, *SLURP1* and *LCE1C* expression levels were much higher in sarcopenia patients and associated with a worse prognosis and weaker immune profile.

3.6. Gene coexpression networks of *TTC39DP, SLURP1*, and *LCE1C*

We identified 103, 57, and 66 genes with strong positive expression correlations with *TTC39DP, SLURP1*, and *LCE1C*, respectively ($R > 0.5$). Interestingly, *SLURP1* and *LCE1C* showed a strong positive expression correlation ($R = 0.9$, $p < 0.001$, Figure 6A). In contrast, no significant correlations existed between *SLURP1* and *LCE1C* with *TTC39DP* (Figures 6B, C). Pathway enrichment analysis identified several significant KEGG pathways associated with *SLURP1* and *LCE1C* expression, including Renin secretion, Ras signaling pathway, Rap1 signaling pathway, Parkinson's disease, inflammatory mediator regulation of TRP channels, GnRH signaling pathway, gastric acid secretion, cGMP-PKG signaling pathway, cAMP signaling pathway, calcium signaling pathway, p53 signaling pathway, neurotrophin signaling pathway, and C-type lectin receptor signaling pathway. These pathways were associated with the 57 and 66 genes strongly correlated with *SLURP1* and *LCE1C*, respectively (Figures 6D, E, FDR < 0.05). However, pathway enrichment analysis with 103 correlated genes with *TTC39DP* did not show significantly enriched pathways.

4. Discussion and conclusion

Sarcopenia is a progressive and generalized skeletal muscle disorder, including the accelerated loss of muscle mass function (23). However, identifying biomarkers that reflect the central pathogenic processes of sarcopenia has remained lacking. The main problem in the diagnosis of sarcopenia is its complex genesis. The pathophysiology of sarcopenia includes inflammatory conditions, endocrine dysfunctions, and glycogen, glucose, and lipid metabolism dysregulation (24). Similarly, muscle-related cytokines and myokines show endocrine actions between muscle and tissues. In addition, many factors related to chronic diseases and lifestyles, such as obesity and low physical activity, may define the development of sarcopenia (25). Regardless of hopeful advances in assessing muscle mass and strength, various mechanisms of sarcopenia have not been fully distinguished, although several biomarkers may be found in both blood and tissue samples (26). Even though histology is the primary standard for recognizing the pathological mechanisms of different sarcopenic stages, biopsy samples are often a complicated process and not acceptable to older adult patients, especially during the follow-up of sarcopenic patients. Therefore, the emerging importance is identifying potential biomarkers for sarcopenia. In this study, we attempted to provide promising biomarkers and pathways that can detect sarcopenia at its early stages.

We found that sarcopenia is associated with increased expression levels of genes related to the neurotrophin, Rap1, Ras, and p53 signaling pathways. Neurotrophin signaling is involved in synaptic plasticity, memory, and neuronal health (27). In addition to sarcopenia and neurotrophin signaling during aging, the accumulation of proinflammatory cytokines generates neurotrophin resistance, increasing cognitive decline and dementia risk (27). *In vivo* studies showed that mice deficient for the tissue-specific Ras signaling, *RasGrf1*, mainly expressed within the pancreatic islets and the hippocampus and hypothalamus regions of the brain, were long-lasting and exhibited better motor coordination in elder animals than in their control (28). In addition, human studies showed that Ras signaling activation is associated with premature aging, including osteoporosis and osteopenia (28). p53 signaling has also been shown to contribute to aging. Preclinical studies showed that naturally aged mice had longevity linked with diminished p53 function, and p53 activation or dysregulation can accelerate aging (29).

In addition, we found that LMM_LP patients showed lower enrichment scores in B-cell receptor signaling, apoptosis, HIF-1 signaling, and adaptive immune response pathways than LMM patients, indicating that immune response and metabolic and biological dysregulation occurred simultaneously with the

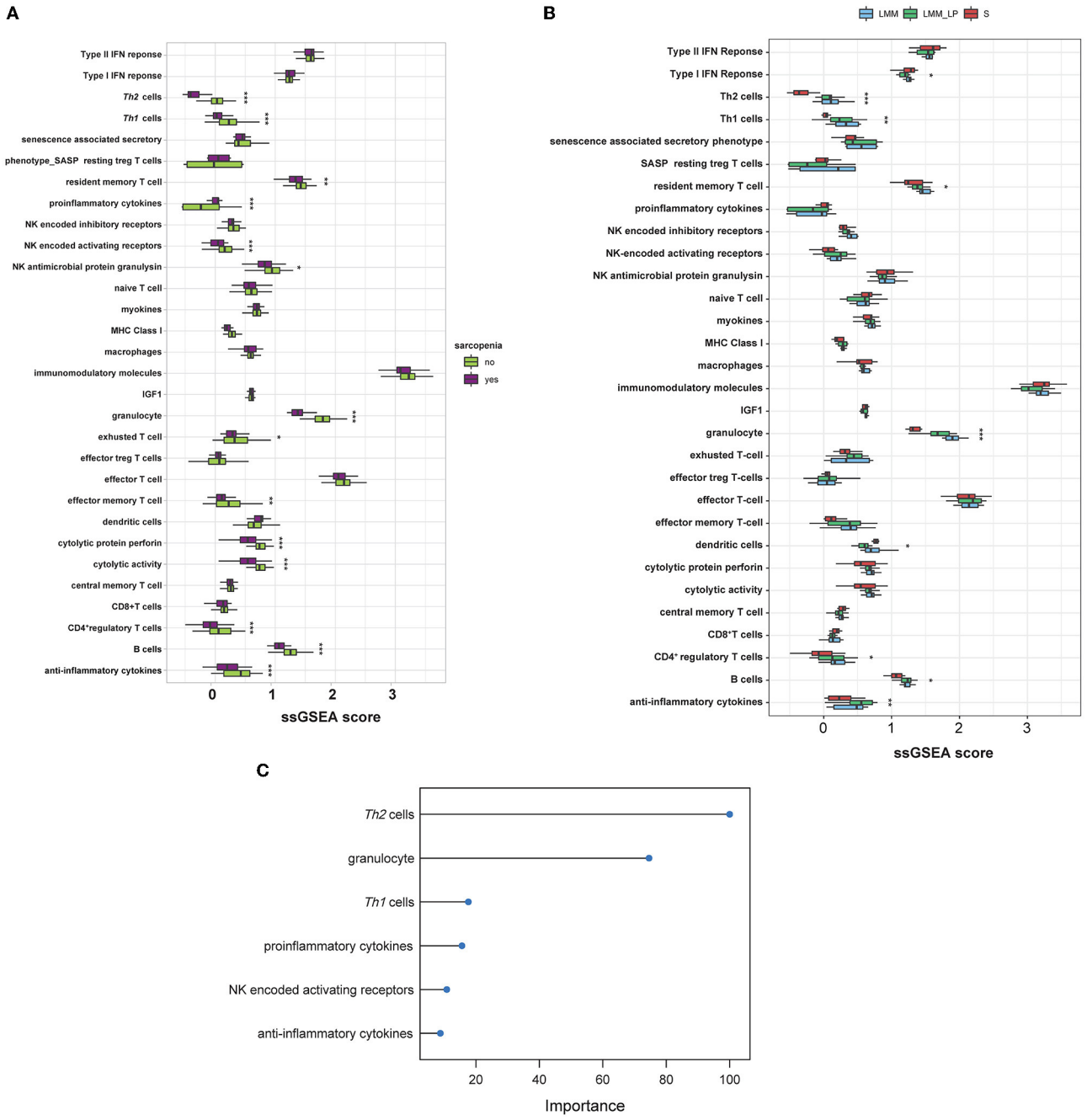

FIGURE 4
Immunological landscape of the S, LMM, and LMM_LP groups. **(A)** Comparison of 30 immune signature expression levels between sarcopenia and healthy controls (*t*-test, *p*-values are shown. $*p < 0.5$, $**p < 0.01$, and $***p < 0.00$). **(B)** Comparison of 30 immune signature expression levels between the S, LMM, and LMM_LP groups (ANOVA test, *p*-values are shown. $*p < 0.5$, $**p < 0.01$, and $***p < 0.00$). **(C)** The elastic regression model discriminates sarcopenia from a healthy control based on 30 immune signature expression profiles. Model importance was identified as regression coefficients.

patient's muscle mass and function deterioration. B cells are involved in the immunosuppressive regulatory process, and during aging, the depletion of the secretion of IL7 and IGF1 causes alterations in the number and function of B cells. Dysfunctional B cells were correlated with the dysregulation of immune aging, affecting the regeneration and strength of the skeletal muscle. It has been suggested that B-cell accumulation in tissues plays a role in sarcopenia obesity, which is related to skeletal muscle inflammation (30). HIF-1 is vital as a mediator in adaptation to hypoxia, which increases oxygen delivery and glucose transporters expression during hypoxia. During sarcopenia, HIF-1 impairs oxidative metabolism and mitochondrial biogenesis, causing muscle mass function deterioration (31).

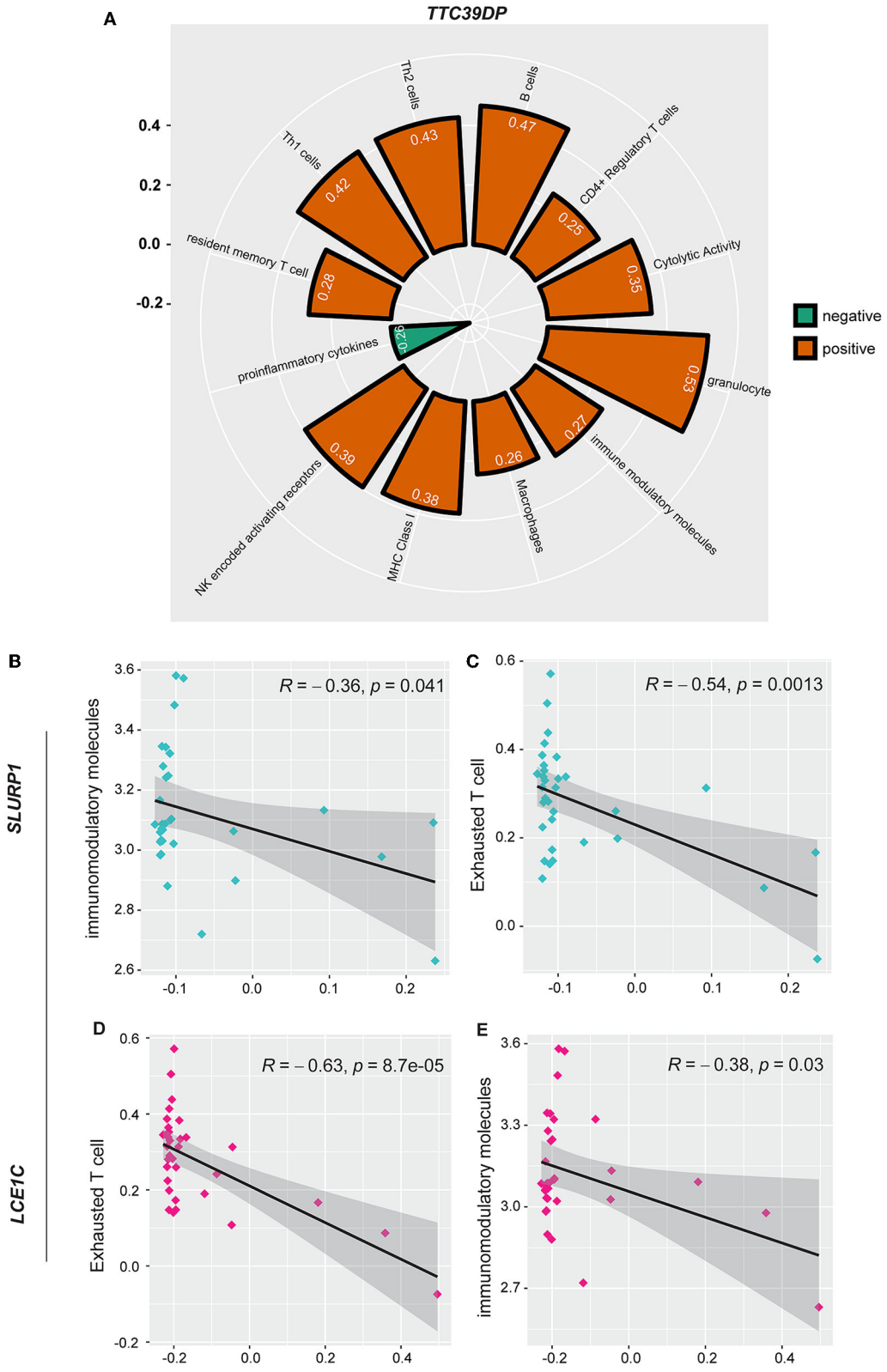

FIGURE 5
Association between *SLURP1*, *LCE1C*, and *TTC39DP* expression levels and immune signatures. **(A)** Significant correlation between *TTC39DP* expression levels and 12 immune signatures. **(B)** Significant correlation between *SLURP1* expression levels with immunomodulatory molecules and **(C)** exhausted T cell. **(D)** Significant correlation between *LCE1C* expression levels with an exhausted T cell. **(E)** Immunomodulatory molecules. Pearson's correlation coefficients (*R*) and *P*-values are shown.

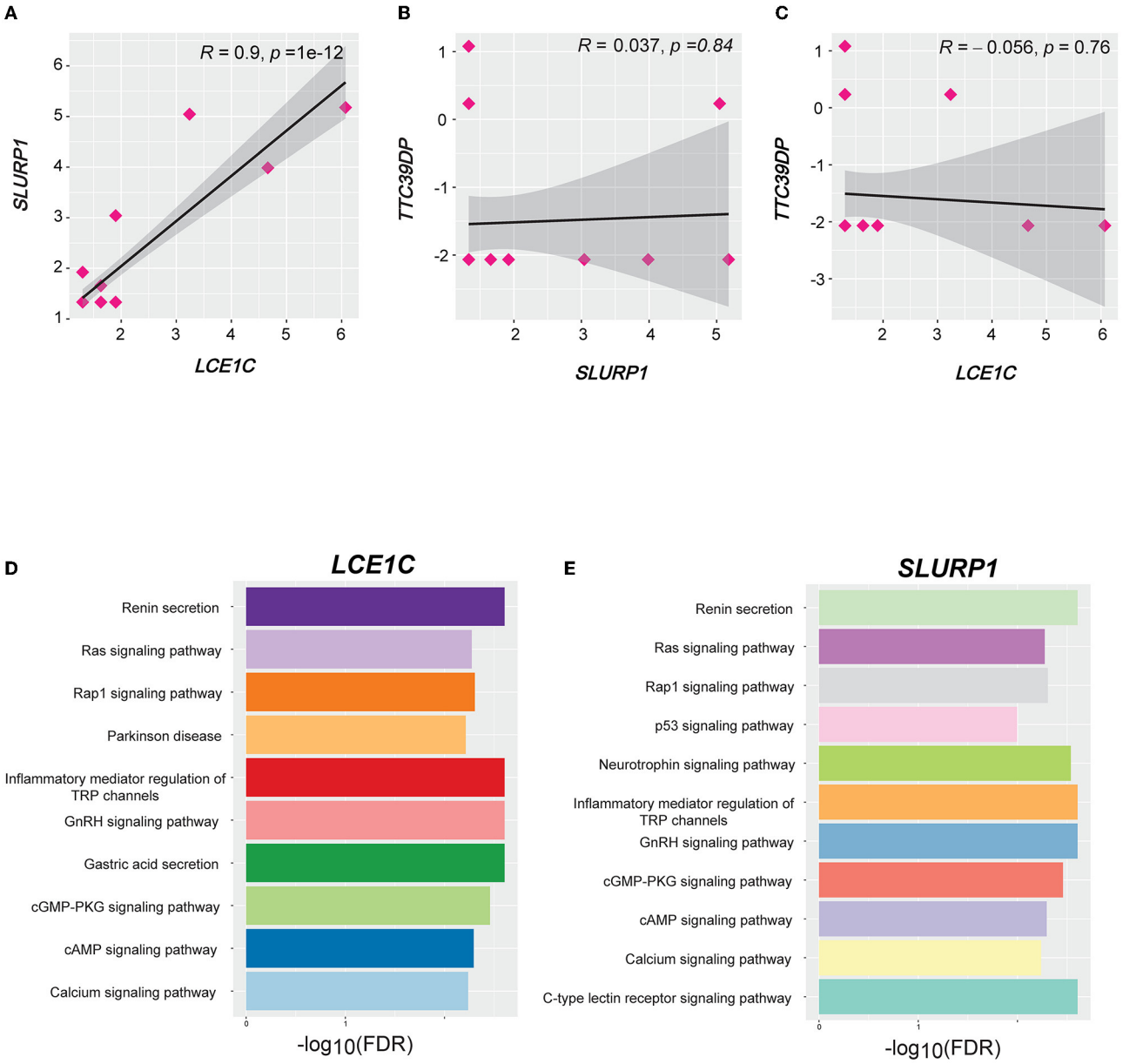

FIGURE 6
Correlation and pathways associated with *LCE1C*, *SLURP1*, and *TTC39DP* expression levels. **(A)** Correlation between *LCE1C* and *SLURP1* expression levels. **(B)** Correlation between *TTC39DP* with *SLURP1* and **(C)** *LCE1C* expression levels. Pearson's correlation coefficients (*R*) and *P*-values are shown. **(D)** The KEGG pathways enriched with *LCE1C* and **(E)** *SLURP1* highly correlated genes ($R > 0.5$) were identified by GSEA (53).

Furthermore, we found five common genes, *TTC39DP*, *SLURP1*, *LCE1C*, *PTCD2P1*, and *OR7E109P*, that can differentiate between sarcopenia patients and healthy controls. *SLURP1* and *LCE1C* show the highest expression levels among sarcopenic Chinese descent than Caucasian and Afro-Caribbean. *TTC39DP* was the highest in LMM_LP than *SLURP1* and *LCE1C* in Afro-Caribbean and Caucasian descents. *SLURP1* is an immunomodulatory protein that promotes corneal immune and angiogenic processes (32). It is expressed in tissues such as skin, palms, soles, and oral and bronchial cells (32). *SLURP1* suppresses TNFα production from T cells and IL1β, IL6, IFNγ, and IL8 secretion, which mimic the anti-inflammatory effect (33). Although we found that proinflammatory cytokines were higher in sarcopenia patients than in healthy control, this could trigger *SLURP1* activation. *SLURP1* was also identified as an epidermal neuromodulator essential for epidermal homeostasis, which indicates this gene's crucial role in aging (34). *LCE1C* and *TTC39DP's* role in aging and sarcopenia development remains lacking, even though reports have identified *LCE1C* as essential in epithelial development (35) and the identification of the skin (36). Although the results report a strong correlation between *LCE1C* and aging, the correlation of this gene with sarcopenia and muscle function deterioration is still needed.

Gene regulatory analysis yields a top-scoring regulon containing GATA1, GATA2, and GATA3 as master regulators and nine predicted direct target genes, and these predicted targets were associated with different pathways. Among these nine targeted genes, *POSTN* and *SLURP1* showed strong associations with locomotion. *POSTN* (periostin) is an extracellular matrix protein crucial in myocardial fibrosis and heart inflammatory processes (37). Periostin was associated with heart aging, and excessive periostin expression contributed to cardiomyocyte senescent (37). In addition, periostin was found to have a maintenance role in muscle mass during muscle regeneration (38). Furthermore, upregulation and secretion of periostin increase muscular dystrophy in mice, and deletion of periostin led to the improvement in skeletal muscle structure and function, suggesting its importance in muscle strength and function (39). However, although our results showed an association of *SLURP1* with locomotion and disease progression, supporting data regarding the role of this gene in muscle function and sarcopenia remain lacking, and further studies are recommended.

As mentioned, sarcopenia is a disease of muscle constrictive dysfunction, metabolic abnormalities, and systemic inflammation (40). Thus, sarcopenia is a disease of more complex networks involving different pathophysiologic mechanisms from dysfunction of neuromuscular junctions, a decline of the endocrine system, imbalance of growth enhancer and suppressor factors, structural alteration of protein synthesis, behavior-mediated pathways (e.g., physical activity and obesity), and inflammation (20). Neuromuscular junction dysfunction could gradually alter muscle action potentials during exercise and be associated with neuromuscular weakness, as shown in Figure 3C (41). In addition, one of the earlier signs of sarcopenic damage would be the early structural alterations of the muscle protein. The essential biomarkers in the evaluation of muscle mass are serum sarcomeric proteins, such as actin, troponin, creatinine, and extracellular matrix proteins (42); in addition, type VI collagen (IC6), MMP-generated degradation fragment of collagen 6 (C6M) (43), the N-terminal peptide (P3NP) (44), and 3-methylhistidine (3MH) (45) are also associated with the pathophysiology of sarcopenia.

In addition, WGCNA analysis revealed that sarcopenia was associated with multiple enriched pathways and was negatively associated with the RISC complex. We also found that the LMM and LMM_LP groups shared two common pathways with the S group, epidermal cell differentiation, and the RISC complex. As observed in sarcopenia, the RISC complex pathway was associated with muscle mass deterioration and function. The RISC pathway involves many biological processes, such as brain aging (21). It has been proven that the dysregulation of miRNA expression (act *via* RISC complex) has been associated with reduced muscle plasticity (22) and will impair skeletal muscle adaptations to exercise (22).

From the immunological perspective, we found that proinflammatory cytokines, type I IFN response, and dendritic cells were significantly upregulated in sarcopenia patients. These cytokines are known to play a critical role in sarcopenia development by causing destructive effects on the skeletal muscle, declined physical performance and muscle strength, and disability in older adults (46). Similarly, inflammatory cytokines such as IL-1 can block the differentiation of myoblasts in the presence of overexpressed activin, and this synergic activity was confirmed in models related to sarcopenia (47). In addition, proinflammatory cytokines highlight the complexity of the inflammaging process. It was shown that patients suffering from obesity who develop sarcopenic obesity in older age show elevated levels of proinflammatory cytokines, indicating the strong association between endocrine, metabolic, and inflammaging (48).

In contrast, Th1&Th2 cells, resident memory T cells, NK activating receptor, NK antimicrobial protein granulysin, granulocytes, exhausted T cells, effector memory T cells, cytolytic protein perforin, cytolytic activity, $CD4^+$ T cells, B cells, and anti-inflammatory cytokines were lower in sarcopenia patients than healthy controls, indicating the strong association of immune system dysregulation with disease progression (49). During immune aging, the reservoir of NK cells in the thymus is nearly depleted, and depleted NK cells were found to be associated with muscle loss in sarcopenia and mortality (50). Furthermore, dendritic cells are related to immune cells' maturation and differentiation, such as Th1 and Th2 cells, and develop an inflammatory environment during immune aging, affecting skeletal muscle regeneration (51). However, there is still a lack of knowledge on the particular role of each immune cell subtype in sarcopenia, but their whole function may work in the skeletal muscle environment modification and affect the physiological homeostasis of skeletal muscle (52).

