REVIEW

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Biomarkers associated with lower limb muscle function in individuals with sarcopenia: a systematic review

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Abstract

Lower limb muscle dysfunction is a key driver for impaired physical capacity and frailty status, both characteristics of sarcopenia. Sarcopenia is the key pathway between frailty and disability. Identifying biological markers for early diagnosis, treatment, and prevention may be key to early intervention and prevention of disability particularly mobility issues. To identify biological markers associated with lower limb muscle (dys)function in adults with sarcopenia, a systematic literature search was conducted in AMED, CINAHL, Cochrane Library, EMBASE, Medline, PubMed, Scopus, SPORTDiscus, and Web of Science databases from inception to 17 November 2021. Title, abstract, and full-text screening, data extraction, and methodological quality assessment were performed by two reviewers independently and verified by a third reviewer. Depending on available data, associations are reported as either Pearson's correlations, regression R^2 or partial R^2, P value, and sample size (n). Twenty eligible studies including 3306 participants were included (females: 79%, males: 15%, unreported: 6%; mean age ranged from 53 to 92 years) with 36% in a distinct sarcopenic subgroup (females: 73%, males: 19%, unreported: 8%; mean age range 55–92 years). A total of 119 biomarkers were reported, categorized into: genetic and microRNAs ($n = 64$), oxidative stress ($n = 10$), energy metabolism ($n = 18$), inflammation ($n = 7$), enzyme ($n = 4$), hormone ($n = 7$), bone ($n = 3$), vitamin ($n = 2$), and cytokine ($n = 4$) markers) and seven lower limb muscle measures predominately focused on strength. Seven studies reported associations between lower limb muscle measures including (e.g. power, force, and torque) and biomarkers. In individuals with sarcopenia, muscle strength was positively associated with free testosterone ($r = 0.40$, $P = 0.01$; $n = 46$). In analysis with combined sarcopenic and non-sarcopenic individuals, muscle strength was positively associated with combined genetic and methylation score (partial $R^2 = 0.122$, $P = 0.03$; $n = 48$) and negatively associated with sarcopenia-driven methylation score (partial $R^2 = 0.401$, $P < 0.01$; $n = 48$). Biomarkers related to genetics ($R^2 = 0.001$–0.014, partial $R^2 = 0.013$–0.122, $P > 0.05$; $n = 48$), oxidative stress ($r = 0.061$, $P > 0.05$; $n \geq 77$), hormone ($r = 0.01$, $\rho = 0.052$ $p > 0.05$, $n \geq 46$) and combined protein, oxidative stress, muscle performance, and hormones ($R^2 = 22.0$, $P > 0.05$; $n \geq 82$) did not report significant associations with lower limb muscle strength. Several biomarkers demonstrated associations with lower limb muscle dysfunction. The current literature remains difficult to draw clear conclusions on the relationship between biomarkers and lower limb muscle dysfunction in adults with sarcopenia. Heterogeneity of biomarkers and lower limb muscle function precluded direct comparison. Use of international classification of sarcopenia and a set of core standardized outcome measures should be adopted to aid future investigation and recommendations to be made.

Keywords Lower limb; Cytokines; Muscle strength; Muscle mass; Inflammation

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Introduction

Sarcopenia is an independent risk factor for poor health outcomes[1–5] including mortality,[6] defined as an age-related loss of skeletal muscle strength, muscle mass, and physical performance.[7] Although poor health status is not a prerequisite,[4,8,9] the presence of multimorbidity nearly doubles the odds of sarcopenia prevalence,[10] which is likely to rise, in line with multimorbidity.[11] Remaining largely undiagnosed,[5,12] sarcopenia affects between 10 and 40% of community-dwelling adults depending on the classification criteria used.[13] There are a variety of classification criteria used for the diagnosis of sarcopenia, including European Working Group on Sarcopenia in Older People (EWGSOP),[14] EWGSOP2,[15] Asian Working Group for sarcopenia criteria (AWG),[16] or International Working Group for Sarcopenia.[13] Sarcopenia classification is normally based on all or a combination of low muscle strength (e.g. knee extensor strength and handgrip strength), low lean mass [bioimpedance analysis, dual-energy X-ray absorptiometry (DEXA)], and low physical performance (gait speed and short physical performance battery score). The EWGSOP in 2019 recommended healthcare professionals treating patients at risk for sarcopenia to take actions to promote early detection and treatment,[15] with diagnosis, treatment and prevention of sarcopenia suggested to become part of routine clinical practice.[7]