This study has several limitations. First, because sarcopenia is a complex disease, obtaining biopsy samples is not usually acceptable to older adult patients, which is why patients' samples are relatively small. Second, we obtained the results by bioinformatics analyses without experimental justification, so further experimental and clinical investigations are required to assess these biomarkers' activity and beneficial effects. In conclusion, this study identified the association between immune responses and biological dysregulation with the patient's muscle mass and function deterioration. Three biomarkers were identified, *TTC39DP*, *SLURP1*, and *LCE1C*, and their expression levels depend on the patient's ethnicity and immune profile. *TTC39DP* was correlated with a better prognosis and stronger immune profile, while *SLURP1* and *LCE1C* were associated with a bad prognosis and weaker immune profile. In addition, a strong association between immune system dysregulation with disease progression and muscle function deterioration was observed. This study provides new insight into sarcopenia's cellular and immunological prospects, which is considered reliable and promising to evaluate the age and sarcopenia-related modifications of skeletal muscle.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE111017.

Ethics statement

Ethics approval was waived since we used only publicly available data and materials in this study.

Author contributions

ZA: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft, writing—review and editing, and visualization. XiW: conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing—original draft, writing—reviewing and editing, visualization, and supervision. DW and TZ: formal analysis and investigation. YZ: methodology and investigation. XuW and ZC: conceptualization, methodology, investigation, writing—reviewing and editing, supervision, project administration, and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.928285/full#supplementary-material

References

1. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing*. (2010) 39:412–23. doi: 10.1093/ageing/afq034

2. Wilson D, Jackson T, Sapey E, Lord JM. Frailty and sarcopenia: the potential role of an aged immune system. *Ageing Res Rev*. (2017) 36:1–10. doi: 10.1016/j.arr.2017.01.006

3. Schiaffino S, Mammucari C. Regulation of skeletal muscle growth by the IGF1-Akt/PKB pathway: insights from genetic models. *Skelet Musc.* (2011) 1:1–14. doi: 10.1186/2044-5040-1-4

4. Markofski MM, Dickinson JM, Drummond MJ, Fry CS, Fujita S, Gundermann DM, et al. Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young and older men and women. *Exp Gerentol.* (2015) 65:1–7. doi: 10.1016/j.exger.2015.02.015

5. Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BBL, Verdijk LB, et al. Aging is accompanied by a blunted muscle protein synthetic response to protein ingestion. *PLoS ONE.* (2015) 10:e0140903. doi: 10.1371/journal.pone.0140903

6. Goronzy JJ, Weyand CM. Understanding immunosenescence to improve responses to vaccines. *Nat Immunol.* (2013) 14:428–36. doi: 10.1038/ni.2588

7. Shaw AC, Joshi S, Greenwood H, Panda A, Lord JM. Aging of the innate immune system. *Curr Opinion Immunol.* (2010) 22:507–13. doi: 10.1016/j.coi.2010.05.003

8. Baylis D, Bartlett DB, Patel HP, Roberts HC. Understanding how we age: insights into inflammaging. *Longevity & healthspan.* (2013) 2:1–8. doi: 10.1186/2046-2395-2-8

9. Migliavacca E, Tay SKH, Patel HP, Sonntag T, Civiletto G, McFarlane C, et al. Mitochondrial oxidative capacity and NAD(+) biosynthesis are reduced in human sarcopenia across ethnicities. *Nat Commun.* (2019) 10:5808. doi: 10.1038/s41467-019-13694-1

10. Chen L-K, Liu L-K, Woo J, Assantachai P, Auyeung T-W, Bahyah KS, et al. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Direct Assoc*. (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025

11. Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformat*. (2013) 14:7. doi: 10.1186/1471-2105-14-7

12. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res.* (2000) 28:27–30. doi: 10.1093/nar/28.1.27

13. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*. (2000) 25:25–9. doi: 10.1038/75556

14. Ulgen E, Ozisik O, Sezerman OU. pathfindR: An R package for comprehensive identification of enriched pathways in omics data through active subnetworks. *Front Genet*. (2019) 10:858. doi: 10.3389/fgene.2019.00858

15. Bland JM, Altman DG. Multiple significance tests: the Bonferroni method. *BMJ.* (1995) 310:170. doi: 10.1136/bmj.310.6973.170

16. Kuhn M. Building predictive models in R using the caret package. *J Stat Softw.* (2008) 28:1–26. doi: 10.18637/jss.v028.i05

17. Janky RS, Verfaillie A, Imrichová H, Van de Sande B, Standaert L, Christiaens V, et al. iRegulon: from a gene list to a gene regulatory network using large motif and track collections. *PLoS Comp Biol.* (2014) 10:e1003731. doi: 10.1371/journal.pcbi.1003731

18. Langfelder P, Horvath S, WGCNA. an R package for weighted correlation network analysis. *BMC Bioinformat.* (2008) 9:1–13. doi: 10.1186/1471-2105-9-559

19. Soikkeli J, Podlasz P, Yin M, Nummela P, Jahkola T, Virolainen S, et al. Metastatic outgrowth encompasses COL-I, FN1, and POSTN up-regulation and assembly to fibrillar networks regulating cell adhesion, migration, and growth. *Am J Pathol.* (2010) 177:387–403. doi: 10.2353/ajpath.2010.090748

20. Curcio F, Ferro G, Basile C, Liguori I, Parrella P, Pirozzi F, et al. Biomarkers in sarcopenia: a multifactorial approach. *Exp Gerontol.* (2016) 85:1–8. doi: 10.1016/j.exger.2016.09.007

21. Grigoriev A, Bonini NM. Age-dependent patterns of microRNA RISC loading. *Aging*. (2014) 6:705–6. doi: 10.18632/aging.100692

22. Margolis LM, Rivas DA. Potential role of microRNA in the anabolic capacity of skeletal muscle with aging. *Exerc Sport Sci Rev*. (2018) 46:86–91. doi: 10.1249/JES.0000000000000147

23. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet.* (2019) 393:2636–46. doi: 10.1016/S0140-6736(19)31138-9

24. Malafarina V, Úriz-Otano F, Iniesta R, Gil-Guerrero LJM. Sarcopenia in the elderly: diagnosis, physiopathology and treatment. *Maturitas.* (2012) 71:109–14. doi: 10.1016/j.maturitas.2011.11.012

25. Biolo G, Cederholm T, Muscaritoli M. Muscle contractile and metabolic dysfunction is a common feature of sarcopenia of aging and chronic diseases: from sarcopenic obesity to cachexia. *Clin Nutr.* (2014) 33:737–48. doi: 10.1016/j.clnu.2014.03.007

26. Kalinkovich A, Livshits G. Sarcopenia–The search for emerging biomarkers. *Ageing Res Rev.* (2015) 22:58–71. doi: 10.1016/j.arr.2015.05.001

27. Cotman CW. The role of neurotrophins in brain aging: a perspective in honor of Regino Perez-Polo. *Neurochem Res.* (2005) 30:877–81. doi: 10.1007/s11064-005-6960-y

28. Slack C. Ras signaling in aging and metabolic regulation. *Nutr Healthy Aging.* (2017) 4:195–205. doi: 10.3233/NHA-160021

29. Wu D, Prives C. Relevance of the p53–MDM2 axis to aging. *Cell Death Different.* (2018) 25:169–79. doi: 10.1038/cdd.2017.187

30. Fan J, Kou X, Yang Y, Chen N. MicroRNA-regulated proinflammatory cytokines in sarcopenia. *Mediat Inflamm.* (2016) 2016. doi: 10.1155/2016/1438686

31. Lee T-W, Liu H-W, Lin Y-F, Lee T-I, Kao Y-H, Chen Y-J. Histone deacetylase inhibition improves metabolism and mitochondrial dynamics: a potential novel therapeutic strategy for sarcopenia coexisting with diabetes mellitus. *Med Hypotheses.* (2022) 158:110724. doi: 10.1016/j.mehy.2021.110724

32. Swamynathan S, Tiwari A, Loughner CL, Gnalian J, Alexander N, Jhanji V, et al. The secreted Ly6/uPAR-related protein-1 suppresses neutrophil binding, chemotaxis, and transmigration through human umbilical vein endothelial cells. *Sci Rep.* (2019) 9:5898. doi: 10.1038/s41598-019-42437-x

33. Chernyavsky AI, Galitovskiy V, Shchepotin IB, Grando SA. Anti-inflammatory effects of the nicotinergic peptides SLURP-1 and SLURP-2 on human intestinal epithelial cells and immunocytes. *BioMed Res Int.* (2014) 2014:609086. doi: 10.1155/2014/609086

34. Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, et al. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. *Hum Mol Genet.* (2003) 12:3017–24. doi: 10.1093/hmg/ddg320

35. Yabuta N, Ota C, Sasakura T, Naito Y, Okuzaki D, Fukushima K, et al. Late cornified envelope 1C (LCE1C), a transcriptional target of TAp63 phosphorylated at T46/T281, interacts with PRMT5. *Sci Rep.* (2018) 8:4892. doi: 10.1038/s41598-018-23045-7

36. Hanson E, Haas C, Jucker R, Ballantyne J. Identification of skin in touch/contact forensic samples by messenger RNA profiling. *For Sci Int Genet Suppl Ser.* (2011) 3:e305–6. doi: 10.1016/j.fsigss.2011.09.015

37. Li Q, Liu X, Wei J. Ageing related periostin expression increase from cardiac fibroblasts promotes cardiomyocytes senescent. *Biochem Biophys Res Commun.* (2014) 452:497–502. doi: 10.1016/j.bbrc.2014.08.109

38. Ito N, Miyagoe-Suzuki Y, Takeda Si, Kudo A. Periostin is required for the maintenance of muscle fibers during muscle regeneration. *Int J Mol Sci.* (2021) 22:3627. doi: 10.3390/ijms22073627

39. Lorts A, Schwanekamp JA, Baudino TA, McNally EM, Molkentin JD. Deletion of periostin reduces muscular dystrophy and fibrosis in mice by modulating the transforming growth factor-β pathway. *Proc Natl Acad Sci USA.* (2012) 109:10978–83. doi: 10.1073/pnas.1204708109

40. Ilich JZ, Kelly OJ, Inglis JE, Panton LB, Duque G, Ormsbee MJ. Interrelationship among muscle, fat, and bone: connecting the dots on cellular, hormonal, and whole body levels. *Ageing Res Rev.* (2014) 15:51–60. doi: 10.1016/j.arr.2014.02.007

41. Rudolf R, Khan MM, Labeit S, Deschenes MR. Degeneration of neuromuscular junction in age and dystrophy. *Front Ageing Neurosci.* (2014) 6:99. doi: 10.3389/fnagi.2014.00099

42. Nedergaard A, Karsdal MA, Sun S, Henriksen K. Serological muscle loss biomarkers: an overview of current concepts and future possibilities. *J Cachexia Sarcop Muscle.* (2013) 4:1–17. doi: 10.1007/s13539-012-0086-2

43. Nedergaard A, Sun S, Karsdal MA, Henriksen K, Kjær M, Lou Y, et al. Type VI collagen turnover-related peptides—novel serological biomarkers of muscle mass and anabolic response to loading in young men. *J Cachexia Sarcop Muscle.* (2013) 4:267–75. doi: 10.1007/s13539-013-0114-x

44. Bhasin S, He EJ, Kawakubo M, Schroeder ET, Yarasheski K, Opiteck GJ, et al. N-terminal propeptide of type III procollagen as a biomarker of anabolic response to recombinant human GH and testosterone. *J Clin Endocrinol Metab.* (2009) 94:4224–33. doi: 10.1210/jc.2009-1434

45. Young VR, Munro HN. Ntau-methylhistidine (3-methylhistidine) and muscle protein turnover: An overview. *Fed Proc.* (1978) 37:2291–300.

46. Cesari M, Penninx BWJH, Pahor M, Lauretani F, Corsi AM, Williams GR, et al. Inflammatory markers and physical performance in older persons: the InCHIANTI study. *J Gerontol Ser A Biol Sci Med Sci.* (2004) 59:M242–8. doi: 10.1093/gerona/59.3.M242

47. Trendelenburg AU, Meyer A, Jacobi C, Feige JN, Glass D. TAK-1/p38/nNFκB signaling inhibits myoblast differentiation by increasing levels of Activin A. *Skelet Muscle.* (2012) 2:1–14. doi: 10.1186/2044-5040-2-3

48. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. *Nat Rev Endocrinol.* (2012) 8:709–16. doi: 10.1038/nrendo.2012.114

49. Nelke C, Dziewas R, Minnerup J, Meuth SG, Ruck TJE. Skeletal muscle as potential central link between sarcopenia and immune senescence. *EBioMedicine.* (2019) 49:381–8. doi: 10.1016/j.ebiom.2019.10.034

50. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol.* (2006) 6:772–83. doi: 10.1038/nri1937

51. Rodrigues LP, Teixeira VR, Alencar-Silva T, Simonassi-Paiva B, Pereira RW, Pogue R, et al. Hallmarks of aging and immunosenescence: Connecting the dots. *Cytokine Growth Factor Rev.* (2021) 59:9–21. doi: 10.1016/j.cytogfr.2021.01.006

52. Zhang X, Li H, He M, Wang J, Wu Y, Li Y. Immune system and sarcopenia: Presented relationship and future perspective. *Exp Gerontol.* (2022) 164:111823. doi: 10.1016/j.exger.2022.111823

53. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Nat Acad Sci USA.* (2005) 102:15545–50. doi: 10.1073/pnas.0506580102

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Association among inflammaging, body composition, physical activity, and physical function tests in physically active women

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Background: Inflammaging is a phenomenon that has been associated with the development and progression of sarcopenia and frailty syndrome. According to the literature, on the one side, the increase in body fat is associated with a systemic pro-inflammatory status, which consequently favors inflammaging, and on the other side, the regular practice of physical exercise can mitigate the development of this scenario. Therefore, here, we aimed to evaluate the association between inflammaging and physical factors, both body and functional, in a group of physically active older women.

Methods: Seventy older women (mean age 72.66 ± 6.17 years) participated in this observational cross-sectional and were separated into the eutrophic, overweight, and obese groups. It was assessed: by bioimpedance—body fat percentage (Fat%) and total (Fat kg), skeletal muscle mass (muscle), and free fat mass both in percentage (FFM%) and total (FFMkg); by the International Physical Activity Questionnaire (IPAQ)—the time of moderate-intensity physical activity per week; by physical tests—handgrip (HG), sit-up-stand-on-the-chair in 5 repetitions (Sit-up) and vertical squat jump test (SJ); in addition to the determination of serum cytokine concentration (IL-6, TNF-α, IL-10, and IL-8), and also body mass index (BMI) and calf circumference (Calf).

Results: Higher FFM% and lower body fat (both kg and %) were found in the eutrophic group than in the other groups. The eutrophic group also performed more weekly physical activity, jumped higher, and presented not only higher serum IL-6 concentration but also an increased ratio of IL-10/IL-6, IL-10/TNF-α, IL-10/IL-8 as compared to the values found in the overweight group. The obese group presented higher body fat (kg and %) and lower FFM% than the other groups and

also higher serum IL-6 concentration than the overweight group. Interestingly, several significant negative and positive correlations between body composition, physical tests, and serum cytokine concentrations were found in the eutrophic and obese groups.

Conclusion: While the eutrophic older women group showed a remarkable regulation of the systemic inflammatory status with positive associations in the physical parameters assessed, the overweight and obese groups presented impairment regulations of the inflammaging, which could be related to less weekly physical activity and higher body fat.

KEYWORDS

aging, inflammaging, physical activity, body composition, functional tests, cytokine profile

Introduction

According to the United Nations (UN) and the World Health Organization (WHO), the decade of 2021–2030 will be committed to improving older adults' quality of life since it had been estimated that, between 2015 and 2050, the proportion of the population aged over 60 would almost double from 12 to 22% (1). The development of Collaborative Healthy Aging was based on it, but the COVID-19 pandemic imposed a new worldwide challenge, particularly to the older adult population, which was one of the populations most suffering from SARS-CoV-2 infection (2–4).

It is known that aging is a natural and heterogeneous process that, in a general way, is associated with a gradual decrease in several body system actions (5, 6). In this respect, it has been described that aged people can significantly lose their functional abilities, whether muscular, mobility, cognitive, psychological, or sensory (7). Of note, it has been highlighted that disablement in older adults can be closely related to the presence of a phenomenon called "inflammaging", which is characterized by chronic, sterile, systemic, and subclinical low-grade inflammation, in which there is an increase in several inflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumor necrosis factor-alpha (TNF-α), in addition to significant alterations in innate and acquired immunity (8, 9). In agreement with the literature, the development of inflammaging is associated with a physiological response to antigenic stress throughout life and can represent an efficient defense mechanism, especially against pathogens already known, as long as it is under control (10). Nonetheless, at the same time, the inflammaging can also be associated with a remarkable impairment in the induction of an effective defense against new antigenic challenges, which leads to this population presenting a reduction of their immune response, a well-known phenomenon observed in older adults named immunosenescence (11, 12). These phenomena, when combined, can favor the development of severe clinical situations, diseases, and syndromes common in the aged population, most notably dementia syndromes, diabetes, cardiovascular disease, cancer, osteoporosis, sarcopenia, and frailty syndrome (13–17).

Among some factors that can contribute to the inflammaging is body fat since alterations in body fat distribution and composition, specifically related to the increase in adipose tissue, a common aspect observed in older adults, promote elevation in the systemic inflammation status as a result of increased production of pro-inflammatory cytokines by infiltrated macrophages in the adipose tissues (18, 19). Raised systemic inflammation is closely associated with insulin resistance in skeletal muscle and liver, which compromises metabolic functions and increases cardiovascular risk by increasing not only circulating fatty acid levels but also lipid oxidation, which, consequently, drives an atherogenic profile (20). Beyond these aspects, it was reported that obese aged people are more vulnerable to developing sarcopenia (21) and also that a systemic pro-inflammatory status can enhance both the development and progression of this clinical condition (22, 23). In relation to sarcopenia, the European Working Group on Sarcopenia in Older People (EWGSOP), in 2019, established that sarcopenia is a muscle disease (muscle failure) characterized by reduced muscle strength, measured by using a hand dynamometer, in which values lower than 16 and 27 kg of force are considered as the cutoff for women and men, respectively, or through the sit-up-stand-of-chair test in five repetitions, in which values above 15 s are considered the cutoff. Furthermore, the EWGSOP also considered low appendicular skeletal muscle mass quantity in the diagnosis of sarcopenia, and the cutoff points with values below 15 and 20 kg, for women and men, respectively, are associated with sarcopenia occurrence (15).

To minimize the inflammaging development and progression, it has been demonstrated that the regular practice of physical exercises is a powerful tool due to its capacity to reduce body fat (24) and regulate the systemic inflammatory status, not only by the increase of the fat "burning" but also by the increase of anti-inflammatory cytokines levels, such as receptor antagonist IL-1 (IL-1ra), IL-10, and the soluble receptor of TNF-α (sTNF-R), produced during and after physical exercise performance (25–28). Another important cytokine is IL-8: This myokine is mainly produced by macrophages and endothelial cells and exerts, indistinctly, a marked chemotactic activity on leukocytes, being also an angiogenic factor (28). Therefore, analyzing the serum concentrations of these cytokines or the ratio between anti-inflammatory and pro-inflammatory ones helps to understand the adaptations of aged people in different scenarios of the aging process, especially even before becoming sarcopenic or

frail (26, 29, 30). Interstingly, the literature pointed out that older adults who regularly practice physical exercises present higher numbers of naive T lymphocytes, lower circulating TNF-α levels, improvement of immune response to the influenza virus vaccination, higher muscle strength and power, and better results in physical functional tests than older adult who are non-practitioners or sedentary (30–32).

In this context, the assessment of strength, power, and balance performance provides information on the quality of the skeletal muscle and the physical conditioning of old people (6, 33, 34). In the aging process, changes occur in the quantity and quality of skeletal muscles, and physical tests can help identify vulnerabilities for the development of frailty and sarcopenia, in addition to being used in monitoring interventions aimed at improving the physical domain and the intrinsic capacity of the aged adults (35–37).

Although it is well-known that both body composition and regular practice of physical exercises can impact inflammaging development and progression (25, 26), there is still no consensus in the literature about the best model to illustrate the inflammaging repercussion within physiological senescence. Thus, in this study, we aimed to identify which factors related to body composition and physical performance could be associated with the inflammaging in physically active older eutrophic, overweight, and obese older women.

Materials and methods

Participants and design of the study

In this observational cross-sectional study, initially, 74 women aged ≥ 60 years were invited to participate voluntarily between March and April 2019. However, as shown in Figure 1, 70 volunteers were included in this study since they met the inclusion and exclusion criteria as described. All volunteers were recruited from the Primary Health Care Program belonging to the Discipline of Geriatrics and Gerontology at the Federal University of São Paulo (UNIFESP). It is worth mentioning that the same geriatric physician was responsible for the clinical and physical examinations. The participants were informed of the risks and benefits of the study before data collection and gave written informed consent for their participation. They signed the informed consent form previously approved by the Ethics Committee of the Federal University of São Paulo (approval number 3.623.247) and by the National Research Ethics Committee (number CAEE:218170619.3.0 000.5505). The study was performed not only in agreement with the Ethical Standards of Exercise Practice (38) but also complied with the Declaration of Helsinki guidelines for research with humans (39).

The inclusion criteria were as follows: (i) the volunteer should regularly practice physical exercises under supervision or orientation, in the last 05 years; (ii) feel fit and have no contraindications for performing physical tests. The exclusion criteria were as follows: (i) weight change >4% in the last 12 months; (ii) use of anti-inflammatory drugs, multivitamins, protein supplements, and hormonal anabolic in the last 2 months; (iii) to present a high risk for fragility fracture in accordance to the Frax index® (40); (iv) being seropositive for HIV, having neoplasms, acute or chronic infections, neurological, cardiovascular, type I diabetes mellitus, and musculoskeletal diseases that prevent physical tests.

Regarding the number of older women volunteers enrolled in the present study, it is important to cite that it was established according to sample size calculation using the G*Power software program (41), considering Student's *t*-test with an effect size (0.30) at α-level (0.05), the statistical power of 0.95, and also a margin of 10% losses or refusal (35). Based on it, a minimum of 70 individuals would be necessary to perform this study.

Study design

According to the flow diagram (Figure 1), the study design was carried out in three stages. First, the volunteers previously invited to participate in this study were robust old women, without clinical alterations in the domains of intrinsic capacity (7), compensated chronic diseases, presented low nutritional risk (assessed by the Mini Nutritional Assessment—MNA) (42), adequate vision and hearing, no change in mood, preserved cognition, preserved strength, and mobility. All volunteers were submitted to clinical and physical examinations by the same geriatric physician, and four women were excluded from the study, by clinical issues.

In addition, it was also applied the International Physical Activity Questionnaire (IPAQ), the physical tests, and the familiarization with the jump test. On the same occasion, the volunteers enrolled were separated, based on BMI criteria presented by WHO (43), into three groups: eutrophic ($n = 27$, $18.5 \leq$ BMI < 25 kg/m^2), overweight ($n = 30$, $25 \leq$ BMI < 30 kg/m^2), and obese ($n = 13$, BMI ≥ 30 kg/m^2). At the second meeting, it was assessed the body composition by bioimpedance and also blood sampling was carried out. After that, the third meeting was dedicated to the performance of the vertical jump test. Finally, 1 week after this last phase, all volunteers were contacted by telephone call not only to communicate the results obtained in the physical test evaluations but also to verify the occurrence of any discomfort or pain as a consequence of the performance of the vertical jump test.

Physical activity

The level of moderate-intensity physical activity level was assessed through the International Physical Activity Questionnaire (IPAQ), validated for the Brazilian population (44, 45). By IPAQ, it is possible to estimate weekly time spent (in minutes) on moderate-intensity physical activities in different contexts of daily life (which may be characterized, subjectively, by the moderate increase in effort): (i) by physical activities of everyday life (e.g., housework); (ii) unsupervised recreational activities (e.g., dancing, cycling, running, and playing sports); and (iii) guided and supervised physical exercises (aerobics, resisted, multi-component, pilates, swimming, and water aerobics).

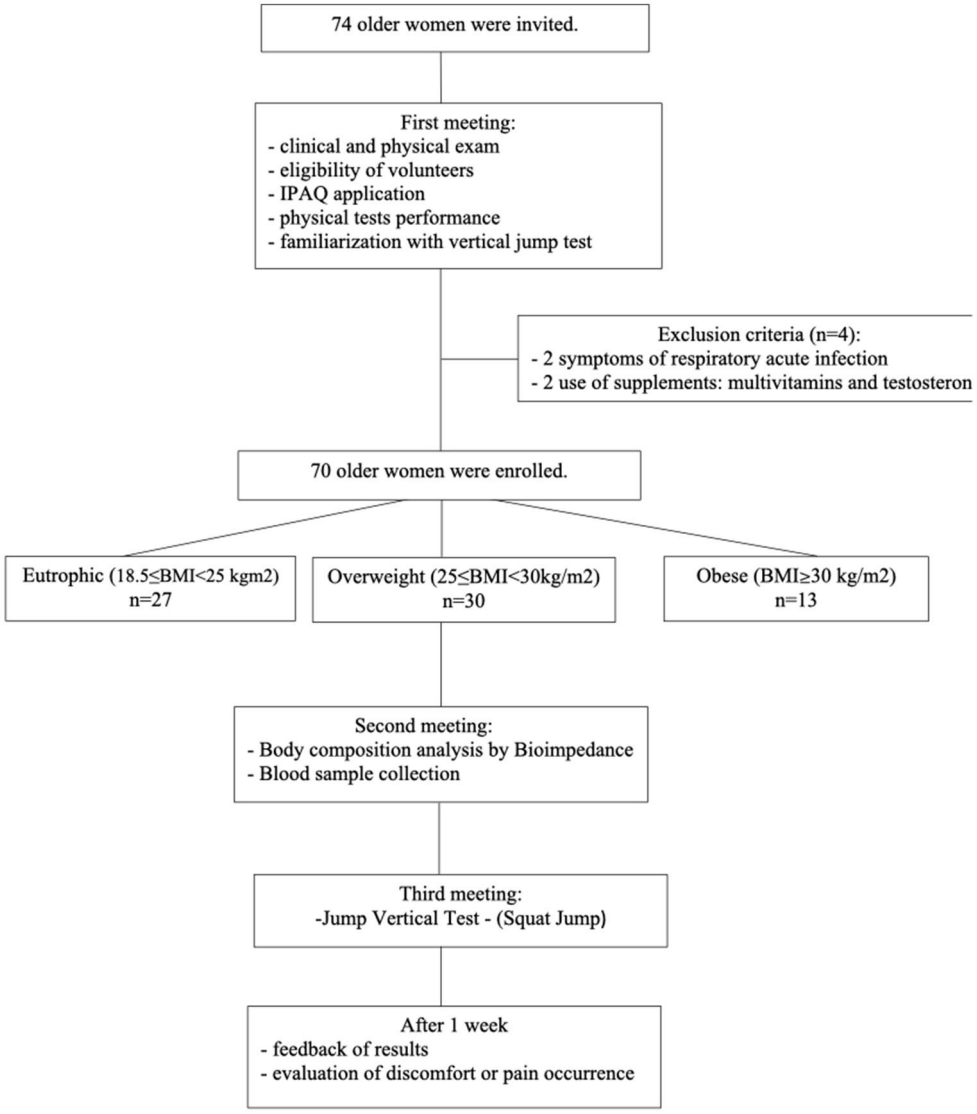

FIGURE 1
Flow diagram and study design.

Anthropometric characteristics and bioimpedance assessment

Data from body weight were carried out using a digital scale (Personal® scale, Filizzola, São Paulo, Brazil) used accurately to the nearest 0.1 kg. Body height was measured using a wall-mounted stadiometer, accurate to the nearest 0.1 cm. To perform these evaluations, the women wore light clothes and no footwear. Body mass index (BMI) was calculated by the equation: weight over height squared (kg/m^2). The left calf circumference was determined by using a measuring tape to the nearest 0.1 cm with the volunteer seated, knees bent at 90 degrees, and feet supported. The body composition was determined using the BIOSCAN 920-2-S® bioimpedance equipment (Matron International Limited, UK), with the volunteer lying supine and resting (46). The bioimpedance assessment was performed in the morning with the volunteers fasting (8 h), with water allowed up to 2 h before the assessment. They were asked to empty their bladder 30 min beforehand. This evaluation was carried out in the morning with the volunteers fasting (8 h) and being allowed to drink water up to 2 h before the evaluation. Then, they were asked to empty their bladders 30 min beforehand. They were also asked not to perform physical exercises the day before, nor to drink coffee or caffeinated beverages (47). The results obtained in the bioimpedance evaluation were as follows: skeletal muscle mass (in kilograms—muscle mass kg), total body fat (in kilograms—Fat kg, and percentage—Fat %), and also fat-free mass (in kilograms—FFM kg, and percentage—FFM %).

Physical tests

The physical tests applied in the present study were the same used in traditional protocols described in the scientific literature

for older adults (35, 48). It was assessed: the sit-to-up test in the chair for five repetitions (sit-up), in which the results were expressed in seconds (s); and the handgrip (HG), in which the results were expressed in kilograms of force (kgf). Regarding the HG test, it was considered the value obtained in best performance out of three attempts, with a 1-min interval, using the dominant hand and a digital dynamometer (Jamar Hydraulic Hand Digital Dynamometer®, Sammons Preston Rolyan, Bolingbrook, IL, USA) (49, 50).

Concerning the evaluation of the vertical jump test, this physical test was performed on a jumping platform (Elite Jump®, S2 Sports, São Paulo, Brazil) (51) after the familiarization carried out during the first meeting. The digital results were expressed in centimeters (cm), which represent the height of the jump, or in watts by body mass (W/kg), which represents the vertical force exerted during the takeoff phase of the jump. Each volunteer was instructed to walk for 5 min before performing the jump tests, in order to activate the muscles of the lower limbs that would be required in this test. The volunteers performed the squat jump (SJ) modality, in which they were oriented to remain static in a knee flexion position, at an angle close to ninety degrees, for 2 s before the jump, without any preparatory movement. Five jumps were performed, with intervals of 15 s between them (35).

Blood samples

Fasting blood samples were collected between 8 and 9 a.m. in a tube without anticoagulant compound to obtain sera aliquots. In brief, after blood coagulation, the tube was submitted to centrifugation (2,000 rpm, 4°C, 10 min), and a minimum of 500 mL of serum was added in Eppendorf's tubes that were stored at −80°C until the cytokines analyses. The volunteers were instructed not to perform physical activities of moderate or vigorous intensity in the 24 h prior to the collection.

Cytokine determination

Cytokine concentrations were determined in the serum samples using multiplex assay (LegendPlex, Biolegend, San Diego, CA, USA) following the manufacturer's instructions. The biomarkers assessed were the tumor necrosis factor-alpha (TNF-α) and the interleukins (IL): IL-6, IL-8, and IL-10. The concentration of these cytokines was calculated using appropriate standard curves (following the manufacturer's instructions). The linearity of multiplex analysis of all cytokines assessed here was, respectively, within the range of 0–10,000 pg/mL, which includes the range of sample determinations. All correlation coefficients of standard curves were in the range of 0.93–0.99, whereas intra-assay coefficients of variance were 2–4% and interassay coefficients of variance were 7–10%. We also calculated the ratio between pro- and anti-inflammatory cytokines to evaluate the systemic inflammatory status (52).

Statistical analysis

Initially, the data obtained in the volunteer groups were compared with the Gauss curve, and the normality of each one was determined using the Shapiro–Wilk test, followed by the evaluation of the homogeneity of variance performed through the Levene test. Parametric variables were presented as mean and standard deviation (X ± SD) and were statistically analyzed using the one-way ANOVA with Tukey's *post-hoc* test. Non-parametric variables were presented as a median and interquartile range, and it was statistically analyzed using the Kruskal–Wallis test with Dunn's *post-hoc* test. A multivariate regression analysis was used to determine the influence of body composition and physical performance variables on the systemic inflammatory status, assessed by serum concentration of both pro- and anti-inflammatory cytokines. Pearson's or Spearman's correlation coefficient analysis was used to assess the association between the parameters obtained in the volunteer groups. The significance level was set at 5% ($p < 0.05$).

Results

Table 1 presents the anthropometric and physical characteristics of the volunteers enrolled in the present study. Based on the one-way ANOVA with Tukey's *post-hoc* test, the statistical analysis of these characteristics in the eutrophic, overweight, and obese groups showed that (1) the group with overweight performed less minutes of moderate-intensity physical activity than the eutrophic group; (2) whereas the BMI, calf circumference, and body fat (both in absolute and relative values) values were lower, the FFM values (both in absolute and relative values) were higher in the eutrophic group than the values found in the other volunteer groups; and (3) overweight group presented lower BMI values and a higher percentage of body fat and FFM than the obese group, as well as a higher muscle mass values than the eutrophic group.

Table 2 shows the descriptive values of the physical tests observed in the volunteer groups. Using the one-way ANOVA analysis with Tukey's *post-hoc* test, it was found that higher values in the vertical jump test performance presented as the average of the height reached in the best jump (in centimeters), in the eutrophic group than the values observed in the overweight group. Interestingly, on average, the eutrophic group presented less strength (expressed in watts per kilogram of corporal mass) than the other volunteer groups (overweight and obese) to perform the vertical jump test. In addition, the obese group showed, on average, higher strength to perform this physical test than the values observed in the overweight group. No significant difference was found in the other physical tests assessed here. It is noteworthy to mention that, in accordance with the results obtained in the physical tests and muscle mass measure, none of the older women who participated in the present study presented sarcopenia, based on the criteria proposed by EWGSOP, formerly cited (15).

Figure 2 shows not only the results obtained in the systemic cytokine analysis but also the value of the ratio between the pro- (IL-6, TNF-α, and IL-8) and anti-inflammatory cytokines (IL-10), presented in the median and interquartile range, in the volunteer

TABLE 1 Mean and standard deviation (X_SD) of age, physical activity time, anthropometric characteristics (BMI and CALF), and body composition (body fat kg, body fat%, muscle mass, FFM kg, and FMM%); as well as the results obtained in the statistical analysis between the volunteers separated into eutrophic, overweight, and obese groups, according to their BMI values.

Variable	All women	18.5 ≤ BMI < 25 (a)	25 ≤ BMI < 30 (b)	BMI ≥ 30 (c)	P-value
	$n = 70$	$n = 27$	$n = q0$	$n = 13$	
Age (year)	72.66 (±6.17)	72.52 (±6.13)	73.30 (±5.68)	71.46 (±7.56)	n.s
Physical activity time (min/week*)	578 (±583)	822 (±722)§	408 (±409)	439 (±412)	$^{\S}p = 0.019$
BMI (kg/m^2)	26.46 (±4.18)	22.5 (±1.48)$^{\S,\#}$	27.17 (±1.48)†	33.00 (±2.75)	$^{\S}p < 0.001$ $^{\#}p < 0.001$ $^{\dagger}p < 0.001$
Calf circumference (cm)	35.25 (±2.82)	33.72 (±2.41)$^{\S,\#}$	36.11 (±2.47)	36.80 (±3.06)	$^{\S}p = 0.009$ $^{\#}p = 0.020$
Fat (kg)	24.82 (±8.86)	17.15 (±3.50)$^{\S,\#}$	26.58 (±5.10)†	38.44 (±5.62)	$^{\S}p < 0.001$ $^{\#}p < 0.001$ $^{\dagger}p < 0.001$
Fat (%)	39.24 (±8.25)	32.66 (±5.15)$^{\S,\#}$	40.8 (±5.33)†	50.84 (±5.02)	$^{\S}p < 0.001$ $^{\#}p < 0.001$ $^{\dagger}p < 0.001$
Muscle mass (kg)	18.13 (±2.74)	17.36 (±2.67)§	18.86 (±2.29)	18.12 (±3.61)	$^{\S}p = 0.030$
FFM (kg)	37.01 (±5.84)	35.67 (±5.8)	38.56 (±5.6)	36.34 (±6.0)	n.s.
FFM (%)	61.09 (±8.0)	67.41 (±5.07)$^{\S,\#}$	58.99 (±5.41)†	50.31 (±5.56)	$^{\S}p < 0.001$ $^{\#}p = 0.005$ $\dagger p < 0.001$

*These values were obtained by using IPAQ (International Physical Activity Questionnaire); BMI, body mass index; FFM, fat-free mass. Significances: §to a-b; $^{\#}$to a-c; †to b-c; n.s, no significant.

TABLE 2 Mean and standard deviation (X_SD) obtained in the physical tests (HG, Sit-Up, SJ cm, SJ W/kg). In addition, the significant differences obtained in these parameters in the volunteers were separated into eutrophic, overweight, and obese groups.

Variable	All women	18.5 ≤ BMI < 25 (a)	25 ≤ BMI < 30 (b)	BMI ≥ 30 (c)	P-value
	$n = 70$	$n = 27$	$n = 30$	$n = 13$	
HG (kgf)	22.16 (±4.6)	21.79 (±3.28)	22.17 (±4.77)	22.88 (±6.56)	n.s
Sit-up (s)	9.86 (±2.73)	9.34 (±2.37)	10.13 (±3.05)	10.37 (±2.71)	n.s
SJ (cm)	11.61 (±4.14)	13.29 (±4.59)§	10.65 (±3.18)	10.33 (±4.22)	$^{\S}p = 0.014$
SJ (W/kg)	22.62 (±5.04)	20.52 (±5.9)$^{\S,\#}$	23.15 (±3.66)†	25.75 (±3.8)	$^{\S}p = 0.048$ $^{\#}p = 0.007$ $^{\dagger}p = 0.042$

HG, handgrip; Sit-up, sit-to-up test in the chair for five repetitions; SJ, squat jump. Significances: § to a-b; $^{\#}$ to a-c; † to b-c; n.s, no significant.

groups (eutrophic, overweight, and obese). Lower systemic levels of IL-6 (Figure 2A) were found in the overweight group than the values observed in the eutrophic and obese groups. No other significant difference was found in TNF- α (Figure 2B), IL-10 (Figure 2C), and IL-8 (Figure 2D) values between the volunteer groups. Based on the ratio analysis, a significant reduction of the ratios was found between IL-10/IL-6 (Figure 2E), IL-10/TNF-α (Figure 2F), and IL-10/IL-8 (Figure 2G) in the overweight group as compared to the values observed in the eutrophic group. The values obtained in the analysis of the systemic cytokine concentrations in the volunteer groups are presented in Supplementary Table S1.

Table 3 shows the results of the multiple linear regression analysis with adjustment for the time of physical activity, anthropometric and body composition measurements, and physical tests, as well as the cytokine analyses in the volunteers who participated in this study, regardless of the BMI values. It was observed negative associations between the time of moderate-intensity physical activity in the week and IL-8 values, between the percentage of body fat and the IL-10/IL-6 ratio, between the free fat mass (in absolute values) and the IL-10/IL-8 ratio, and between vertical jump (cm) test and TNF-α. Conversely, positive associations were found between the time of moderate-intensity physical activity in the week and TNF-α values, between the percentage of fat-free mass and IL-10 values, between IL-6 and handgrip or vertical jump (cm) tests, and between IL-8 and vertical jump (cm) test.

Since significant associations were found in multiple linear regression analysis between anthropometric and body composition, as well as physical tests and the cytokines assessed here, we followed the correlation analysis between these data obtained in each volunteer group. Figures 3, 4 present the significant results found in this analysis in eutrophic and obese groups,

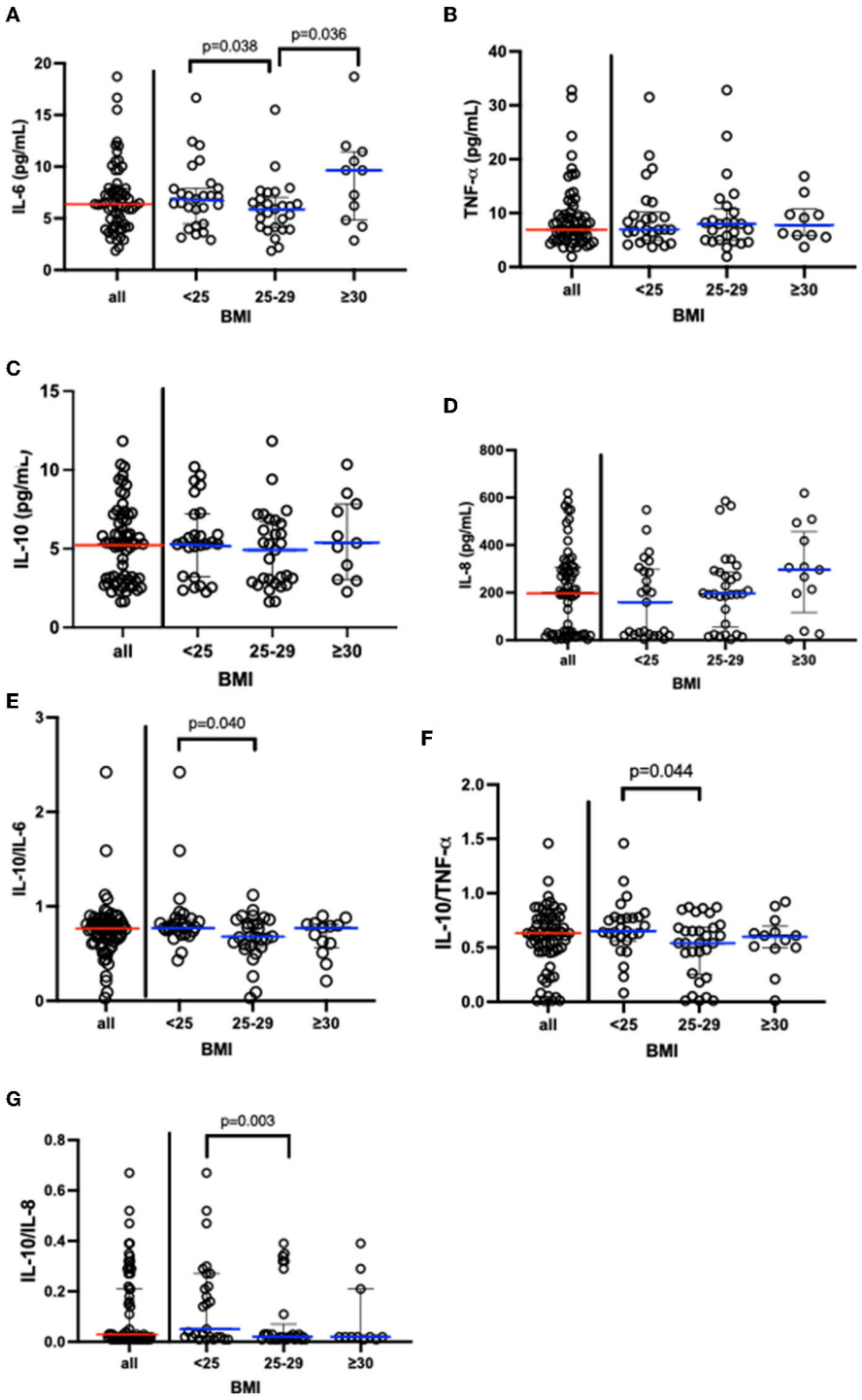

FIGURE 2
Results [median and interquartile range (X_25–75)] concerning of systemic cytokine concentration **(A)** IL-6, **(B)**TNF-α, **(C)** IL-10, **(D)** IL-8; and also the ratio between **(E)** IL-10/IL-6, **(F)** IL-10/TNF-α, **(G)** IL-10/IL-8. In addition, the data obtained when the volunteers were separated into eutrophic, overweight, and obese groups. IL, interleukin; BMI, body mass index; TNF-α, alpha tumor necrosis factor.

respectively. There were no significant correlations in the overweight group.

In the eutrophic group (Figure 3), it was found negative correlations between the IL-10/IL-8 ratio and BMI (Figure 3A) or body fat (in kg, Figure 3B), as well as between IL-6 values and muscle mass (in kg, Figure 3C) or FFM (in kg, Figure 3D). Conversely, a positive correlation was found between the vertical jump test (W/kg) and IL-8 (Figure 3E). Concerning the results obtained in the obese group (Figure 4), negative associations were found between the values of age and the IL-10/IL-8 ratio

TABLE 3 Significant results obtained in the multiple linear regression analysis between the parameters related to the physical activity time, anthropometric measurements, body composition measurements and the physical tests or the cytokine profile assessed in all volunteers enrolled in the study.

Variables	ß-value	95% CI	P-value	R^2
Physical activity time*–TNF-α	0.009789	0.002813 to 0.01676	0.007	0.872
Physical activity time*–IL-8	−0.008502	−0.01541 to −0.001596	0.017	0.797
Fat %—IL-10/IL-6	−16.92	−31.95 to −1.891	0.028	0.815
FFM kg—IL-10/IL-8	−29.47	−54.21 to −4.739	0.021	0.922
FFM%—IL-10	0.3489	0.05762 to 0.6401	0.020	0.990
HG—IL-6	0.03975	0.003141 to 0.07636	0.034	0.915
SJ cm—IL-6	0.04248	0.009323 to 0.07564	0.013	0.914
SJ cm—TNF-α	−0.01333	−0.02553 to −0.00112	0.033	0.872
SJ cm—IL-8	0.01217	0.0001551 to 0.02419	0.047	0.802

*These values were obtained by using IPAQ (International Physical Activity Questionnaire); FFM, fat-free mass; HG, handgrip; SJ, squat jump; IL, interleukin, TNF-α, alpha tumor necrosis factor.