Sarcopenia constitutes one of the main components of frailty and plays a key etiological role in the frailty process.[17] Although sarcopenia is defined by low muscle mass, strength, and function, these do not always align with the functional aspect more relevant to frailty, physical disability including mobility issues, and falls.[17] Physical functional limitations including immobility have detrimental effects on an individual's independence and quality of life, often leading to falls, disability, and subsequent adverse health outcomes.[18] As such, the Society of Sarcopenia, Cachexia and Wasting Disorders suggested that sarcopenia with limited mobility should be considered as an important clinical entity.[19] Early diagnosis of sarcopenia may enable early targeted interventions to prevent or alleviate physical functional limitations, frailty, and disability. Therefore, identifying novel targets may be key to reducing frailty and disability and improving physical function associated with sarcopenia.

Sarcopenia is a complex geriatric syndrome of multifactorial pathogenesis,[1] including neuromuscular degeneration, changes in muscle protein turnover, hormone levels and sensitivity, chronic inflammation, oxidative stress, and behaviour/lifestyle factors.[20] Recently, there has been particular interest in identifying effective biomarkers to accurately diagnose[21] and treat sarcopenia through targeted, nutritional, pharmacological, and rehabilitation interventions.[7] This has resulted in the identification of numerous biomarkers associated with sarcopenic muscle function[21] and the establishment of a new disease code in ICD-10-CM. High levels of circulating inflammatory markers associated with lower skeletal muscle measures,[22,23] mammalian target of rapamycin, hormones including insulin-like growth factor-1 (IGF-1), and insulin through muscle protein synthesis and breakdown[24] have been identified. As such, the identification of biomarkers associated with muscle function for the diagnosis, treatment, and prevention could aid in the development of targeted therapeutic therapies for treatment and prevention of sarcopenia and subsequent physical functional capacity and disability.

Despite the high prevalence of sarcopenia in older adults,[15,25] the potential for biological markers as tools to aid diagnosis, treatment, and prevention of sarcopenia is poorly understood. It is important to understand how any identified prognostic biomarkers are associated with skeletal muscle function, specifically lower limb skeletal muscle dysfunction as potential targets for early disease identification and diagnosis, and prevention of sarcopenia and subsequent disability through the development of new therapeutics. Although research is progressing in terms of the identification of prognostic biomarkers, previous studies provide variable results leading to inconsistent associations with muscle function and the use of biomarkers as indicators of change following treatment. Accordingly, the present review of literature aimed to identify associations between biological markers and lower limb muscle function in adults with sarcopenia.

Methods

Search strategy and selection criteria

The review was designed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses[26] with the protocol was predefined and registered on PROSPERO (CRD42020197544). Nine databases (AMED 1887–, CINAHL 1981–, Cochrane Library 1973–, EMBASE 1974–, Medline 1946–, PubMed 1801–, Scopus 1939-SPORTDiscus 1969–, Web of Science 1921–) were searched to identify original research articles published in peer-reviewed journals published before 17 November 2021. Review articles, conference abstracts, and grey literature were excluded. Articles were limited to English language only. A systematic search strategy was developed in PubMed (*Table* S1) and was replicated as closely as possible in other bibliographic databases. The predefined search strategy was expanded to include all known subgroups of biomarkers. The search strategy inclusion and exclusion criteria were considered in line with Population, Intervention, Comparator, Outcomes, and Study design (*Table* 1). Studies included participants or a subgroup of participants with sarcopenia not related to other conditions, for example, renal cachexia to prevent diseases status influencing associations. A bio-

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Table 1 Population, intervention or exposure, comparator, outcomes, and study design (PICOS) criteria