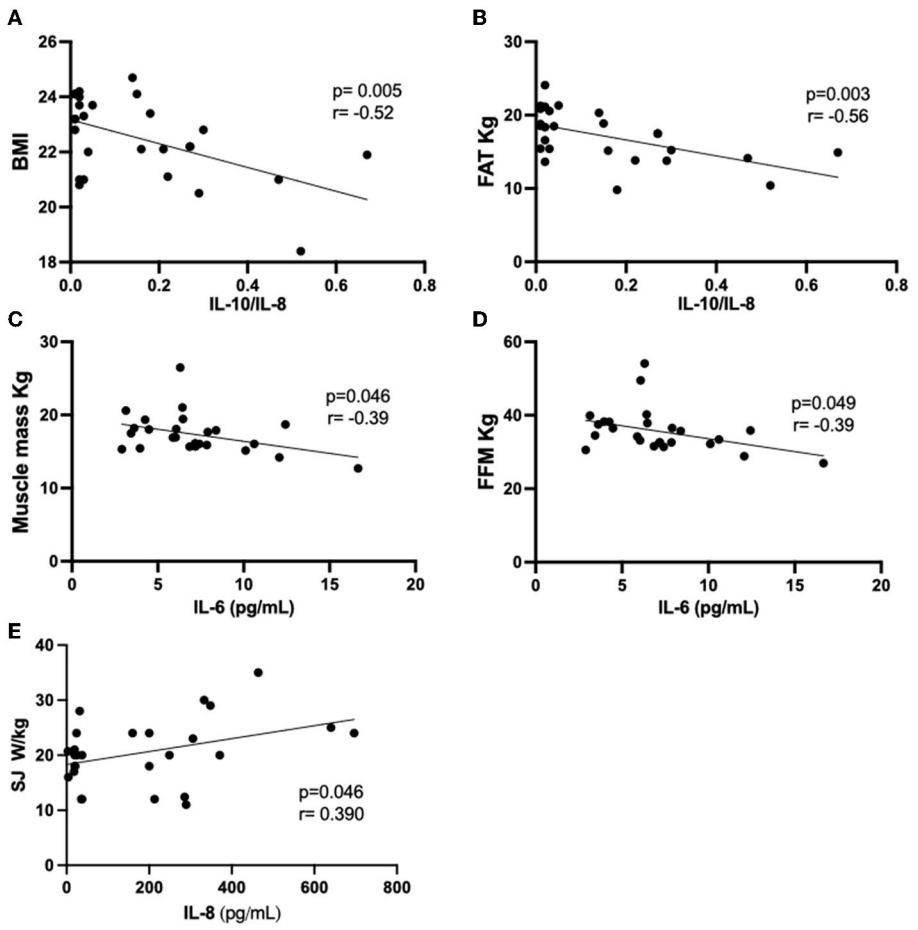

FIGURE 3
Results of the Pearson's coefficient correlation analysis of anthropometric or body composition characteristics with cytokines or cytokine ratio in the eutrophic group. **(A)** BMI AND IL-10/IL-8; **(B)** FAT kg and IL-10/IL-8; **(C)** Muscle mass and IL-6; **(D)** FFM and IL-6; **(E)** SJ W/Kg and IL-8. IL, interleukin; BMI, body mass index; FFM, fat-free mass; SJ, squat jump.

(Figure 4A) and also between the values of the sit-up test and the IL-10/TNF-α ratio (Figure 4C). On the other hand, positive correlations were found between the muscle mass (in kg) and IL-10/IL-6 ratio (Figure 4B) and between the vertical jump test (in cm) and the IL-10/IL-8 ratio (Figure 4D). Supplementary Table S1 presents the correlations between the minutes by the week of

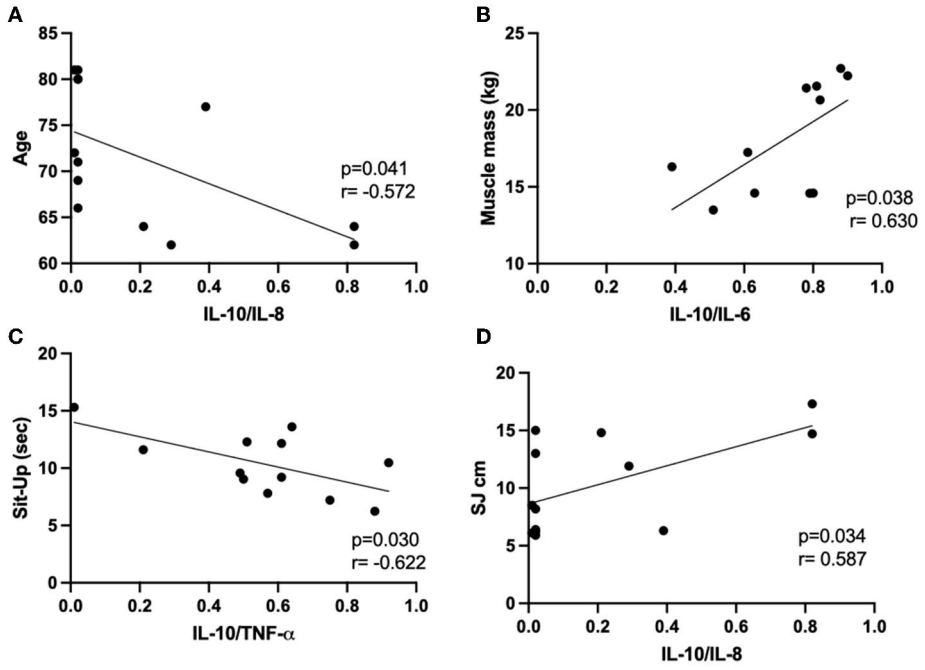

FIGURE 4
Results of the Pearson's coefficient correlation analysis of anthropometric or body composition characteristics with cytokines or cytokine ratio in the obese group. **(A)** age and IL-10/IL-8; **(B)** muscle mass and IL-10/IL-6; **(C)** sit-up and IL-10/TNF-α; **(D)** SJ cm and IL-10/IL-8. IL, interleukin; Sit-Up, sit-up test in the chair for five repetitions; TNF-α, alpha tumor necrosis factor; SJ, squat jump.

moderate physical activities, assessed by IPAQ, and the values of systemic cytokine concentration or their ratios in the eutrophic, overweight, and obese groups. It was possible to observe that there were no significant associations between them.

Concerning the evaluation of the volunteers' physical safety in this study, falls and physical accidents during their performances were not verified. In addition, contact was made by telephone 1 week following the performance test, and we verified that there was no occurrence of discomfort or pain related to vertical jump tests.

Discussion

First of all, it is essential to highlight that the older women who participated in the present study performed on the time of moderate-intensity physical activity, expressed in minutes per week, above the minimum value recommended by the WHO (53), as well as that the clinical evaluations, in association with body composition and physical tests analysis, showed that none of the volunteers had sarcopenia, following the criteria proposed by the EWGSOP (15).

It is utmost of importance to point out that, until now, there are no references that allow classifying "normal" or "health" concentrations of systemic cytokines, particularly in the older adult population (25, 26). Thus, we opted to compare our results with the data available in the literature. For instance, compared with the data presented in the study performed by Lavin et al., which evaluated the serum concentrations of the cytokines in a group of eutrophic older adults who performed combined-exercise training (both aerobics and resistances exercises) and in a group with healthy non-exercisers older adults, the basal values (pg/mL) of IL-6 were 2.0 ± 0.2 and 3.9 ± 1.2, and the basal values of TNF-α were 1.7 ± 0.2 and 1.3 ± 0.2, respectively (54), which were different from the values found in the present study (Supplementary Table S1). Conversely, the serum IL-6 concentration (pg/mL) presented by a healthy population of British women over 50 years was 5.67 ± 2.02 (55), which is similar to the values observed in this study. Beyond these data found in healthy individuals, in studies that evaluated older adults with clinical conditions of evident inflammatory vulnerability, the values of systemic cytokines concentration were different from our findings. For instance, octogenarians admitted with a hip fracture at a hospital in Amsterdam presented 1.61 (1.08–2.42, pg/mL) of serum IL-8 concentration (56), whereas, in an Italian study, the serum mean values of IL-8 and IL-10 (pg/mL), in 18 seniors admitted in a hospital for elective surgeries, were 10.8 ± 4.4 and 5.3 ± 3.7, respectively (57). Moreover, Brazilian men aged 60–80 with systemic arterial hypertension and type 2 diabetes mellitus, both eutrophic and overweight, had serum IL-10 concentrations (pg/mL) with mean values of 90.13 ± 24.37 and 64.34 ± 23.81, TNF-α concentrations of 1.49 ± 0.378 and 3.01 ± 0.448, and IL-10/TNF-α ratios of 8.38 ± 3.06 and 32.49 ± 18.81, respectively (29). In another study, an adult Spanish women group with symptomatic fibromyalgia submitted to a physical exercise program showed, before the intervention, a serum IL-8 concentration of 157 ± 35 pg/mL (58), which were values similar to find in the present study. Hence, in conjunct, these pieces of information corroborate the previous mention that, until now, there are no reference values for the concentrations of the systemic cytokines, which allows us to define "normal" or healthy condition for the older adult population.

Regarding inflammaging development, this phenomenon is closely associated with body fat (12–14) and, interestingly, can be mitigated by regular practice of physical exercise (9, 10). Based on it, to better assess these aspects in an older women population, we grouped the volunteers into three subgroups, based on the WHO body mass index classification (43), and several clinical parameters related to anthropometry, body composition, and physical tests were evaluated. As expected, elevations in body fat values were found in the groups with higher BM, in contrast to the reductions in the FFM percentages when the BMI increases. Interestingly, the overweight group presented a higher muscle mass, in absolute values (kg), than the values found in the eutrophic group. Corroborating this finding, it was reported in the literature that people with raised body fat also showed an increased amount of skeletal muscle mass (59). In this respect, it had been suggested that this associative aspect is related to the fact that these people perform their simple day-to-day tasks, or daily physical activities in a similar way to strength training (60), but with infiltrated fat in their muscular architecture (61, 62).

Beyond these observations, we also observed that the calf circumference in the eutrophic group was lower than the values found in the other volunteer groups. Although calf circumference has been associated with skeletal muscle reserve (62, 63), our finding that the eutrophic group performed better in the vertical jump test, specifically the squat jump (SJ), reaching greater heights (in cm), in association with the lower values found in the power (W/kg) applied to perform this physical test, particularly in comparison with the overweight group, can putatively indicate that the increased time dedicated to the regular practice of physical exercise, observed in this group, could be useful to optimize their performance.

Another point that needs to be cited is related to the observation that the eutrophic group showed increased systemic IL-6 concentration as compared to the values found in the overweight group. Although we cannot affirm, this difference could be putatively associated with the higher time of moderate-intensity weekly physical activity reported by the eutrophic group (2-fold more) since it was reported that IL-6 can be acutely produced during exercise training and it can act as a molecule implicated in the energy metabolism regulation and indirectly mediate an anti-inflammatory effect by enhancing the IL-10 levels (64–66). Based on its prominent capacity to regulate energy metabolism, we can suggest that the higher IL-6 concentration in the eutrophic group could be useful to maintain their anthropometric and body composition since they presented less body fat values and a higher percentage of fat-free fraction as compared to the values found in the overweight group. Furthermore, this higher IL-6 concentration found in the eutrophic group allows us to also suggest that a systemic anti-inflammatory status was generated since this group showed an increased ratio between IL-10, a classical anti-inflammatory cytokine, and the IL-6, TNF-α, and IL-8, all classified as pro-inflammatory cytokines as compared to the values observed in the overweight group, as previously reported in the literature (25, 29).

Conversely, the elevated and chronic maintenance of systemic IL-6 concentration is closely implicated in several unhealthy endpoints, particularly favoring the development of diseases and comorbidities (25, 67, 68). According to the literature, one of the possible sites of IL-6 production is the adipose tissue, which is mainly associated with obesity development (10, 12, 69). These pieces of information can support our findings, in which the obese group presented increased systemic IL-6 concentration that could be derived from their higher mass fat (∼40 and 25% in absolute and relative values, respectively) as compared to the values found in the overweight group. Beyond these differences, interestingly, we also observed that the average vertical jump values measured in terms of the ratio of the power (W) to body mass (kg) were higher in the obese group, demonstrating that they were able to generate greater vertical force in a fraction of a second in the takeoff of the squat jump (35, 70), even though they did not achieve the best performance, assessed by the jump height (in cm). Similarly, when comparing the overweight and eutrophic groups, the former exerted greater power and worse performance (at the height of the jump) in performing the vertical jump. Interestingly, the overweight women had a greater amount of muscle mass than the eutrophic women. These results can reinforce the importance of assessing muscle power in the older adult population, even though the fact that aged people with higher BMI presented a worse performance in the vertical jump height is not a novelty (35, 71). However, until now, it has been not reported that older women with overweight and who regularly practice physical exercises are able to generate greater vertical force and worse performance at jump height than eutrophic women. In this respect, the suggestive explanations for these data obtained in the overweight and obese group could be summarized in two points: (i) during physiological aging, these individuals showed an increase in fatty infiltration in the skeletal muscles, which accentuates the worse quality of muscle fiber contraction and the loss of muscle strength and power, resulting in a reduction in movement performance (61, 72, 73) (ii) and that this condition drives a chronic adaptation in which older adults with more body mass generate a greater amount of fast-twitch muscle fibers (type II fibers) that allowed them to exercise more strength and muscle power to perform their daily activities (60, 74).

Other remarkable findings reported here were obtained through the multiple linear regression analysis. Based on these data, it was possible to prove that the best adaptations to the inflammaging, assessed by the cytokine concentration, were associated with more weekly time dedicated to moderate-intensity physical activity, a lower percentage of body fat, a higher percentage of fat-free mass, and better performance in the physical tests, both handgrip and in the vertical jump test (maximum height in cm). Moreover, the result of a negative association between physical activity time in moderate intensity and IL-8, a pro-inflammatory cytokine, reinforces the literature, indicating that physical exercise is able to favor the generation of a systemic anti-inflammatory status (69, 75). It is also worth mentioning that, in agreement with the studies, elevations in the IL-8 during physical exercise performance can be involved not only in an inflammatory response elicited by muscle contraction (28, 76) but also can be related to angiogenic phenomena that can improve the circulation in the muscle tissue (77). Another negative association found was between the values of FFM (in kg) and the IL-10/IL-8 ratio, which could indicate that a rise in fat-free mass would be associated with an

increase in IL-8 level, corroborating the idea that this interleukin is associated with the emergence of new capillary blood vessels in active skeletal muscle, however, remembering that this cytokine can depict a pro-inflammatory status too (76, 78).

Regarding the aging process, the scientific literature has suggested that the follow-up of adult athletes or even individuals who maintain a regular practice of physical exercises throughout life can be the best way to assess the physiological process of aging (26, 63, 78).

Based on it, the results obtained in the older adult population who participated in this study could be useful to demonstrate some significant aspects of aging, mainly associated with inflammaging. Particularly, the results obtained in the intragroup correlation analysis of the eutrophic group showed significant negative associations between systemic IL-6 concentration and muscle mass or FFM (absolute values in kg), as well as between the IL-10/IL-8 ratio and BMI or body fat (absolute values in kg), which together reveals that the better anthropometric and body composition was associated with a prominent regulation of the systemic inflammatory status. In addition, it was also observed another significant positive correlation between IL-8 and jump power, which can indicate that better performance in the vertical jump test is associated with this inflammatory molecule that can act as an important cytokine involved in the angio-protection of skeletal muscle, as formerly cited (28, 77).

In the obese group, it was observed that the lower weekly time dedicated to physical activity was associated with a higher percentage of body fat and a lower percentage of FFM. Furthermore, negative correlations between age and IL-10/IL-8 and also between the sit-up test (in seconds) and IL-10/TNF-α, as well as positive correlations between muscle mass (absolute values in kg) and IL-10/IL-6 and also between vertical jump and IL-10/IL-8 were found and together can indicate that a regulated systemic inflammatory status is essential to achieve a better performance in some physical tests and improve skeletal muscle mass. Even though the obese group had presented an increase in their systemic IL-6 concentration, which chronically can increase the vulnerability to unhealthy clinical outcomes related to inflammaging (13, 15), our data corroborate the expectation that among older women with obesity, the body fat loss should be desirable (8, 9, 25) and also that better control of inflammatory status can positively impact on physical activity performance.

It is paramount to point out that the absence of significant correlations in the intragroup analysis in the overweight group could be putatively attributed to the fact that this group is composed of older women with adaptive characteristics closer to eutrophic or obesity, which led us to obtain very discrepant results and no significant associations could be found.

In the same way, despite the multivariate analysis showing, in general, positive correlations between higher weekly time dedicated to performing moderate-intensity physical activity and better inflammatory adaptations, in an interesting way, the correlation analysis of volunteer groups did not show statistically significant values between the physical activity time in moderate intensity and the absolute cytokines values or cytokines ratio (Supplementary Table S2), which could be putatively associated with a possible heterogeneity of the results found in the volunteer groups.

Limitations of the study

In the present study, we can highlight as limitations of the study: (1) the lack of results concerning the abdominal circumference, which could be used to better characterize the volunteer groups; (2) the lack of a sedentary group, which could allow us to compare our results and improve our understanding about the inflammaging; (3) even though the IPAQ, used in this study, allowed us to measure the different ways of performing daily physical activities of moderate intensity performed by the volunteers, the use of more technological tools, such as the pedometer and global positioning system (GPS), could improve the measurement of physical activities and occupational factors that could impact on weekly energy expenditure; (4) similarly, despite the clinical evaluation and use of the MNA allowed us to rule out nutritional risk in the volunteers, a more assertive assessment of dietary habits could add relevant information about the nutritional profile of the older adults participating in this study.

Conclusion

Based on the results obtained in this study, we can suggest that (1) long-standing physical exercise performed by older women can favor the regulation of the systemic inflammatory status, which was associated with some positive effects in the anthropometric, body composition, and physical tests; (2) the eutrophic group presented the better physical parameters than overweight (more time of moderate-intensity physical activity, lower amount of body fat, and better performance in vertical jump height), which can be putatively associated with pivotal adaptations of inflammaging (expressed here in terms of pro- and anti-inflammatory cytokine associations); and (3) the obese group presented lower regulation of systemic inflammatory status compared to the overweight group, which can be associated with higher body fat. Further studies including older adult populations with different intrinsic capacities are necessary to improve our understanding of the inflammaging phenomenon.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of the Federal University of Sáo Paulo (approval number 3.623.247) and by the National Research Ethics Committee (number CAEE:218170619.3.0 000.5505). The patients/participants provided their written informed consent to participate in this study.

Author contributions

CS is responsible for managing the study and writing of the article and he was the doctor responsible for the physical and clinical evaluations of the volunteers. GA was responsible for training and physical test familiarization. VP, EA, and JD contributed equally in the collection of blood samples and dosage of interleukins in the blood. FM and TR contributed equally in the evaluation of bioimpedance and performance of physical tests. YJ and NN contributed equally to article writing and literature review. MR and AA-S contributed equally to the statistical analyzes. RV contributed to the writing and revision of the English language. AB and AJ contributed equally to conception and design of the study. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1206989/full#supplementary-material

References

1. WHO. Clinical Consortium on Healthy Ageing 2019. In: *Proceedings of the Report of Consortium Meeting*. Geneva. Available online at: https://www.who.int/PUBLICATIONS/I/ITEM/9789240009752 2019 (accessed March 12, 2023).

2. Levin AT, Hanage WP, Owusu-Boaitey N, Cochran KB, Walsh SP, Meyerowitz-Katz G. Assessing the age specificity of infection fatality rates for COVID-19: systematic review, meta-analysis, and public policy implications. *Eur J Epidemiol.* (2020) 35:1123–38. doi: 10.1007/s10654-020-00698-1

3. Cezario K, Santos C, Filho CMA, Amirato GR, Paixao VD, Almeida EB, et al. Older women who practiced physical exercises before the COVID-19 pandemic present metabolic alterations and worsened functional physical capacity after one year of social isolation. *Healthcare.* (2022) 10:1939. doi: 10.3390/healthcare10091736

4. WHO. *Health Organization: WHO's Work on the UN Decade of Healthy Ageing* (2021–2030). Available online at: https://www.who.int/initiatives/decade-of-healthy-ageing 2019 (accessed March 12, 2023).

5. Calcinotto A, Kohli J, Zagato E, Pellegrini L, Demaria M, Alimonti A. Cellular senescence: aging, cancer, and injury. *Physiol Rev.* (2019) 99:1047–78. doi: 10.1152/physrev.00020.2018

6. Cavanaugh EJ, Richardson J, McCallum CA, Wilhelm M. The predictive validity of physical performance measures in determining markers of preclinical disability in community-dwelling middle-aged and older adults: a systematic review. *Phys Ther.* (2018) 98:1010–21. doi: 10.1093/ptj/pzy109

7. Beard JR, Jotheeswaran AT, Cesari M, Araujo de Carvalho I. The structure and predictive value of intrinsic capacity in a longitudinal study of ageing. *BMJ Open.* (2019) 9:e026119. doi: 10.1136/bmjopen-2018-026119

8. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci.* (2000) 908:244–54. doi: 10.1111/j.1749-6632.2000.tb06651.x

9. Franceschi C, Capri M, Monti D, Giunta S, Olivieri F, Sevini F, et al. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev.* (2007) 128:92–105. doi: 10.1016/j.mad.2006.11.016

10. Fulop T, Larbi A, Dupuis G, Le Page A, Frost EH, Cohen AA, et al. Immunosenescence and inflamm-aging as two sides of the same coin: friends or foes? *Front Immunol.* (2017) 8:1960. doi: 10.3389/fimmu.2017.01960

11. Santoro A, Bientinesi E, Monti D. Immunosenescence and inflammaging in the aging process: age-related diseases or longevity? *Ageing Res Rev.* (2021) 71:101422. doi: 10.1016/j.arr.2021.101422

12. Fulop T, Larbi A, Pawelec G, Khalil A, Cohen AA, Hirokawa K, et al. Immunology of aging: the birth of inflammaging. *Clin Rev Allergy Immunol.* (2023) 64:109–22. doi: 10.1007/s12016-021-08899-6

13. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. *J Gerontol A Biol Sci Med Sci.* (2014) 69 Suppl 1:S4–9. doi: 10.1093/gerona/glu057

14. Baylis D, Bartlett DB, Patel HP, Roberts HC. Understanding how we age: insights into inflammaging. *Longev Healthspan.* (2013) 2:8. doi: 10.1186/2046-2395-2-8

15. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* (2019) 48:16–31. doi: 10.1093/ageing/afz046

16. Kirk B, Miller S, Zanker J, Duque G. A clinical guide to the pathophysiology, diagnosis and treatment of osteosarcopenia. *Maturitas.* (2020) 140:27–33. doi: 10.1016/j.maturitas.2020.05.012

17. Chiles Shaffer N, Ferrucci L, Shardell M, Simonsick EM, Studenski S. Agreement and predictive validity using less-conservative foundation for the National Institutes of health sarcopenia project weakness cutpoints. *J Am Geriatr Soc.* (2017) 65:574–9. doi: 10.1111/jgs.14706

18. Barchetta I, Cimini FA, Ciccarelli G, Baroni MG, Cavallo MG. Sick fat: the good and the bad of old and new circulating markers of adipose tissue inflammation. *J Endocrinol Invest.* (2019) 42:1257–72. doi: 10.1007/s40618-019-01052-3

19. Priest C, Tontonoz P. Inter-organ cross-talk in metabolic syndrome. *Nat Metab.* (2019) 1:1177–88. doi: 10.1038/s42255-019-0145-5

20. Tarkowski A, Bjersing J, Shestakov A, Bokarewa MI. Resistin competes with lipopolysaccharide for binding to toll-like receptor 4. *J Cell Mol Med.* (2010) 14:1419–31. doi: 10.1111/j.1582-4934.2009.00899.x

21. da Silva Alexandre T, Scholes S, Ferreira Santos JL, de Oliveira Duarte YA, de Oliveira C. Dynapenic abdominal obesity increases mortality risk among english and brazilian older adults: a 10-year follow-up of the ELSA and SABE studies. *J Nutr Health Aging.* (2018) 22:138–44. doi: 10.1007/s12603-017-0966-4

22. de Carvalho DHT, Scholes S, Santos JLF, de Oliveira C, Alexandre TDS. Does abdominal obesity accelerate muscle strength decline in older adults? Evidence from the English longitudinal study of ageing. *J Gerontol A Biol Sci Med Sci.* (2019) 74:1105–11. doi: 10.1093/gerona/gly178

23. Maximo RO, de Oliveira DC, Ramirez PC, Luiz MM, de Souza AF, Delinocente MLB, et al. Combination of dynapenia and abdominal obesity affects long-term physical performance trajectories in older adults: sex differences. *Am J Clin Nutr.* (2022) 115:1290–9. doi: 10.1093/ajcn/nqac023

24. Turcotte LP, Fisher JS. Skeletal muscle insulin resistance: roles of fatty acid metabolism and exercise. *Phys Ther.* (2008) 88:1279–96. doi: 10.2522/ptj.20080018

25. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol.* (2011) 11:607–15. doi: 10.1038/nri3041

26. Minuzzi LG, Chupel MU, Rama L, Rosado F, Munoz VR, Gaspar RC, et al. Lifelong exercise practice and immunosenescence: master athletes cytokine response to acute exercise. *Cytokine.* (2019) 115:1–7. doi: 10.1016/j.cyto.2018.12.006

27. Fujio K, Okamura T, Yamamoto K. The Family of IL-10-secreting CD4+ T cells. *Adv Immunol.* (2010) 105:99–130. doi: 10.1016/S0065-2776(10)05004-2

28. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nat Rev Endocrinol.* (2012) 8:457–65. doi: 10.1038/nrendo.2012.49

29. Goncalves CV, Ribeiro IS, Galantini MPL, Muniz IPR, Lima PHB, Santos GS, et al. Inflammaging and body composition: new insights in diabetic and hypertensive elderly men. *Exp Gerontol.* (2022) 170:112005. doi: 10.1016/j.exger.2022.112005

30. Almeida EB, Santos JMB, Paixao V, Amaral JB, Foster R, Sperandio A, et al. L-glutamine supplementation improves the benefits of combined-exercise training on oral redox balance and inflammatory status in elderly individuals. *Oxid Med Cell Longev.* (2020) 2020:2852181. doi: 10.1155/2020/2852181

31. Bachi AL, Suguri VM, Ramos LR, Mariano M, Vaisberg M, Lopes JD. Increased production of autoantibodies and specific antibodies in response to influenza virus vaccination in physically active older individuals. *Results Immunol.* (2013) 3:10–6. doi: 10.1016/j.rinim.2013.01.001

32. Amirato GR, Borges JO, Marques DL, Santos JMB, Santos CAF, Andrade MS, et al. L-glutamine supplementation enhances strength and power of knee muscles and improves glycemia control and plasma redox balance in exercising elderly women. *Nutrients.* (2021) 13:1025. doi: 10.3390/nu13031025

33. Santana MG, de Lira CA, Passos GS, Santos CA, Silva AH, Yoshida CH, et al. Is the six-minute walk test appropriate for detecting changes in cardiorespiratory fitness in healthy elderly men? *J Sci Med Sport.* (2012) 15:259–65. doi: 10.1016/j.jsams.2011.11.249

34. Mijnarends DM, Meijers JM, Halfens RJ, ter Borg S, Luiking YC, Verlaan S, et al. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. *J Am Med Dir Assoc.* (2013) 14:170–8. doi: 10.1016/j.jamda.2012.10.009

35. Santos CAF, Amirato GR, Jacinto AF, Pedrosa AV, Caldo-Silva A, Sampaio AR, et al. Vertical jump tests: a safe instrument to improve the accuracy of the functional capacity assessment in robust older women. *Healthcare.* (2022) 10:323. doi: 10.3390/healthcare10020323

36. Lauretani F, Russo CR, Bandinelli S, Bartali B, Cavazzini C, Di Iorio A, et al. Age-associated changes in skeletal muscles and their effect on mobility: an operational diagnosis of sarcopenia. *J Appl Physiol (1985).* (2003) 95:1851–60. doi: 10.1152/japplphysiol.00246.2003

37. Bautmans I, Knoop V, Amuthavalli Thiyagarajan J, Maier AB, Beard JR, Freiberger E, et al. WHO working definition of vitality capacity for healthy longevity monitoring. *Lancet Healthy Longev.* (2022) 3:e789–e96. doi: 10.1016/S2666-7568(22)00200-8

38. Shephard RJ. Ethics in exercise science research. *Sports Med.* (2002) 32:169–83. doi: 10.2165/00007256-200232030-00002

39. Petrini C. Helsinki 50 years on. *Clin Ter.* (2014) 165:179–81. doi: 10.7417/ct.2014.1729

40. Zerbini CA, Szejnfeld VL, Abergaria BH, McCloskey EV, Johansson H, Kanis JA. Incidence of hip fracture in Brazil and the development of a FRAX model. *Arch Osteoporos.* (2015) 10:224. doi: 10.1007/s11657-015-0224-5

41. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* (2007) 39:175–91. doi: 10.3758/BF03193146

42. Bauer JM, Kaiser MJ, Anthony P, Guigoz Y, Sieber CC. the mini nutritional assessment–its history, today's practice, and future perspectives. *Nutr Clin Pract.* (2008) 23:388–96. doi: 10.1177/0884533608321132

43. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation on Obesity*. Geneva: WHO (1997).

44. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* (2003) 35:1381–95. doi: 10.1249/01.MSS.0000078924.61453.FB

45. Hallal PC, Victora CG. Reliability and validity of the International Physical Activity Questionnaire (IPAQ). *Med Sci Sports Exerc.* (2004) 36:556. doi: 10.1249/01.MSS.0000117161.66394.07

46. Sipers W, de Blois W, Schols J, van Loon LJC, Verdijk LB. Sarcopenia is related to mortality in the acutely hospitalized geriatric patient. *J Nutr Health Aging.* (2019) 23:128–37. doi: 10.1007/s12603-018-1134-1

47. Paixao V, Almeida EB, Amaral JB, Roseira T, Monteiro FR, Foster R, et al. Elderly subjects supplemented with L-glutamine shows an improvement of mucosal immunity in the upper airways in response to influenza virus vaccination. *Vaccines.* (2021) 9:107. doi: 10.3390/vaccines9020107

48. Guralnik JM, Seeman TE, Tinetti ME, Nevitt MC, Berkman LF. Validation and use of performance measures of functioning in a non-disabled older population: MacArthur studies of successful aging. *Aging.* (1994) 6:410–9. doi: 10.1007/BF03324272

49. Abizanda P, Navarro JL, Garcia-Tomas MI, Lopez-Jimenez E, Martinez-Sanchez E, Paterna G. Validity and usefulness of hand-held dynamometry for measuring muscle strength in community-dwelling older persons. *Arch Gerontol Geriatr.* (2012) 54:21–7. doi: 10.1016/j.archger.2011.02.006

50. Alley DE, Shardell MD, Peters KW, McLean RR, Dam TT, Kenny AM, et al. Grip strength cutpoints for the identification of clinically relevant weakness. *J Gerontol A Biol Sci Med Sci.* (2014) 69:559–66. doi: 10.1093/gerona/glu011

51. Loturco I, Pereira LA, Kobal R, Kitamura K, Cal Abad CC, Marques G, et al. Validity and usability of a new system for measuring and monitoring variations in vertical jump performance. *J Strength Cond Res.* (2017) 31:2579–85. doi: 10.1519/JSC.0000000000002086

52. Goswami B, Rajappa M, Mallika V, Shukla DK, Kumar S. TNF-alpha/IL-10 ratio and C-reactive protein as markers of the inflammatory response in CAD-prone North Indian patients with acute myocardial infarction. *Clin Chim Acta.* (2009) 408:14–8. doi: 10.1016/j.cca.2009.06.029

53. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. *Br J Sports Med.* (2020) 54:1451–62. doi: 10.1136/bjsports-2020-102955

54. Lavin KM, Perkins RK, Jemiolo B, Raue U, Trappe SW, Trappe TA. Effects of aging and lifelong aerobic exercise on basal and exercise-induced inflammation. *J Appl Physiol (1985).* (2020) 128:87–99. doi: 10.1152/japplphysiol.00495.2019

55. Wei J, Xu H, Davies JL, Hemmings GP. Increase of plasma IL-6 concentration with age in healthy subjects. *Life Sci.* (1992) 51:1953–6. doi: 10.1016/0024-3205(92)90112-3

56. van Munster BC, Bisschop PH, Zwinderman AH, Korevaar JC, Endert E, Wiersinga WJ, et al. Cortisol, interleukins and S100B in delirium in the elderly. *Brain Cogn.* (2010) 74:18–23. doi: 10.1016/j.bandc.2010.05.010

57. CapriM, Yani SL, Chattat R, Fortuna D, Bucci L, Lanzarini C, et al. Pre-operative, high-IL-6 blood level is a risk factor of post-operative delirium onset in old patients. *Front Endocrinol.* (2014) 5:173. doi: 10.3389/fendo.2014.00173

58. Ortega E, Garcia JJ, Bote ME, Martin-Cordero L, Escalante Y, Saavedra JM, et al. Exercise in fibromyalgia and related inflammatory disorders: known effects and unknown chances. *Exerc Immunol Rev.* (2009) 15:42–65.

59. Vilaca KH, Carneiro JA, Ferriolli E, Lima NK, de Paula FJ, Moriguti JC. Body composition, physical performance and muscle quality of active elderly women. *Arch Gerontol Geriatr.* (2014) 59:44–8. doi: 10.1016/j.archger.2014.02.004

60. Hickey MS, Carey JO, Azevedo JL, Houmard JA, Pories WJ, Israel RG, et al. Skeletal muscle fiber composition is related to adiposity and in vitro glucose transport rate in humans. *Am J Physiol.* (1995) 268:E453–7. doi: 10.1152/ajpendo.1995.268.3.E453

61. Manini TM, Clark BC. Dynapenia and aging: an update. *J Gerontol A Biol Sci Med Sci.* (2012) 67:28–40. doi: 10.1093/gerona/glr010

62. Reid KF, Fielding RA. Skeletal muscle power: a critical determinant of physical functioning in older adults. *Exerc Sport Sci Rev.* (2012) 40:4–12. doi: 10.1097/JES.0b013e31823b5f13

63. Barbosa-Silva TG, Menezes AM, Bielemann RM, Malmstrom TK, Gonzalez MC, Grupo de Estudos em Composicao Corporal e N. Enhancing SARC-F: improving sarcopenia screening in the clinical practice. *J Am Med Dir Assoc.* (2016) 17:1136–41. doi: 10.1016/j.jamda.2016.08.004

64. Wei J, Jiao J, Chen CL, Tao WY, Ying YJ, Zhang WW, et al. The association between low calf circumference and mortality: a systematic review and meta-analysis. *Eur Geriatr Med.* (2022) 13:597–609. doi: 10.1007/s41999-021-00603-3

65. Chow LS, Gerszten RE, Taylor JM, Pedersen BK, van Praag H, Trappe S, et al. Exerkines in health, resilience and disease. *Nat Rev Endocrinol.* (2022) 18:273–89. doi: 10.1038/s41574-022-00641-2

66. Wedell-Neergaard AS, Lang Lehrskov L, Christensen RH, Legaard GE, Dorph E, Larsen MK, et al. Exercise-induced changes in visceral adipose tissue mass are regulated by IL-6 signaling: a randomized controlled trial. *Cell Metab.* (2019) 29:844–55 e3. doi: 10.1016/j.cmet.2018.12.007

67. Pedersen BK. Exercise-induced myokines and their role in chronic diseases. *Brain Behav Immun.* (2011) 25:811–6. doi: 10.1016/j.bbi.2011.02.010

68. Tyrrell DJ, Goldstein DR. Ageing and atherosclerosis: vascular intrinsic and extrinsic factors and potential role of IL-6. *Nat Rev Cardiol.* (2021) 18:58–68. doi: 10.1038/s41569-020-0431-7

69. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. *Physiol Rev.* (2008) 88:1379–406. doi: 10.1152/physrev.90100.2007

70. Loturco I, Pereira LA, Kobal R, Abad CCC, Komatsu W, Cunha R, et al. Functional screening tests: interrelationships and ability to predict vertical jump performance. *Int J Sports Med.* (2018) 39:189–97. doi: 10.1055/s-0043-122738

71. Moore BA, Bemben DA, Lein DH, Bemben MG, Singh H. Fat mass is negatively associated with muscle strength and jump test performance. *J Frailty Aging.* (2020) 9:214–8. doi: 10.14283/jfa.2020.11

72. Larsson L, Sjodin B, Karlsson J. Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22–65 years. *Acta Physiol Scand.* (1978) 103:31–9. doi: 10.1111/j.1748-1716.1978.tb06187.x

73. Argaud S, Pairot de Fontenay B, Blache Y, Monteil K. Explosive movement in the older men: analysis and comparative study of vertical jump. *Aging Clin Exp Res.* (2017) 29:985–92. doi: 10.1007/s40520-016-0660-0

74. Tanner CJ, Barakat HA, Dohm GL, Pories WJ, MacDonald KG, Cunningham PR, et al. Muscle fiber type is associated with obesity and weight loss. *Am J Physiol Endocrinol Metab.* (2002) 282:E1191–6. doi: 10.1152/ajpendo.00416.2001

75. Severinsen MCK, Pedersen BK. Muscle-organ crosstalk: the emerging roles of myokines. *Endocr Rev.* (2020) 41:594–609. doi: 10.1210/endrev/bnaa016

76. Keane MP, Arenberg DA, Lynch JP 3rd, Whyte RI, Iannettoni MD, Burdick MD, et al. The CXC chemokines, IL-8 and IP-10, regulate angiogenic activity in idiopathic pulmonary fibrosis. *J Immunol.* (1997) 159:1437–43. doi: 10.4049/jimmunol.159.3.1437

77. Fousek K, Horn LA, Palena C. Interleukin-8: a chemokine at the intersection of cancer plasticity, angiogenesis, and immune suppression. *Pharmacol Ther.* (2021) 219:107692. doi: 10.1016/j.pharmthera.2020.107692

78. Mikkelsen UR, Couppe C, Karlsen A, Grosset JF, Schjerling P, Mackey AL, et al. Life-long endurance exercise in humans: circulating levels of inflammatory markers and leg muscle size. *Mech Ageing Dev.* (2013) 134:531–40. doi: 10.1016/j.mad.2013.11.004

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Prevalence of associations among sarcopenia, obesity, and metabolic syndrome in Brazilian older adults

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Background: Although aging is a process associated with the development of obesity, metabolic syndrome (MetS), and sarcopenia, the prevalence of these conditions in older adults from São Paulo, Brazil, is unclear.

Methods: Therefore, the current study aimed to investigate the prevalence of obesity, sarcopenia, and MetS, both separately and together, in a community-based sample of older adults from São Paulo, Brazil. Data from the medical records of 418 older adults of both genders, aged 60 years or older (mean age 69.3 ± 6.5 years), who were not physically active, were used to conduct this retrospective cross-sectional study. Anthropometric variables were used to determine both body mass index (BMI) and Conicity index (C index). Sarcopenia and MetS were defined according to the criteria of the European Working Group on Sarcopenia in Older People and by the Brazilian Society of Endocrinology and Metabolism, respectively.

Results: Based on BMI, the group of older men ($n = 91$) showed a predominance of adequate weight ($n = 49$) and the group of older women ($n = 327$) showed a predominance of obesity ($n = 181$). In association with obesity, while only the group of older women presented with sarcopenia ($n = 5$), 52 older women and 9 older men presented with MetS, and two older women presented with sarcopenia + MetS [prevalence ratio = 0.0385, 95% CI (0.007;0.1924)]. Based on the C index, 58 older women and 11 older men presented with MetS, while the occurrence of sarcopenia or MetS + sarcopenia was found in 32 and 5 older women, respectively [prevalence ratio = 0.0910, 95% CI (0.037;0.2241)].

Discussion: Our results suggest that obesity, as measured by BMI or the C Index, was more closely associated with the occurrence of MetS than sarcopenia, regardless of gender, and also that sarcopenic obesity was only found in the group of older women. Additionally, the prevalence ratio of obesity, sarcopenia, and MetS evidenced using the C index was 2.3 times higher than the values found using the BMI classification.

KEYWORDS

metabolic syndrome, sarcopenia, sarcopenic obesity, Conicity index, body mass index, obesity prevalence, aging

1. Introduction

Population aging is one of the most impactful global changes in different societies. For instance, in 1991, the number of people aged 60 and over represented 7.3% of the total population, whereas in 2025, this group will represent 15%. According to global projections, the number of older adults could reach 2 billion individuals in 2050, representing 21.5% of the world's population (1, 2).

Among several characteristics, it is widely accepted that aging or senescence is a natural, dynamic, progressive, and therefore inevitable phenomenon in which morphological, functional, biochemical, and psychological alterations can be observed, resulting from the interaction of a series of variables, such as genetics, lifestyle, and diseases (3). With regard to lifestyle, there is convincing evidence that physical inactivity can promote metabolic syndrome (MetS), which predisposes to increased risk factors for the development of chronic diseases and comorbidities associated with aging, such as metabolic syndrome (4).

According to the World Health Organization (WHO), MetS can be fundamentally defined by clinical and laboratory data, and its worldwide prevalence is approximately 25 to 35% (5, 6). The literature points out that one of the factors that may potentiate the occurrence of MetS in older adults is the development of obesity associated with aging (7). The excessive increase in body weight due to the accumulation of fat in adipose tissue, a fact that characterizes obesity, varies between 20 and 40% in the older adult population, depending on the evaluation model used. It has been emphasized that the increase in the manifestation of obesity in older adults is associated, among other factors, with a significant reduction in the level of daily physical activity, which can even lead individuals to become sedentary (8).

In particular, the decline in physical activity observed in the older adult population is closely associated with the progressive loss of skeletal muscle mass, which can vary between 10 and 40%. Corroborating this information, studies have reported a 30 to 50% decrease in muscle mass in individuals between the ages of 40 and 80. This reduction is linked to a significant loss of functional capacity of approximately 3% per year after the age of 60. According to the literature, the reduction in skeletal muscle mass and loss of muscle strength (defined as dynapenia) associated with reduced physical mobility characterize the occurrence of the geriatric syndrome called sarcopenia. Sarcopenia is related to clinical outcomes such as loss of mobility, increased risk of falls, frailty syndrome, cardiovascular disease, neurodegenerative disease, and osteoporosis, and is also a predictor of mortality in older adults (9).

Recent epidemiologic studies have highlighted the possible coexistence of a sarcopenic condition in older adults with obesity, a situation called sarcopenic obesity (OS), which occurs mainly in individuals over 55 years of age (10).

Since obesity is a prominent factor in both MetS and sarcopenia in older adults, it is very important to correctly define its occurrence. According to the WHO, obesity can be defined as abnormal or excessive fat accumulation that poses a health risk. In addition to the BMI index, central obesity, which is common in older adults, can be estimated by the conicity index (C index), proposed by Valdez (11) in the early 1990s, as an indicator of adiposity and body fat distribution, especially in older adults (12).

Although it is possible to find reports on MetS and sarcopenia in the older adult population, studies aimed at comparing different models of obesity assessment and their association with the occurrence of MetS and sarcopenia in this population are still scarce in the literature. Therefore, the aim of the current study was, first, to evaluate the presence of obesity using two different models and, second, to correlate the presence of obesity with the manifestations of MetS and sarcopenia, either alone or together, in older adults.

2. Methods

2.1. Study population

This study has a retrospective cross-sectional design, based on the medical records of older adults attending the "Centro de Referência do Idoso – Casa do Idoso," an institution belonging to the Municipal Social Assistance Office in São José dos Campos City, São Paulo, Brazil. The sample consisted of 418 individuals of both genders, aged 60 years and older at the time of data collection, without a diagnosis of chronic degenerative disease, type 1 diabetes, neoplasm, respiratory, renal, or liver disease, autoimmune, infectious, and/or neurological disease, and who were not engaged in any exercise training program.

It is paramount to mention that we obtained data from the population aged 60 years and older because, according to the Brazilian Ministry of Health, these individuals are considered older adults. Moreover, in the present study, we did not include older adults who reported regular engagement in any exercise training program, since it is widely known that the regular practice of exercise training is beneficial for everybody, particularly the older adult population, and can improve several physiological, metabolic, and physical characteristics. In addition, the older adults who were physically active reported regular engagement in a wide range of exercise training programs, which included types, intensities, and modalities that did not allow us to adequately separate them into one or even two or three groups.

The primary endpoint was the diagnosis of MetS, and the secondary endpoint was sarcopenia. To assess the associations between MetS and sarcopenia, the BMI was measured at baseline, and the prevalence of the association between MetS and sarcopenia was determined for ranges of BMI. Figure 1 shows the analysis process performed.

2.2. Anthropometric measurements

The older adult participants were clinically evaluated by the same geriatrician responsible for each "Centro de Referência do Idoso – Casa do Idoso." Data on the age, gender, race, body weight (kg), height (cm), and waist circumference (cm) of each subject were recorded in the initial database. Body composition was assessed using body mass index (BMI), which represents a diagnostic criterion for obesity, and muscle mass, which was defined using Lee's equation (13), as shown below.

2.3. Body mass index calculation

Older adults were categorized by BMI, according to the stratification developed by Adams et al. (14) as follows:

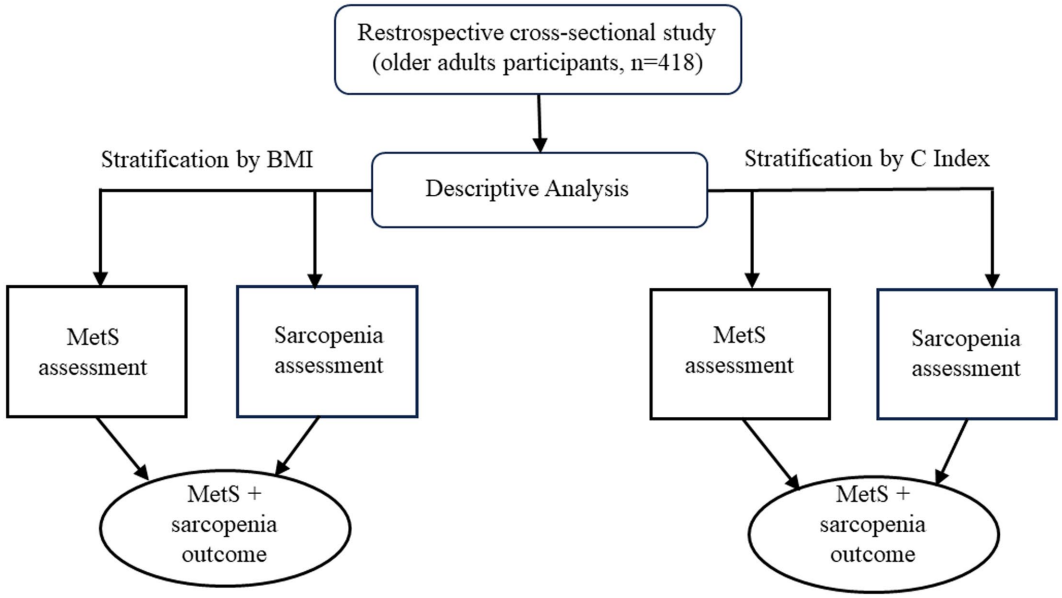

FIGURE 1
Flow diagram of the study design. BMI, Body Mass Index; C Index, Conicity Index; MetS, Metabolic Syndrome.

underweight = BMI < 18.5 kg/m^2; adequate weight = BMI between 18.5 and 24.9 kg/m^2; overweight = BMI between 25 and 29.9 kg/m^2; and obesity = BMI > 30 kg/m^2.

2.4. Conicity index calculation

The Conicity index (C index) was used to assess the visceral fat adipose mass as a measure of central adiposity obesity. This index was chosen because of the ease of interpretation of the values obtained. For instance, a C index of 1.25 indicates that the individual has a waist circumference 25% greater than the circumference of a cylinder with the same height, weight, waist circumference, and human body density. This index also offers the highest level of discrimination between MetS and sarcopenia.

The cut-off points were C Index values >1.25 for men and C Index >1.18 for women (15).

2.5. Assessment of metabolic syndrome

To determine the manifestation of metabolic syndrome (MetS), the criteria proposed by the Brazilian Society of Endocrinology and Metabolism were adopted (16), as also discussed in Freitas et al. (4).

2.6. Assessment of sarcopenia

The assessment of the occurrence of sarcopenia followed the criteria recently presented by the European Working Group on Sarcopenia in Older People (EWGSOP) (9). Muscle strength was determined using the handgrip test, with values < 29 kg for men and < 17 kg for women indicating low muscle strength. Muscle mass was estimated using appendicular skeletal muscle mass (aSMM) according to Lee's equation (13), as described below:

$$aSMM = 0.244 * body\ weight + 7.8 * height + 6.6 * gender - 0.098 * age + (race - 3.3)$$

where body weight was measured in kilograms and height in meters; regarding gender, a value of 0 was used for women and 1 for men; regarding race, 0 was used for white people or Hispanics, 1.4 for African-Americans, and − 1.2 for Asian people. After applying the equation, the values obtained were divided by the square of the height (m^2) of each subject to calculate the muscle mass index for each participant. The characterization of the presence of low muscle mass was defined when the values reached < 7 kg/m^2 for men and < 5.5 kg/m^2 for women.

2.7. Statistical analysis

Continuous variables were expressed as mean and standard deviation (X ± SD), and the number of subjects with MetS and sarcopenia was used to express prevalence data.

A McNemar's test with continuity correction was used to test the hypothesis of an association between MetS and sarcopenia in the older adult population based on the BMI classification and C Index. In addition, both the BMI and C Index were used to compute the prevalence ratios (P_R) for each group of participants (older women and older men), separated by their BMI and C Index values, showing an association with MetS and sarcopenia in comparison to those who did not show an association.

The statistical analysis was carried out using GraphPad Prism version 10.0 at a significance level of alpha = 5.0% ($p < 0.05$).

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TABLE 1 Anthropometric, body composition, and clinical characteristics data of the older adult participants in this study.

Groups	Participants		
Variables	Total *n* = 418 (100.0%)	Men *n* = 91 (21.7%)	Women *n* = 327 (78.3%)
Age (years)	69.3 ± 6.5	69.9 ± 6.4	69.1 ± 6.6
Weight (kg)	68.9 ± 13.7	77.0 ± 16.6	66.9 ± 12.1
Height (cm)	157 ± 9.0	167 ± 7.0	154 ± 6.0
Body Mass Index (BMI, kg/m^2)	27.9 ± 4.7	27.4 ± 4.6	28.1 ± 4.8
Waist Size (cm)	92.97 ± 13.8	96.45 ± 13.9	92.12 ± 14.1
Conicity Index (CI)	1.29 ± 0.14	1.31 ± 0.12	1.28 ± 0.15
Appendicular Skeletal Muscle Mass (aSMM, kg/m^2)	8.19 ± 1.55	10.13 ± 1.20	7.71 ± 1.21
Handgrip (kg)	23.6 ± 9.1	33.8 ± 11.8	20.8 ± 5.8
Clinical Aspects	27.9 ± 4.7	27.4 ± 4.6	28.1 ± 4.8
Type 2 Diabetes	98 (23.4%)	21 (23.08%)	77 (23.5%)
Hypertension	268 (64.1%)	53 (58.24%)	215 (65.8%)
Altered Cholesterol	184 (44.2%)	31 (34.06%)	153 (46.8%)

Values are presented as mean ± standard deviation (SD), and the clinical variables represent the number of subjects presenting with each clinical aspect.

TABLE 2 The absolute number (*n*) and the percentage (%), total and categorized by gender, of subjects with metabolic syndrome (MetS), sarcopenia, or both (MetS + sarcopenia).

Groups	Total *n* (%)		Older men n (%)		Older women n (%)	
Clinical manifestations	*418* (100.0)		*91* (100.0)		*327* (100.0)	
	NO	YES	NO	YES	NO	YES
Sarcopenia	*371* (88.8%)	*47* (11.2%)	*91* (100%)	*0* (0.0%)	*280* (85.6%)	*47* (14.4%)
MetS	*342* (81.8%)	*76* (18.2%)	*78* (85.7%)	*13* (13.3%)	*264* (80.7%)	*63* (19.3%)
MetS + Sarcopenia	*412* (98.6%)	*6* (1.4%)	*91* (100%)	*0* (0.0%)	*321* (98.1%)	*6* (1.8%)
McNemar's value of *p**	$p = 0.2944$	$p = 0.0116$	$p = 0.3560$	–	$p = 0.5201$	$p = 0.1527$

The significant value of p was set at $p < 0.05$.
*McNemar's test with continuity correction.

3. Results

Table 1 shows the anthropometric, physical, and clinical characteristics of the older adult participants in the present study, both pooled (total) and separated by gender.

Table 2 shows the number of participants with or without MetS and sarcopenia, both pooled (total) and separated by gender, in addition to their prevalence ratio. It was demonstrated that most of the subjects did not present with MetS and/or sarcopenia. It is worth mentioning that the absolute number of older women with MetS was higher than the value found in the group of older men, and the proportional occurrence of this syndrome in the group of older women was almost 1:4 (19.3%), whereas in the group of older men it was 1:6 (13.3%). In addition, because none of the older men presented with sarcopenia, the concomitant occurrence of MetS and sarcopenia was observed only in the group of older women, with a percentage of 1.8%. Interestingly, a McNemar's test performed on the pooled subjects (total group) showed a significant value of p, which suggests an association between the occurrence of sarcopenia and MetS.

Table 3 presents the classification of the participants according to their BMI values, both pooled (total) and separated by gender. According to the values found, it is possible to demonstrate that the majority of older men were classified as having adequate weight, while older women were classified as obese. The table also shows the prevalence of MetS and sarcopenia in the older adult subjects categorized as underweight, adequate weight, or obese according to their BMI values. Regarding the group of older men, it was found that the participants with adequate weight or obesity had MetS, and the number of older adults with obesity with MetS was almost two times higher than those with adequate weight. As previously noted, none of these subjects presented with sarcopenia. However, in relation to the older women group, the occurrence of MetS was verified in all groups when separated by their nutritional status. It is important to point out that the number of older women with MetS increased concomitantly with the increase in BMI values; thus, the obese subgroup showed the highest number of individuals with MetS. Specifically, regarding the occurrence of sarcopenia, the adequate weight subgroup had the highest incidence of this clinical condition, followed by the underweight group and the obese group. Interestingly, the number of older women with MetS and sarcopenia was similar between the subgroups. Finally, in addition to the significant p-values shown in McNemar's analysis, except in the group of older men with adequate weight, the prevalence ratios observed in the group of older women with obesity were 17- and

TABLE 3 The absolute number (*n*) and the percentage (%) of subjects with or without metabolic syndrome (MetS), sarcopenia, or both (MetS + sarcopenia), separated by gender and categorized by the BMI classification into underweight (< 18.5 kg/m2), adequate weight (between 18.5 and 24.9 kg/m2), and obese (> 30 kg/m2).

Groups	Volunteers (*n* = 418)					
	Underweight *n* (%)		Adequate weight *n* (%)		Obesity *n* (%)	
Clinical manifestations	Older men 4 (4.4)	Older women 24 (7.34)	Older men 49 (53.85)	Older women 122 (37.31)	Older men 38 (41.76)	Older women 181 (55.35)
Sarcopenia	*0* (0.0)	*16* (4.9)	*0* (0.0)	*26* (7.9)	*0* (0.0)	*5* (1.53)
MetS	*0* (0.0)	*2* (0.6)	*4* (4.4)	*9* (2.75)	*9* (9.9)	*52* (15.9)
MetS + Sarcopenia	*0* (0.0)	*2* (0.6)	*0* (0.0)	*2* (0.61)	*0* (0.0)	*2* (0.6)
None	*4* (100)	*8* (2.45)	*45* (49.45)	*90* (27.5)	*29* (31.8)	*126* (38.53)
McNemar's value of *p**	––	*p = 0.0022*	*p = 0.1336*	*p = 0.0058*	*p = 0.0077*	*p = 0.0001*
Prevalence Ratio [95% CI]	––	$P_R = 1.5$ [0.54;4.16]	––	$P_R = 0.2343$ [0.06;0.86]	––	$P_R = 0.0385$ [0.008;0.22]

The significant value of *p* was set at $p < 0.05$.
*McNemar's test with continuity correction.

TABLE 4 The absolute number (*n*) and percentage (%) of subjects, separated by gender, with C index values above the cut-offs of 1.25 for the older men group and above 1.18 for the older women group, and who also had or did not have metabolic syndrome (MetS), sarcopenia, or both (MetS + sarcopenia).

Groups	Volunteers (*n* = 418)	
	Older men *n* (%)	Older women *n* (%)
Clinical manifestations	C Index > 1.25 *n* = 68 (74.73)	C Index > 1.18 *n* = 284 (86.85)
Sarcopenia	*0* (0.0)	*32* (9.8)
MetS	*11* (12.1)	*59* (18.0)
MetS + Sarcopenia	*0* (0.0)	*5* (1.5)
None	*57* (62.6)	*193* (59.0)
McNemar's value of *p**	*p* = 0.0026	*p* = 0.0064
prevalence ratio	––	$P_R = 0.0910$ [95% CI (0.087; 0.2011)]

The significant value of *p* was set at $p < 0.05$.
*McNemar's test with continuity correction.

6-fold lower than those found in the underweight and adequate weight groups, respectively.

Table 4 shows the prevalence of MetS and sarcopenia in both groups classified as obese according to the C Index cut-off of 1.25 for men and 1.18 for women. It can be observed that only 11 older men presented with MetS, whereas 59 older women had been diagnosed with this syndrome. In addition, while 32 older women had sarcopenia, only five presented with both MetS and sarcopenia. Finally, the McNemar analysis showed significant associations between the outcomes in the two groups of subjects separated by the respective C Index cut-offs for men and women, and the prevalence ratio found in the group with older women was approximately 0.09.

Figure 2 summarizes the main data found in the study.

4. Discussion

In the present study, we demonstrated that when MetS was the primary outcome, among a total of 76 older adults who presented with this syndrome (Table 2), 63 older women and 13 older men also presented with obesity based on their BMI classification (Table 3). In addition, when sarcopenia was the primary outcome, it was found that, among the total of 47 older adults who had this clinical condition (Table 2), only five also had obesity based on BMI classification (Table 3), and they were found exclusively in the group of older women. Finally, it was demonstrated that the concomitant occurrence of MetS and sarcopenia was only observed in six older women, of whom two were underweight, two were of adequate weight, and two were obese (Table 3). Beyond these results, we also showed that, among the total subjects with obesity ($n = 347$), according to the C index (Table 4), the majority of individuals who also presented with MetS were in the group of older women ($n = 59$) compared to that of older men ($n = 11$). Moreover, in the same population that presented with obesity, the occurrence of sarcopenia was detected in 32 older women, while the concomitant occurrence of MetS and sarcopenia was detected in five older women. It is worth highlighting that the values of the prevalence ratio (P_R) found in the group of older women classified as obese by the C index, who also had concomitant MetS and sarcopenia were 2.3 times higher than the values found in the group of older women classified as obese by the BMI values who also had concomitant MetS and sarcopenia. In addition, the significant results obtained in the McNemar test suggest

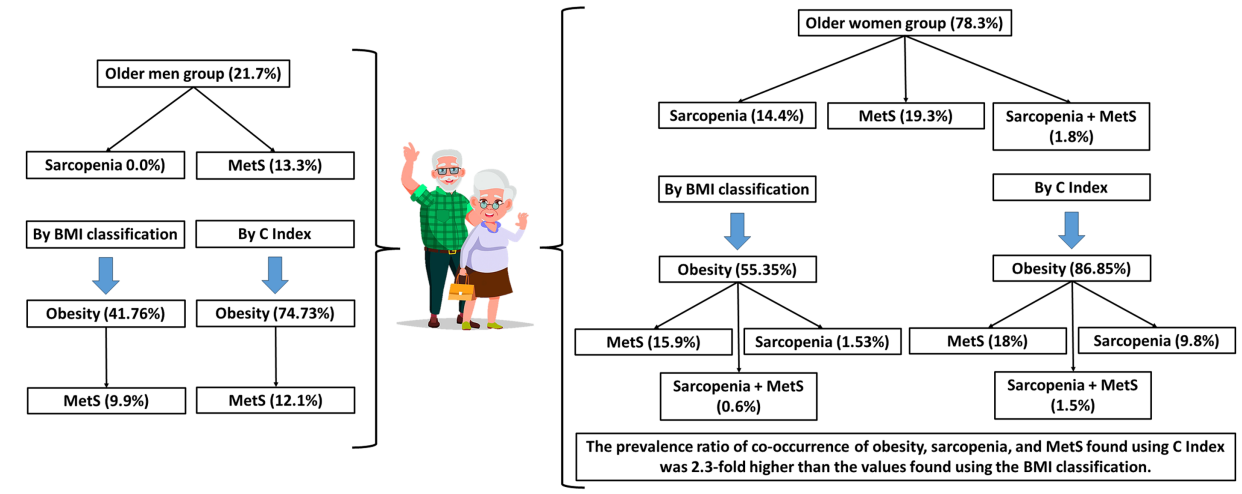

FIGURE 2
Summarized representation of the main results in this study. BMI, Body Mass Index; C Index, Conicity Index; MetS, Metabolic Syndrome.

the existence of a remarkable association between the occurrence of MetS and sarcopenia in the older adult population of participants in this study.

According to the WHO, obesity is a disease in which the accumulation of excess fat in the body can significantly affect human health. In fact, the prevalence of obesity worldwide is so high that the WHO considers it to be the global epidemic of the 21st century. Whether or not urgent action is taken to prevent and treat obesity, it is predicted that more than 50% of the world's population will be obese in 2025. In this respect, the WHO reports that the occurrence of obesity can be defined through the BMI, and this index can also be used to estimate the prevalence of obesity in a population (17). However, it should be noted that although there is a good correlation between BMI and body fat mass, this index does not consider the variation in body fat distribution and may not correspond to the same degree of obesity or associated risks in different individuals and populations. Therefore, the WHO itself advises that BMI values should be interpreted with caution (18, 19).

Particularly in the context of clinical practice aimed at older adults, the evaluation of abdominal obesity by measuring waist circumference may be putatively considered a more important anthropometric measure than BMI to assess the risk of mortality. In agreement with the literature, the presence of visceral obesity is closely associated with the occurrence of dyslipidemia, arterial hypertension, endothelial dysfunction, polycystic ovary syndrome, coronary heart disease, and cerebral vascular disease. In addition, visceral obesity is also directly related to the development of insulin resistance and leads to the development of metabolic syndrome and death (20). In this sense, the use of the C index, as elaborated by Valdez et al. (11), is configured as an effective assessment of visceral obesity since it considers the distribution of central fat. It is paramount to point out that the C index evaluation not only takes into account weight, height, and waist circumference but is also based on the proposition that the accumulation of fat around the waist similar to "cone figure" in the human body (11, 21).

Pereira et al. (22) demonstrated that the C index showed a better association with the presence of MetS in both older men and older women compared to the results obtained with the BMI (22).

As appealing as genetic predisposition may be in determining the higher accumulation of fat in the body in some individuals, it is well known that this accumulation can also occur regardless of the individual's genetics, and, in these situations, a lifestyle with an evident excess of energy intake associated with reduced physical activity leads to fat accumulation, especially in the abdominal region (18).

Reduced levels of daily physical activity or physical inactivity not only increase the risk of obesity but also the risk of sarcopenia (8, 9). With regard to sarcopenia, epidemiologic studies conducted in Brazil showed a prevalence of 14 and 16% in older men and older women, respectively (23). Furthermore, sarcopenia is closely associated with several clinical outcomes, such as loss of mobility, an increased risk of falls, frailty syndrome, cardiovascular disease, neurodegenerative disease, and osteoporosis, and is also a relevant predictor of mortality (9).

Among the various biological processes that induce sarcopenia, we can highlight the decrease in protein synthesis and/or increase in protein degradation, loss of neuromuscular integrity, and increased intramuscular fat content. In fact, the remarkable reduction in muscle mass observed in sarcopenia can be attributed to some factors, such as reduced growth hormone release and physical inactivity, which can influence a lower expression of key proteins involved in protein synthesis and, consequently, an increase in protein degradation, leading to muscle atrophy (9, 24).

The data obtained regarding the presence of sarcopenia in the studied individuals, particularly when stratified by the BMI and C indexes, revealed interesting results. In this sense, it is important to note that: (1) the occurrence of sarcopenia was only detected in the group of older women; (2) most of the older women who presented

with sarcopenia were classified in the group as having adequate weight compared to the obese group, according to the BMI; and (3) the number of older women with obesity, according to the C index, who presented with sarcopenia was higher than the amount of participants found in the group of older women with adequate weight and obesity, both classified according to their BMI. Furthermore, the prevalence ratio [$P_R = 0.0910$ (95% CI 0.037, 0.2211)] observed in the group of older women with obesity, according to the C index, compared to the prevalence ratio [$P_R = 0.0385$ (95% CI [0.0077, 0.1924)] observed in the group of older women with obesity classified based on BMI, allows us to putatively suggest that the C index was more effective in defining the presence of sarcopenia in the older adult population participating in the study.

Specifically in relation to the association between sarcopenia and obesity, the literature shows the occurrence of a phenotype known as *sarcopenic obesity* (SO), especially during aging (10).

It is widely accepted that the manifestation of SO is related to genetic, physiological, and environmental factors. However, studies have demonstrated that some molecular mechanisms associated with SO are dependent on a dynamic balance between positive and negative mediating substances for muscle growth and that this balance impacts the maintenance of mass and skeletal muscle functions (25). Thus, it has been proposed that the occurrence of an imbalance in the following factors may lead to *sarcopenic obesity*: (i) primary metabolic abnormalities leading to increased systemic and muscular oxidative stress, with increased inflammation and insulin resistance; (ii) a consequent decrease in the hormonal balance, which may putatively stimulate a cascade of negative events, such as an increase in muscle catabolic potential; (iii) ectopic lipid deposition, which compromises protein turnover; (iv) mitochondrial dysfunction, causing an increase in oxidative stress, a reduction in the production of ATP, low production of muscle strength, and resistance to the prolonged exercise; (v) functionally altered muscle stem cells that can differentiate from adipocytes with a concomitant increase in inflammation; and (vi) physical inactivity, which is directly related to the control of positive energy balance, muscle oxidation, and protein turnover (26, 27).

Since OS translates as a phenotype caused by an imbalance of several factors, it is worth noting that its occurrence has a deleterious effect on the life of the individual, as it favors both the increased incidence of non-communicable chronic diseases and the low quality of life in these individuals (28). Thus, our observation that the C index was more sensitive than BMI in detecting the occurrence of OS may guide further studies to better define its prevalence.

Based on this information, there is no doubt that the manifestation of sarcopenia and MetS has a negative impact on the quality of life of the older adult population. Therefore, the concomitant manifestation of MetS and sarcopenia in the older adult population with obesity leads to an increased risk of the occurrence of adverse health events when compared to individuals who do not have both of these conditions or even those who have only MetS or sarcopenia (29). In this sense, in the meta-analytic study by Zhang and collaborators (30), around 35% of the non-obese individuals who presented with sarcopenia also manifested MetS, whereas only approximately 22% of the population studied without sarcopenia presented with MetS. In addition, the same authors also reported the existence of a significantly positive odds ratio (OR) between MetS and sarcopenia in the population studied, particularly in those with adequate weight (30).

Even though the above information demonstrates that an association between MetS and sarcopenia can be found in different populations, which could provide important data for medical assistance, a recent study highlighted that heterogeneous aspects of individuals, related to social, biological, and clinical characteristics, in conjunction with other aspects, such as the location and conditions in which the evaluations are conducted, represent key points in the decisions regarding the variables to be used in epidemiological studies since they undoubtedly impact the results obtained in a real context, particularly in relation to clinical practice (31). For instance, in the epidemiological studies that have provided data on the risk factors associated with the occurrence of both sarcopenia and MetS, it is possible to observe a large number of different factors reported in association with a diversity of criteria used, which inevitably generates greater heterogeneity in the results, which in turn can preclude the attainment of consistent conclusions (31–33).

Considering the above, we can emphasize that this study has some strengths related to the sample size and the robust statistical analysis. However, some limitations should be pointed out, such as: (i) the lack of body composition assessment using Dual-energy X-ray Absorptiometry (DXA), which is considered the gold standard for this measure, or even bioelectrical impedance analysis (BIA), which is an alternative and low-cost method often used in a large population; (ii) the lack of comparison of the data obtained in this study with other older adult groups who regularly engage in physical exercise; (iii) the lack of a dietary assessment, which could provide us with relevant information regarding the dietary habits of the older adult participants in this study; and (iv) the cross-sectional nature of this study, which does not allow us to establish cause-and-effect relationships between the data evaluated.

5. Conclusion

Based on the results obtained in the present study, we were able to demonstrate that the occurrence of MetS is higher in older adults who present with obesity, regardless of their gender and the use of the BMI or C index, which agrees with the literature. In addition, we also showed interesting results regarding the presence of sarcopenia, not only because t it was found exclusively in the group of older women but also by the fact that the highest number of participants with this clinical condition belonged to the group with adequate weight and not to the group with obesity, specifically when BMI was used. Additionally, taking into account the data obtained in the analysis of the prevalence ratios, as far as we were able to establish, this is the first study to demonstrate that the C index was more effective than the BMI in identifying the prevalence estimates of the occurrence of obesity and the clinical conditions assessed here (MetS and sarcopenia), both individually and in combination, in an older adult population. Finally, further studies are needed, both to confirm the results presented here and to better understand the use of the C index in relation to the prevalence of obesity, MetS, and sarcopenia in the older adult population.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the patients/ participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

AB conceived the study, analyzed the data, and wrote the first draft together with CS, SV, RP, and CF. LP, CS, AF, LO, and CF participated in the design and development of this study. LP, YJ, JA, and MR participated in the data analysis. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Zaslavsky C, Gus I. Idoso: doença cardíaca e comorbidade. *Arq Bras Cardiol*. (2002) 79:635–9.

2. Martín MAC, Silleras BM, Enciso LC, Marcos SC, de la Torre AM, Río MPR. Changes in body composition in relation to the stage of demaletia in a group of institutionalized elderly. *Nutr Hosp*. (2013) 28:1093–01.

3. SBGG. XIV Congresso Brasileiro de Geriatria e Gerontologia – GERON. Adriano Gordilho (coord.), Salvador – BA; Brazil, (2004).

4. Freitas ED, Fernandes AC, Maledes LL, Pimaleta AM, Velásquez-Meléndez G. Metabolic syndrome: a review on diagnostic criteria. *Rev Min Enferm*. (2008) 12:403–1.

5. Lyra R, Oliveira M, Lins D, CavalcantI N, Gross JL, Maia FFR, et al. Diretrizes Sociedade Brasileira de. *Diabetes*. (2003) 5:21–5.

6. Haffner S, Taegtmeyer H. Obesidade Epidêmica e Síndrome Metabólica. *Circulation*. (2003) 108:1541–5. doi: 10.1161/01.CIR.0000088845.17586.EC

7. Mathus-Vliegen EM. Obesity and the elderly. *J Clin Gastroenterol*. (2012) 46:533–4. doi: 10.1097/MCG.0b013e31825692ce

8. Jiang BC, Villareal DT. Therapeutic and lifestyle approaches to obesity in older persons. *Curr Opin Clin Nutr Metab Care*. (2019) 22:30–6. doi: 10.1097/MCO.0000000000000520

9. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing*. (2019) 48:16–31. doi: 10.1093/ageing/afy169

10. Merriwether EN, Host HH, Sinacore DR. Sarcopenic indices in community-dwelling older adults. *J Geriatr Phys Ther*. (2012) 35:118–5. doi: 10.1519/JPT.0b013e31823c4bef

11. Valdez RA. Simple model-based index of abdominal adiposity. *J Clin Epidemiol*. (1991) 44:955–6. doi: 10.1016/0895-4356(91)90059-I

12. Saklayen MG. The global epidemic of the metabolic syndrome. *Curr Hypertens Rep*. (2018) 20:12. doi: 10.1007/s11906-018-0812-z

13. Lee RC, Wang Z, Heo M, Ross R, Janssen I, HeyMetSfield SB. Total-body skeletal muscle mass: development and cross validation of anthropometric prediction models. *Am J Clin Nutr*. (2000) 72:796–3. doi: 10.1093/ajcn/72.3.796

14. AdaMetS KF, Schatzkin A, Harris TB, Kipnis V, Mouw T, Ballard-Barbash R, et al. Overweight, obesity, and mortality in a large prospective cohort of persons 50 to 71 years old. *N Engl J Med*. (2006) 355:763–8. doi: 10.1056/NEJMoa055643

15. Pimaletel GMC, Wanderley PTQC, Tavares FCLP. Excesso de peso e índice de conicidade em idosos com diabetes mellitus. *R Assoc bras Nutr*. (2020) 11:59–71. doi: 10.47320/rasbran.2020.1662

16. Sociedade Brasileira de Endocrinologia e Metabologia. Síndrome Metabólica, 2020, [acesso em 25abr2020] Disponível em: https://www.endocrino.org.br/a-sindrome-metabolica/

17. WHO (2021). Available at: https://www.who.int/news-room/facts-in-pictures/detail/6-facts-on-obesity#:~:text=Body%20mass%20index%20(BMI)E28093,toormorethan2030.

18. Afonso C IndexPN. Saúde, atividade física e peso corporal: contributo para o seu conhecimento numa amostra da população adulta Portuguesa. Mestrado em Saúde Pública. Faculdade de Medicina e Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto (1999).

19. Abeso. Diretrizes brasileiras de obesidade 2016/ABESO. 4.ed – São Paulo, SP (2016). 1–8.

20. Silveira RE, Santos AS, Sousa MC, Monteiro TSA. Expenses related to hospital admissions for the elderly in Brazil: perspectives of a decade. *Einstein*. (2013) 11:514–0. doi: 10.1590/s1679-45082013000400019

21. Milagres LC, Martinho KO, Milagres DC, Franco FS, Ribeiro AQ, Novaes JF. Waist-to-height ratio and the conicity index are associated to cardiometabolic risk factors in the elderly population. *Cien Saúde Colet*. (2019) 24:1451–61. doi: 10.1590/1413-81232018244.12632017

22. Pereira MWM, Arruda AL, MetS L, KmetS M, KG D, GSA A, et al. Indicadores antropométricos associados a fatores de risco cardiovasculares em idosos. *Rev Eletr Gest Saúde do Idoso*. (2014) 5:3115–31.

23. Alexandre TDS, Duarte YAO, Santos JLF, Wong R, Lebrão ML. Prevalence and associated factors of sarcopenia among elderly in Brazil: findings from the SABE study. *J Nutr Health Aging*. (2014) 18:284–0. doi: 10.1007/s12603-013-0413-0

24. Glass DJ. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat Cell Biol*. (2003) 5:87–90. doi: 10.1038/ncb0203-87

25. Lozano-Montoya I, Correa-Pérez A, Abraha I, Soiza RL, Cherubini A, O'Mahony D, et al. Nonpharmacological interventions to treat physical frailty and sarcopenia in older patients: a systematic overview - the SENATOR project ONTOP series. *Clin Interv Aging*. (2017) 12:721–0. doi: 10.2147/CIA.S132496

26. Kwon H, Pessin JE. Adipokines mediate inflammation and insulin resistance. *Front Endocrinol (Lausanne)*. (2013) 4:71. doi: 10.3389/fendo.2013.00071

27. Trouwborst I, Verreijen A, Memelink R, Massanet P, Boirie Y, Weijs P, et al. Exercise and nutrition strategies to counteract Sarcopenic obesity. *Nutrients*. (2018) 10:605. doi: 10.3390/nu10050605

28. Zamboni M, Mazzali G, Fantin F, Rossi A, Di Francesco V. Sarcopenic obesity: a new category of obesity in the elderly. *Nutr Metab Cardiovasc Dis*. (2008) 18:388–5. doi: 10.1016/j.numecd.2007.10.002

29. Rubio-Ruiz ME, Guarner-Lans V, Pérez-Torres I, Soto ME. Mechanisms underlying metabolic syndrome-related sarcopenia and possible therapeutic measures. *Int J Mol Sci*. (2019) 20:647. doi: 10.3390/ijMetS20030647

30. Zhang H, Lin S, Gao T, Zhong F, Cai J, Sun Y, et al. Association between sarcopenia and metabolic syndrome in middle-aged and older non-obese adults: a systematic review and Meta-analysis. *Nutrients*. (2018) 10:364. doi: 10.3390/nu10030364

31. Zupo R, Moroni A, Castellana F, Gaspari C, Catino F, Lampignano L, et al. A machine-learning approach to target clinical and biological features associated with

sarcopenia: findings from northern and southern Italian aging populations. *Meta*. (2023) 13:565. doi: 10.3390/metabo13040565

32. Tan A, Thomas RL, Campbell MD, Prior SL, Bracken RM, Churm R. Effects of exercise training on metabolic syndrome risk factors in post-menopausal women – a systematic review and meta-analysis of randomized controlled trials. *Clin Nutr*. (2023) 42:337–1. doi: 10.1016/j.clnu.2023.01.008

33. Nishikawa H, Asai A, Fukunishi S, Nishiguchi S, Higuchi K. Metabolic syndrome and sarcopenia. *Nutrients*. (2021) 13:3519. doi: 10.3390/nu13103519

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Prospective sarcopenia outcomes associated with physical performance in individuals aged 55 years and over in Malaysia

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Background: While the potential of physical performance tests as screening tools for sarcopenia is evident, limited information on relevant reference values for sarcopenia detection. In this study, we aimed to establish the prospective relationship between physical performance tests, including time up and go (TUG), functional reach (FR), gait speed (GS), and hand grip strength (HGS) with five-year sarcopenia risk and to determine suitable cut-off values for screening activities.

Method: This was a prospective study utilizing data from the Malaysian Elders Longitudinal Research (MELoR) study, which involved community-dwelling older adults aged 55 years and above at recruitment. Baseline (2013–2015) and wave 3 (2019) data were analyzed. Sarcopenia risk was determined using the strength, assistance walking, rising from a chair, climbing stairs, and falls (SARC-F) tool, with SARC-F ≥ 4 indicating sarcopenia. Baseline physical performance test scores were dichotomized using ROC-determined cut-offs.

Result: Data were available from 774 participants with mean age of 68.13 (SD = 7.13) years, 56.7% women. Cut-offs values for reduced GS, TUG, FR, and HGS were: <0.7 m/s (72.9% sensitivity and 53% specificity), >11.5 s (74.2%; 57.2%), <22.5 cm (73%; 54.2%) and HGS male <22 kg (70.0%; 26.7%) and female <17 kg (70.0%; 20.3%) respectively. Except for FR = 1.76 (1.01–3.06), GS = 2.29 (1.29–4.06), and TUG = 1.77 (1.00–3.13) were associated with increased sarcopenia risk after adjustments for baseline demographics and sarcopenia.

Conclusion: The defined cut-off values may be useful for the early detection of five-year sarcopenia risk in clinical and community settings. Despite HGS being a commonly used test to assess strength capacity in older adults, we advocate alternative strength measures, such as the sit-to-stand test, to be included in the assessment. Future studies should incorporate imaging modalities in the classification of sarcopenia to corroborate current study findings.

KEYWORDS

sarcopenia, physical performance test, SARC-F, older adults, cut-off value

1. Introduction

Sarcopenia has been reported in up to 29 percent of community-dwelling older persons worldwide, though prevalence varies according to definitions, study setting, and population selection (1). The prevalence of sarcopenia is higher among nursing home residents with a 33 percent prevalence previously reported (2, 3). Among older persons with disabilities or those who receive rehabilitation, the prevalence rises to 78 percent (4, 5). The highest prevalence of sarcopenia has been reported in low- and middle-income countries (LMICs) (6) with a 7 to 44 percent prevalence reported in Malaysia (7).

Sarcopenia negatively impacts afflicted older adults' quality of life. The reduction in muscle quality and quantity leads to decreased mobility, and in the longer term, increased dependency. Moreover, sarcopenia has been recognized as an independent condition by the World Health Organization through its listing in the International Classification of Diseases ICD-10 suggesting the need for early prevention and management strategies (8). Loss of functional capacity in older adults manifests as greater difficulties in completing basic tasks such as walking at a regular pace, loss of muscle strength, and mobility impairments. Poor physical performance is associated with sarcopenia (9–11), frailty (12, 13), and cognitive impairment (14–16).

While sarcopenia is a common age-related issue with calls for opportunistic screening in the primary care setting (17), the availability of published cut-off scores for physical performance tests commonly used to predict sarcopenia remains limited. Further, healthcare practitioners currently lack the knowledge or training necessary to identify and manage physical capacity losses as people age (18). Previous studies have utilized short physical performance battery (SPPB), hand grip strength (HGS), and timed up-and-go TUG tests to predict the risk of sarcopenia (11, 19). Poor physical performance in older persons is also related to adverse outcomes in older persons such as falls (12, 20) disability (21) and poor quality of life (22).

In the Asian population, physical performance test cut-off values are largely determined from published studies conducted in the East Asian population (23). Further research from other parts of the continent is needed. In addition, previous studies have addressed cross-sectional detection of the presence of sarcopenia rather than prediction of future risk of sarcopenia. Thus, in this study, we sought to evaluate and establish the predictive ability of the prospective relationship between physical performance tests, including time up and go (TUG), functional reach (FR), gait speed (GS), and hand grip strength (HGS) with five-year sarcopenia for adults aged 55 years and over in Malaysia, using newly established cut-off values. We formulated a hypothesis that the physical performance tests (HGS, TUG, GS, and FR) could accurately predict the risk of sarcopenia after 5-years of follow-up.

2. Materials and methods

2.1. Study design and data source

This prospective observational study utilized baseline (2013 to 2015) and wave 3 follow-up (2019) data from the Malaysian Elders and Longitudinal Research (MELoR) study. The MELoR study is now the Transforming Cognitive Frailty to Later Life Self-sufficiency (AGELESS) study, which was funded by the Ministry of Higher Education Malaysia Long Term Research Grant Scheme (LR005-2019) LRGS/1/2019/UM//1/1.

2.2. Study population

The study population comprised community-dwelling individuals aged 55 years and above identified from the electoral rolls of three neighboring parliamentary constituencies using simple stratified sampling. Further details on recruitment strategies for the MELoR study have been published elsewhere (24). Baseline measurements of physical performance and potential influencing factors were utilized in this study. A total of 1,311 participants (n = 1,311) were initially collected for this study at the baseline. Following a 5-year follow-up period, 537 participants were lost to follow-up, declined or died. Out of the 774 data initially managed for follow-up, 21 were found to be incomplete. We collected a total of 747 complete data entries for analysis. Telephone follow-up was conducted in 2019 by trained interviewers. Sarcopenia outcomes were obtained from the telephone follow-up interviews (Figure 1).

2.3. Baseline assessments

Figure 1 depicts a flowchart of participants and recruitment. The socio-demographic data of age, gender, marital status, medical history, comorbidities, and anthropometric data were collected at baseline ($n = 1,311$). Weight was measured using a digital scale (TANITA type TBF-400). Height was measured using a calibrated standing stadiometer into the nearest centimeter. The body mass index (BMI) was then calculated by weight divided by the body weight in kilograms with the height in meters (kg/m2). Waist and hip circumference were obtained in the standing position using a measuring tape and the waist-to-hip ratio was calculated by dividing the waist measurements with the hip measurements. Older adults aged 55 years and above, residing in the community, demonstrated the ability to stand independently for at least 1 min without any support, walk 7 meters, and have the capability to get in and out of a chair with or without an assistive device were included in the study. However, older adults who were unable to comprehend and follow the instructions for the physical performance tests, those experiencing acute illnesses, and individuals taking medications that could potentially impact their balance during the assessment were excluded from the study.

2.4. Sarcopenia screening tools

In this study, Sarcopenia was determined during telephone follow-up using the SARC-F tool, which consists of the five questions: Strength (S), Assistance walking (A), Rising from a chair (R), Climbing stairs (C), and Falls (F), rated from "not at all" to "extremely difficult" on a scale of 0 to 2. The suggested cut-off for the presence of sarcopenia is four points out of the maximal total score of 10 (25, 26). SARC-F

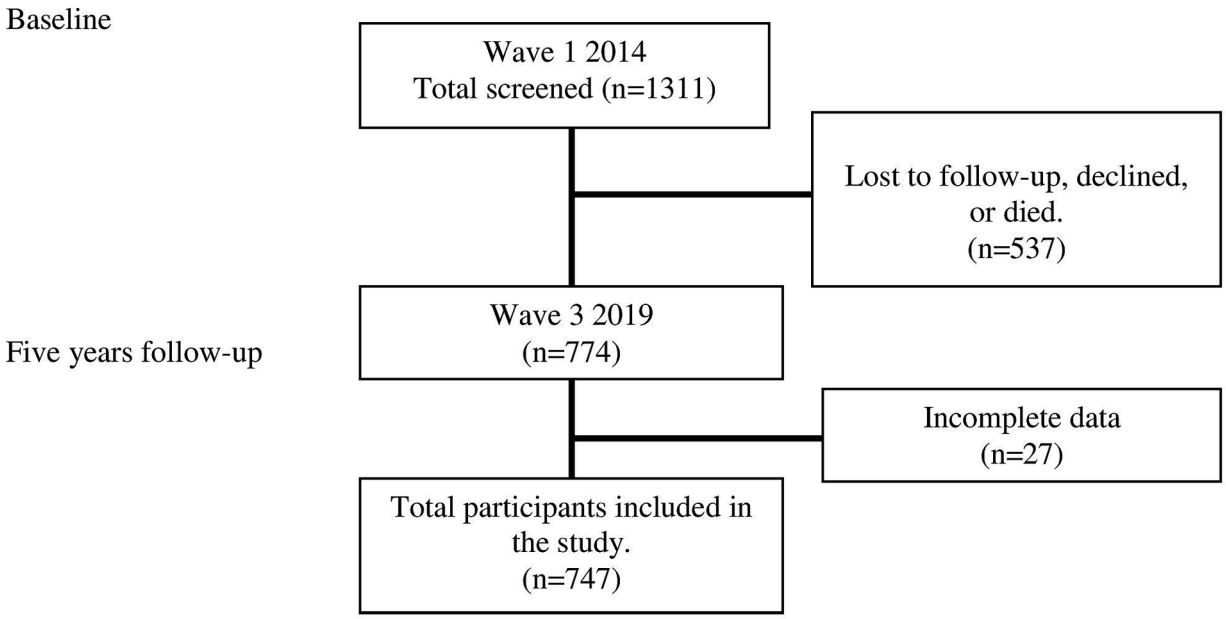

FIGURE 1
Flowchat of participant recruitment and follow-up.

scores were determined both at baseline and at five-year follow-up for all participants. SARC-F at baseline involved the substitution of sex-specific lowest quintile for hand grip strength for strength, as the question was not available for the baseline questionnaire.

2.5. Physical performance tests

2.5.1. Gait speed

A 10-meter walking path (2-meter acceleration, 6-meter walk, and 2-meter deceleration) was used in this study. The participant was asked to walk using their casual walking speed. The stopwatch was started when the foot first crossed the marked acceleration point and stopped when the foot crossed the first marked deceleration point. The time taken was recorded in seconds. The test was then repeated and the mean of two trials was taken as the result (27).

2.5.2. Time up and go

To conduct the TUG test, a wooden solid chair with its seat at a height of 46 cm above the ground was used. The participant was given verbal instruction prior to the test. The stopwatch is started as soon as the participant's bottom leaves the chair. Participants then walked three meters at their usual walking speed, turned around, and walked back to the chair and sat down. The stopwatch stopped when the participant's bottom touched the chair once again. The average time (in seconds) of two performances was used as the result (28).

2.5.3. Functional reach

The functional reach test was performed in the standing position. The participant stood against the wall with one arm positioned at 90° of forward flexion with a measuring tape placed at shoulder height. Participant was instructed to maintain their base of support with legs shoulder width apart while trying to reach forward as far as they could. The difference in length between the initial position of the tip of the middle finger (in cm) and the furthest position was considered the FR (29).

2.5.4. Hand grip strength

To assess HGS, a Jamar™ hand dynamometer (Patterson Medical, United States) was used. Participants were seated with shoulders adducted, elbow flexed to 90 degrees, with the forearm and the wrist maintained in neutral position, while the hand gripping the dynamometer. The best result from the dominant hand was taken as the result for the present study (30).

2.6. Data analysis

Statistical analyses were conducted using the Statistical Package for Social Science for Windows, version 2,626 (IBM Corp., Armonk, N.Y., United States). The Independent t-test was used to generate descriptive characteristics for continuous variables while the Chi-squared test was used for categorical variables. The association between physical performance tests and the risk of sarcopenia was assessed using multiple logistic regressions. The physical performance test data were dichotomized based on the outcome value of the receiver operating characteristic curve (ROC) analysis. By categorizing participants as having or not having a risk of sarcopenia at 5 years, this approach creates a plot of sensitivity (true positive rate) vs. 1-specificity (false positive rate) at each test value. The test value with the highest sensitivity and specificity was then selected as a cut-off to classify whether the participant has the condition (31, 32). The SARC-F cumulative incidence was calculated by dividing new cases of SARC-F during the follow-up period by the number of participants at risk in the population at baseline. The incidence rate was obtained by dividing

TABLE 1 Characteristics of participants with SARC-F ≥ 4 and SARC-F < 4 at Baseline and 5 Years follow up ($N = 747$).

Characteristic	Baseline data		*p* value	5 years follow up		*p* value
	Normal (SARC-F < 4) $n = 661$	With SARC-F ≥ 4 (SARC-F ≥ 4) $n = 86$		Normal (SARC-F < 4) $n = 632$	With SARC-F ≥ 4 (SARC-F ≥ 4) $n = 115$	
Age, Year, Mean ± SD	67.52 ± 6.86	72.00 ± 6.98	**<0.001**	67.53 ± 6.52	70.77 ± 8.84	**0.001**
Gender, Female, *n* (%)	372 (56.3%)	51 (59.3%)	0.595	346 (54.7%)	77 (67.0%)	**0.015**
BMI, Mean ± *SD*	24.62 ± 4.18	26.18 ± 5.00	**0.002**	24.56 ± 4.15	26.15 ± 4.90	**<0.001**
Marital status, single or no partner, *n* (%)	140 (21.3%)	25 (29.1%)	0.136	130 (20.6%)	35 (30.5%)	0.067
Educational level, primary or below, n (%)	113 (17.2%)	22 (25.6%)	0.085	89 (14.2%)	46 (40%)	**<0.001**
Comorbidities, *n* (%)						
Hypertension	267 (52.9%)	43 (72.9%)	**0.002**	260 (54.4%)	50 (58.1%)	0.520
Diabetes mellitus	147 (22.4%)	34 (40.5%)	**<0.001**	138 (21.4%)	48 (40.7%)	**0.001**
Heart disease	46 (4.3%)	11 (6.3%)	0.225	30 (6.3%)	9 (10.5%)	0.162
Stroke	7 (1.1%)	0 (0.0%)	0.429	9 (1.9%)	1 (1.2%)	0.256
CKD	14 (2.1%)	3 (3.6%)	0.301	14 (2.2%)	3 (2.6%)	0.806
COPD	2 (0.3%)	1 (1.2%)	0.303	2 (0.3%)	1 (0.9%)	0.393
High cholesterol	349 (53.1%)	55 (65.5%)	**0.021**	333 (53.2%)	71 (61.7)	0.091

Bold values of *p*-value <0.05 as indicated. CKD=Chronic Kidney Disease, COPD = Chronic obstructive pulmonary disease.

new cases of SARC-F by the total-person-time observed between the two assessments.

3. Results

3.1. Participants' characteristics and incidence of SARC-F ≥ 4 in 5 years

Baseline and follow-up SARC-F scores were available for 747 participants. Participants' baseline characteristics are presented in Table 1. Eighty-six (11.1%) fulfilled the SARC-F cut-off of four or more points and were classified as sarcopenic at baseline while 6.8% (n = 51) women from the total population were categorized as sarcopenia group, mean age (SD) 72.0 ± 6.98, $p < 0.001$. From the data analysis at follow-up, 632 (84.6%) participants were categorized as normal (SARC-F < 4), and 115 (15.4%) participants were in the sarcopenia category (SARC-F ≥ 4). The mean age for normal group participants was 67.5 ± 6.52 years, and 54.7% (n = 346) were women. The participant's age range in the sarcopenic group was 70.8 ± 8.84 years, of which 67% (n = 77) were women. There were significant differences in body mass index (BMI), educational level, and presence of diabetes mellitus (DM) between groups ($p < 0.001$) (Table 1). The five-year incidence of possible sarcopenia according to SARC-F was 17 per 100 person-years.

3.2. Physical performance among participants with and without SARC-F≥4

Physical performance among participants with and without sarcopenia in the baseline and after five years of follow-up is summarized in Table 2. The TUG, GS, and FR were significantly correlated with the SARC-F scores ($p < 0.001$) at the baseline and follow-up. However, HGS in this study was not significant in predicting the risk of sarcopenia in both groups (Table 2; Figure 2).

3.3. Cut off values for physical performance measures

The area under the ROC curve (AUC) was used to assess the performance of baseline GS, TUG, HGS, and FR in predicting probable sarcopenia in 5 years. The reference values set for GS were ≤ 0.7 m/s (72.9% sensitivity and 53% specificity). Meanwhile, ≥11.5 s was set for TUG (74.2% sensitivity and 57.2% specificity). We proposed a cut-off value of ≤22.5 cm for FR (73% sensitivity and 54.2% specificity). The cut-off values for male and female HGS proposed were < 22 kg and < 17 kg, respectively, (70% sensitivity for both genders, 26.7% specificity for male HGS, and 20.3% for female HGS) (Table 3).

3.4. Physical performance and sarcopenia

The association was significant for TUG, GS, and FR even after adjustment for baseline sarcopenia except for HGS. The rate Ratio (RR) of sarcopenia at five years follow-up among older people with poor physical performance was measured by an unadjusted model, adjusted Model 1 (model adjusted for age, gender, marital status, and educational level), adjusted Model 2 [adjustment made in adjusted model 1 + comorbidities (chronic kidney disease (CKD), emphysema/chronic objective pulmonary

TABLE 2 Physical Performance according to SARC-F.

Physical performance measures	Baseline (Mean ± SD)		*p*-value	After 5 years follow-up (Mean ± SD)		*p* value
	Normal	With SARC-F ≥ 4		Normal	With SARC-F ≥ 4	
Gait speed, m/s	0.84 ± 0.19	0.66 ± 0.16	**<0.001**	0.85 ± 0.18	0.67 ± 0.17	**<0.001**
Time up and go, sec	11.42 ± 2.86	15.13 ± 4.07	**<0.001**	11.37 ± 2.65	14.43 ± 4.59	**<0.001**
Hand grip strength, kg						
Male	27.19 ± 8.31	25.30 ± 8.05	0.066	27.65 ± 8.65	25.99 ± 7.89	0.263
Female	21.83 ± 7.10	20.66 ± 7.44	0.131	22.12 ± 6.93	20.59 ± 7.93	0.089
Functional reach, cm	26.28 ± 7.29	23.15 ± 7.52	**<0.001**	26.74 ± 7.17	21.39 ± 6.86	<0.001

Bold values of *p*-value <0.05 as indicated.

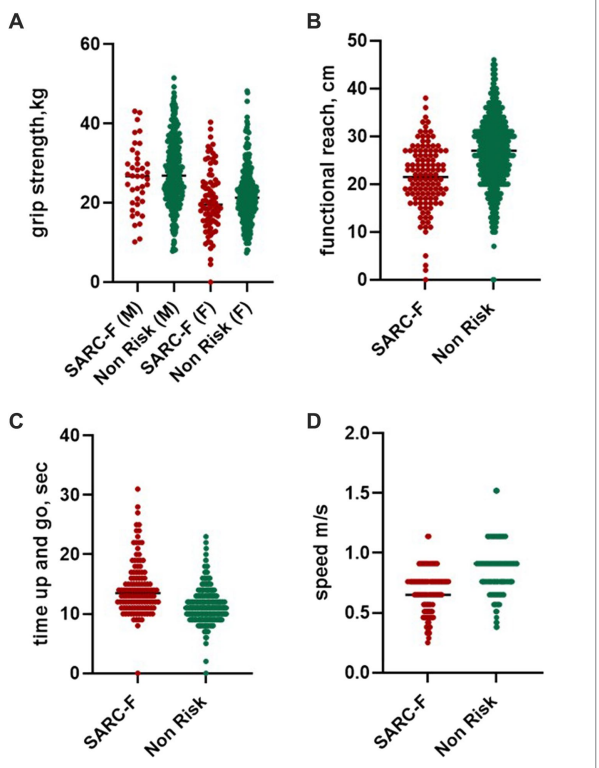

FIGURE 2
Physical performance score in non-risk participants and with a SARC-F score of 4 and above. Graph figure of physical performance in participants with SARC-F ≥ 4. SARC-F: S=Strength, A = Assistance in walking, R = Rising from a chair, C=Climbing stairs, F = Falls history.

disease (COPD), diabetes, hypertension, cholesterol, stroke)] and adjusted Model 3 (adjustment made in adjusted model 2 + baseline SARC-F) as depicted in Table 3. The unadjusted model of GS was 4.28 (2.85; 6.42); TUG was 3.83 (2.48; 5.93) meanwhile FR 3.24 (2.17;4.82) and HGS 1.71 (1.15; 2.56). The HGS was the only parameter that was not significant after being adjusted with models 1, 2, and 3: 1.21 (0.77;1.99), 1.07 (0.62;1.84), and 1.04 (0.60;1.81) consecutively (Table 3).

4. Discussion

Aging is associated with balance impairment, and it is estimated that 13% of older people ages 65–69 and 46% of older adults ages 85 and above self-report having balance deficits (33). Lack of balance, unsteadiness while walking, and weak muscles are the main internal factors that increase the risk of falling in older adults (34). Older individuals with sarcopenia often experience weakened muscles, which can significantly impact their balance while walking. Early detection of sarcopenia is crucial in mitigating the progression of muscle loss and maintaining better health outcomes for older individuals. Implementing straightforward physical performance assessments in clinical practice becomes imperative to effectively prevent the occurrence of sarcopenia. Knowing the cut-off value of physical performance will empower health practitioners to establish targeted sarcopenia prevention programs effectively. Research conducted by Pepera et al. in 2021 revealed compelling evidence regarding the efficacy of a two-month multicomponent exercise training (MCEP) program in enhancing mobility among older adults. This comprehensive exercise regimen encompasses a combination of balance and muscle-strengthening exercises, strategically designed to amplify both balance performance and gait ability. Sarcopenia notably exerted a significant impact on these aspects in older adults.

This study identified the cut-off values for physical performance tests to predict the risk of sarcopenia in 5-years among individuals aged 55 years and over in Malaysia. The proposed cut-off value of gait speed was at ≤0.7 m/s, ≤11.5 s for TUG, and < 22.5 cm for FR. The cut-off of sex-specific hand grip strength for men was <22 kg women was <17 kg. Several studies have been conducted to predict probable sarcopenia utilizing SARC-F but were focused mainly on studying prevalence (35–39) and were not focused on the physical performance's cut off values to predict sarcopenia risk in the Southeast Asian population.

The ability of TUG to predict sarcopenia has been mentioned in one study (11) with the cut-off set at 10.85 s (sensitivity of 67% and specificity of 88.7%). This result was slightly different from the present study which set the cut-off at ≤11.5 s (sensitivity of 74.2% and specificity of 57.2%). As physical performance is correlated with physiological and anthropometric measurements such as height and limb length that vary according to ethnicity, body morphology differences between European and Asian populations probably play a

TABLE 3 Rate ratio of SARC-F ≥ 4 at 5 years follow up among older people with poor physical performance measured using different type of test.

Physical Performance	Cut-off value	Sensitivity (%)	Specificity (%)	SARC-F ≥ 4, Rate Ratio, RR (95% CI)				
				Unadjusted model	Age-adjusted	Adjusted model 1	Adjusted model 2	Adjusted model 3
Gait Speed, m/s	0.70	79.2	53.0	**4.28 (2.85. 6.42)**	**3.65 (2.38–5.60)**	**2.69 (1.64–4.11)**	**2.45 (1.40–4.29)**	**2.29 (1.29–4.06)**
Time Up and Go, sec	11.5	74.2	57.2	**3.83 (2.48; 5.93)**	**3.33 (2.13; 5.21)**	**2.70 (1.69; 4.29)**	**1.97 (1.08; 3.25)**	**1.77 (1.00; 3.13)**
Functional reach, cm	22.5	73.2	54.2	**3.24 (2.17; 4.82)**	**2.85 (1.89; 4.29)**	**2.00 (1.28; 3.13)**	**1.76 (1.02; 3.05)**	**1.76 (1.01; 3.06)**
Hand grip strength, kg	M: <22	70.0	26.7	**1.71 (1.15; 2.56)**	**1.72 (1.14; 2.58)**	1.21 (0.77; 1.99)	1.07 (0.62; 1.84)	1.04 (0.60; 1.81)
	F: <17	70.0	20.3					

Bold fonts indicate significant at $p < 0.05$, CI = confidence interval.
Adjusted Model 1: Adjustment of age, Gender, Marital status, and Education level.
Adjusted Model 2: Adjustment made in Adjusted model 1 + Comorbidities (CKD, Emphysema/COPD, Diabetes, Hypertension, Cholesterol, Stroke).
Adjusted Model 3: Adjustment made in Adjusted model 2+ Baseline SARC-F.

role in the dissimilarity of step length and walking speed resulting in the majority of the older Asian population having slower TUG (40).

In terms of gait speed, the cut-off point set in this study ≤0.7 m/s (sensitivity 79.2% and specificity 53%) was lower than the value set by the Asian Working Group of Sarcopenia (AWGS) which is <0.8 m/s (41). However, this cut-off was within a range value presented by Cawthon in his study which set the cut-off points in walking speed of 0.60 m/s and 0.75 m/s discriminated older adults with mobility limitation related to sarcopenia (42). Another study by Kang et al. (43) denoted healthy older adult walking speed was 1.23 ± 0.26 m/s, which is 0.08 m/s faster compared to sarcopenic people (1.15 ± 0.25 m/s, value of p <0.001).

In this study, the specificity for HGS cut-offs was quite low. This cut-off value of HGS was the lowest among the cut-off set by other working definition sarcopenia such as AWGS (<26 kg for men and < 18 kg for women) and EWGSOP (<30 kg for men and < 20 kg for women). The lack of association between HGS and sarcopenia defined using SARC-F at 5-year follow-up may be attributed to the limitation of SARC-F. A previous study has, however, suggested that SARC-F showed a high level of overlap with established definitions of sarcopenia of up to 54% with the IWGS definition (36). In addition, in the more recent study, it was suggested that SARC-F is better suited to rule out sarcopenia in case-finding. Since HGS just measures upper body strength, its value in determining whole-body strength is probably limited (44). However, according to research conducted by Laudisio et al. (45), a correlation exists between muscle strength, as quantified through handgrip strength, and the overall physical and mental well-being of older adults. Furthermore, enhancing muscle performance has the potential to contribute to an improved quality of life for this population.

4.1. Strength and limitation

This study represents the first longitudinal investigation into the relationship between physical performance measures (PPMs) including GS, TUG, HGS, and FR, and adverse health namely Sarcopenia in older Malaysian adults. However, this study lacked objective measurements of body composition and imaging (i.e., dual-energy X-ray absorptiometry and magnetic resonance imaging) to classify sarcopenia, which may have led to inaccuracies. Various imaging techniques, encompassing both qualitative and quantitative approaches, are utilized for evaluating muscle mass and body composition. These methods include computed tomography (CT), nuclear magnetic resonance (MRI), dual-energy X-ray absorptiometry (DXA), bioelectrical impedance analysis (BIA), and muscle ultrasound. CT and MRI are regarded as the benchmark due to their ability to provide accurate assessments of distinct body tissues, but a consensus regarding the specific cutoff values for defining sarcopenia remains absent (46). Secondly, the MELoR study targeted Malaysian adults living in an urban area which will limit the generalization to those living in rural areas whose occupations and lifestyles may be different. Future studies should include older people living in rural areas and compare their performance to evaluate whether geographical factors may affect the results. Finally, no physical assessment was conducted during the follow-up visit. Hence, we were not able to evaluate the influence of changes in physical performance on sarcopenia. Nevertheless, this study revealed the temporal associations between physical performance tests on five-year sarcopenia risk in older adults in Malaysia.

5. Conclusion

This study proposed the cut-off values for physical performance tests that may be useful for early detection of sarcopenia risk within the older Malaysian population. Future studies should seek to confirm our findings using more accurate sarcopenia measurements which should ideally include imaging modalities. In addition, the value of screening for five-year sarcopenia risk using physical performance tests should also be evaluated.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the research ethics committee of the university of Malaya. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

IM, SM, DKAS, and MPT conceived the study, contributed to the study design, obtained the funding for the study and were responsible for the conduct of the study. IM was involved in data collection. SM and MPT contributed to data analysis. All authors contributed to the writing of the manuscript and approved the final submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Meier NF, Lee DC. Physical activity and sarcopenia in older adults. *Aging Clin Exp Res*. (2020) 32:1675–87. doi: 10.1007/s40520-019-01371-8

2. Beaudart C, McCloskey E, Bruyere O, Cesari M, Rolland Y, Rizzoli R, et al. Sarcopenia in daily practice: assessment and management. *BMC Geriatr*. (2016) 16:170. doi: 10.1186/s12877-016-0349-4

3. Cruz-Jentoft AJ, Landi F, Schneider SM, Zúñiga C, Arai H, Boirie Y, et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the international sarcopenia initiative (EWGSOP and IWGS). *Age Ageing*. (2014) 43:748–59. doi: 10.1093/ageing/afu115

4. Doi T, Tsutsumimoto K, Ishii H, Nakakubo S, Kurita S, Shimada H. Association between sarcopenia, its defining indices, and driving cessation in older adults. *J Nutr Health Aging*. (2021) 25:462–6. doi: 10.1007/s12603-020-1554-6

5. Shimokata H, Shimada H, Satake S, Endo N, Shibasaki K, Ogawa S, et al. Chapter 2 epidemiology of sarcopenia. *Geriatr Gerontol Int*. (2018) 18:13–22. doi: 10.1111/ggi.13320

6. Smith L, Jacob L, Barnett Y, Butler LT, Shin JI, López-Sánchez GF, et al. Association between food insecurity and sarcopenia among adults aged ≥65 years in low- and middle-income countries. *Nutrients*. (2021) 13. doi: 10.3390/nu13061879

7. Af ML, Clark B, Shahar S, Abdul Manaf Z, DKA Singh, Lau H, et al. Characterisation of sarcopenia in Malaysian elders using the Asian working group on sarcopenia and the European working group on sarcopenia in older people consensus definitions introduction figure 1. Prevalence of sarcopenia among Malaysian elderly according to Asian working Group for Sarcopenia References (2015).

8. Anker S, Morley J, von Haehling S. Welcome to the ICD-10 code for sarcopenia. *J Cachexia Sarcopenia Muscle*. (2016) 7:512–4. doi: 10.1002/jcsm.12147

9. Bahat G, Kilic C, Eris S, Karan MA. Power versus sarcopenia: associations with functionality and physical performance measures. *J Nutr Health Aging*. (2021) 25:13–7. doi: 10.1007/s12603-020-1544-8

10. Frisoli A, Paes AT, Borges J, Ingham SM, Cartocci MM, Lima E, et al. The association between low lean mass and osteoporosis increases the risk of weakness, poor physical performance and frailty in Brazilian older adults: data from SARCOS study. *Eur J Clin Nutr*. (2021) 75:446–55. doi: 10.1038/s41430-020-00753-w

11. Martinez BP, Gomes IB, de Oliveira CS, Ramos IR, Rocha MDM, Júnior LAF, et al. Accuracy of the timed up and go test for predicting sarcopenia in elderly hospitalized patients. *Clinics*. (2015) 70:369–72. doi: 10.6061/clinics/2015(05)11

12. Binotto MA, Lenardt MH, Rodríguez-Martínez MDC. Physical frailty and gait speed in community elderly: a systematic review. *Rev Esc Enferm USP*. (2018) 52:e03392. doi: 10.1590/s1980-220x2017028703392

13. Menezes K, Auger C, Barbosa JFS, Gomes CS, Menezes WRS, Guerra RO. Trajectories and predictors of functional capacity decline in older adults from a Brazilian northeastern hospital. *J Geriatr Phys Ther*. (2021) 44:82–7. doi: 10.1519/jpt.0000000000000255

14. Chang KV, Hsu TH, Wu WT, Huang KC, Han DS. Association between sarcopenia and cognitive impairment: a systematic review and Meta-analysis. *J Am Med Dir Assoc*. (2016) 17:1164.e7–1164.e15. doi: 10.1016/j.jamda.2016.09.013

15. Peng TC, Chen WL, Wu LW, Chang YW, Kao TW. Sarcopenia and cognitive impairment: a systematic review and meta-analysis. *Clin Nutr*. (2020) 39:2695–701. doi: 10.1016/j.clnu.2019.12.014

16. Sui SX, Williams LJ, Holloway-Kew KL, Hyde NK, Pasco JA. Skeletal muscle health and cognitive function: a narrative review. *Int J Mol Sci*. (2020) 22. doi: 10.3390/ijms22010255

17. Lenchik L, Boutin RD. Sarcopenia: beyond muscle atrophy and into the new Frontiers of opportunistic imaging, precision medicine, and machine learning. *Semin Musculoskelet Radiol*. (2018) 22:307–22. doi: 10.1055/s-0038-1641573

18. Beaudart C, Rolland Y, Cruz-Jentoft AJ, Bauer JM, Sieber C, Cooper C, et al. Assessment of muscle function and physical performance in daily clinical practice: a position paper endorsed by the European Society for Clinical and Economic Aspects of osteoporosis, osteoarthritis and musculoskeletal diseases (ESCEO). *Calcif Tissue Int*. (2019) 105:1–14. doi: 10.1007/s00223-019-00545-w

19. Choi KM. Sarcopenia and sarcopenic obesity. *Korean J Intern Med*. (2016) 31:1054–60. doi: 10.3904/kjim.2016.193

20. Hicks C, Levinger P, Menant JC, Lord SR, Sachdev PS, Brodaty H, et al. Reduced strength, poor balance and concern about falls mediate the relationship between knee pain and fall risk in older people. *BMC Geriatr*. (2020) 20:94. doi: 10.1186/s12877-020-1487-2

21. Aartolahti E, Lönnroos E, Hartikainen S, Häkkinen A. Long-term strength and balance training in prevention of decline in muscle strength and mobility in older adults. *Aging Clin Exp Res*. (2020) 32:59–66. doi: 10.1007/s40520-019-01155-0

22. Groessl EJ, Kaplan RM, Rejeski WJ, Katula JA, Glynn NW, King AC, et al. Physical activity and performance impact long-term quality of life in older adults at risk for major mobility disability. *Am J Prev Med*. (2019) 56:141–6. doi: 10.1016/j.amepre.2018.09.006

23. Chen LK, Liu LK, Woo J, Assantachai P, Auyeung TW, Bahyah KS, et al. Sarcopenia in Asia: consensus report of the Asian working Group for Sarcopenia. *J Am Med Dir Assoc*. (2014) 15:95–101. doi: 10.1016/j.jamda.2013.11.025

24. Alex D, Khor HM, Chin AV, Hairi NN, Othman S, Khoo SPK, et al. Cross-sectional analysis of ethnic differences in fall prevalence in urban dwellers aged 55 years and over in the Malaysian elders longitudinal research study. *J BMJ Open*. (2018) 8:e019579. doi: 10.1136/bmjopen-2017-019579

25. Nishikawa H, Asai A, Fukunishi S, Takeuchi T, Goto M, Ogura T, et al. Screening tools for sarcopenia. *In Vivo*. (2021) 35:3001–9. doi: 10.21873/invivo.12595

26. Vidal-Cuellar C, Mas G, Ayamamani-Torres P, Yazawa T, Rosas-Carrasco O, Tello T. Identification of probable sarcopenia based on SARC-F and SARC-CalF in older adults from a low-resource setting. *J Frail Sarcop Falls*. (2022) 7:222–30. doi: 10.22540/JFSF-07-222

27. Karpman C, Lebrasseur NK, Depew ZS, Novotny PJ, Benzo RP. Measuring gait speed in the out-patient clinic: methodology and feasibility. *Respir Care*. (2014) 59:531–7. doi: 10.4187/respcare.02688

28. Ziegl A, Hayn D, Kastner P, Löffler K, Weidinger L, Brix B, et al. Quantitative falls risk assessment in elderly people: results from a clinical study with distance based timed up-and-go test recordings. *Physiol Meas*. (2020) 41:115006. doi: 10.1088/1361-6579/abc352

29. Scena S, Steindler R, Ceci M, Zuccaro SM, Carmeli E. Computerized functional reach test to measure balance stability in elderly patients with neurological disorders. *J Clin Med Res*. (2016) 8:715–20. doi: 10.14740/jocmr2652w

30. Abdul Mutalib S, Mace M, Seager C, Burdet E, Mathiowetz V, Goldsmith N. Modernising grip dynamometry: inter-instrument reliability between GripAble and Jamar. *BMC Musculoskelet Disord*. (2022) 23:80. doi: 10.1186/s12891-022-05026-0

31. Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Caspian J Intern Med*. (2013) 4:627–35.

32. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem*. (1993) 39:561–77. doi: 10.1093/clinchem/39.4.561

33. Osoba MY, Rao AK, Agrawal SK, Lalwani AK. Balance and gait in the elderly: a contemporary review. *Laryngosc Investig Otolaryngol*. (2019) 4:143–53. doi: 10.1002/lio2.252

34. Lacroix A, Kressig RW, Muehlbauer T, Gschwind YJ, Pfenninger B, Bruegger O, et al. Effects of a supervised versus an unsupervised combined balance and strength training program on balance and muscle power in healthy older adults: a randomized controlled trial. *Gerontology*. (2016) 62:275–88. doi: 10.1159/000442087

35. Bahat G, Yilmaz O, Kiliç C, Oren MM, Karan MA. *Performance of SARC-F in regard to sarcopenia definitions muscle mass and functional measures*. Berlin: Springer Science and Business Media LLC (2018).

36. Kemmler W, Sieber C, Freiberger E, von Stengel S. The SARC-F questionnaire: diagnostic overlap with established sarcopenia definitions in older German men with sarcopenia. *Gerontology*. (2017) 63:411–6. doi: 10.1159/000477935

37. Malmstrom TK, Miller DK, Simonsick EM, Ferrucci L, Morley JE. SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. *J Cachexia Sarcopenia Muscle*. (2016) 7:28–36. doi: 10.1002/jcsm.12048

38. Piotrowicz K, Głuszewska A, Czesak J, Fedyk-Łukasik M, Klimek E, Sánchez-Rodríguez D, et al. SARC-F as a case-finding tool for sarcopenia according to the EWGSOP2. National validation and comparison with other diagnostic standards. *Aging Clin Exp Res*. (2021) 33:1821–9. doi: 10.1007/s40520-020-01782-y

39. Sacar DE, Kilic C, Karan MA, Bahat G. Ability of SARC-F to find probable sarcopenia cases in older adults. *J Nutr Health Aging*. (2021) 25:757–61. doi: 10.1007/s12603-021-1617-3

40. Ibrahim A, Singh DKA, Shahar S. 'Timed up and Go' test: age, gender and cognitive impairment stratified normative values of older adults. *PLoS One*. (2017) 12:e0185641. doi: 10.1371/journal.pone.0185641

41. Locquet M, Beaudart C, Reginster J-Y, Petermans J, Bruyere O. Comparison of the performance of five screening methods for sarcopenia. *Clin Epidemiol*. (2018) 10:71–82. doi: 10.2147/CLEP.S148638

42. Cawthon PM, Patel SM, Kritchevsky SB, Newman AB, Santanasto A, Kiel DP, et al. What cut-point in gait speed best discriminates community-dwelling older adults with mobility complaints from those without? A pooled analysis from the sarcopenia definitions and outcomes consortium. *J Gerontol A Biol Sci Med Sci*. (2021) 76:e321–7. doi: 10.1093/gerona/glab183

43. Kang M-G, Kim K-I, Kang JK, Kang S-J, Roh H-K, Jung H-Y. Association between gait speed measured using a wearable device and sarcopenia. *Innov Aging*. (2019) 3:S885–6. doi: 10.1093/geroni/igz038.3242

44. Bohannon RW. Grip Strength: An Indispensable Biomarker For Older Adults. *Clin Interv Aging*. (2019) 14:1681–91. doi: 10.2147/cia.S194543

45. Laudisio A, Giovannini S, Finamore P, Loreti C, Vannetti F, Coraci D, et al. Muscle strength is related to mental and physical quality of life in the oldest old. *Arch Gerontol Geriatr*. (2020) 89:104109. doi: 10.1016/j.archger.2020.104109

46. Giovannini S, Brau F, Forino R, Berti A, D'Ignazio F, Loreti C, et al. Sarcopenia: diagnosis and management, state of the art and contribution of ultrasound. *J Clin Med*. (2021) 10:5552. doi: 10.3390/jcm10235552

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