Population	Individuals were required to be human adults (aged >18 years) with all, or a distinct subgroup of participants diagnosis/classification of sarcopenia
Intervention or exposure	Individuals or a distinct subgroup of individuals were required to have a diagnosis/classification of sarcopenia. All definitions of sarcopenia were included within this review. Studies including at-risk population without a diagnosis/classification of sarcopenia, and/or sarcopenia/cachexia related to co-morbidities, for example, cancer, were excluded
Comparator	Examining the relationship between biological markers (biomarkers) and measurement of lower limb muscle function (e.g. strength, mass, and power)
Outcomes	Report on a biomarker (defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention,[27] excluding imaging-based biomarkers) and measurement of lower limb muscle function (e.g. muscle strength, muscle mass, or power) regardless of measurement modality
Study design	Only original peer-reviewed research articles in English language were included, with review articles, conference abstracts, and grey literature excluded. Any study design that included the information described above was considered for inclusion

marker (defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention,[27] excluding imaging-based biomarkers) was required to be reported. Studies were also required to report a lower limb measure, for example, muscle strength or mass. Following the removal of duplicates, a two-phase screening strategy was undertaken by two independent reviewers (SLS and RLJ) based on (i) title and abstract, (ii) full-text appraisal. Any discrepancies were resolved by consensus, where consensus could not be obtained a third reviewer (LP) was consulted. Additional studies were identified by manual searches of the references of included articles completed by SLS and RLJ. Reports were downloaded into Rayyan.[28]

Data extraction

Data were extracted independently by two authors (SLS, RLJ) and verified by a third (LP). All data were extracted using a standardized piloted data extraction form. The information included the following for baseline only: first author; year of publication; study design; population; sex ratio; age; study origin; body mass index; physical activity levels; measure of biomarkers; and lower limb muscle measure. Associations between muscle dysfunction and biomarkers were extracted as adjusted and unadjusted correlations and regression coefficients. Additional data were requested from authors if they were not reported fully in the papers, and these studies were not included if the requested data were not provided.

Assessment of methodological quality (risk of bias)

The methodological quality of the papers was assessed independently by two reviewers (SLS, RLJ) using the Joanna Briggs Institute checklist for analytical cross-sectional studies.[29] Regardless of study design, data were treated as cross-sectional with only baseline data extracted. The primary measures extracted were cross-sectional associations between biomarkers and lower limb muscle measures, rather than the likely cause of sarcopenia; therefore, prognosis criteria were deemed unsuitable. Each criterion was scored as 'Yes', 'No', 'Unclear', and 'Not applicable', and overall determined 'include', 'exclude', and 'seek further information' (*Table* 2).

Evidence synthesis

Summary statistics are presented as frequencies (%). Data were synthesized separately for (i) descriptive study data identifying biomarkers (all eligible studies) and (ii) associations between biomarkers and lower limb muscle measures (all studies reporting associations). Citations were categorized based on the biomarker's primary role and lower limb muscle measure extracted from the original source into Excel for tabulation. The included studies were heterogeneous with regard to associations between individual and categories of biomarkers and muscle dysfunction measures. Meta-regression was considered inappropriate, and narrative data analysis was performed.[52] Depending on available data associations are reported as either correlation coefficients (Pearson or Spearman) or regression R^2 or partial R^2, P value, and sample size.

Results

In total, 7282 articles were identified through database and manual searches; following the removal of duplicates, 3859 articles were included for title and abstract screening, with 120 articles sought for full-text screening (*Figure* 1). One article could not be sought; 97 were excluded with 22 studies meeting the inclusion criteria. Following risk of bias assessment, two studies[41,44] were excluded based on lack of clear

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Table 2 Check list for study quality from Joanna Briggs Institute for cross-sectional studies; scoring yes = +, no = −, unclear =?, not applicable = N/A

Author	Were the criteria for inclusion in the study clearly defined?	Were the study subjects and the setting described in detail?	Was the exposure measured in a valid and reliable way?	Were objective; standard criteria used for measurement of the condition	Were confounding factors identified?	Were strategies to deal with confounding factors stated	Were the outcomes measured in a valid and reliable way?	Was appropriate statistical analysis used?	Overall appraisal
Chen *et al*. (2019)[30]	+	?	+	+	+	−	+	+	Include
Chen *et al*. (2017)[31]	+	+	+	+	−	−	+	+	Include
He *et al*. (2020)[32]	+	+	+	+	+	−	+	+	Include
He *et al*. (2020)[33]	+	+	+	+	+	+	+	+	Include
Hinkley *et al*. (2020)[34]	+	?	+	+	+	+	+	+	Include
Hofmann *et al*. (2015)[35]	+	+	+	+	+	+	+	+	Include
Khanal *et al*. (2021)[36]	+	+	+	+	+	+	+	+	Include
Kim *et al*. (2016)[37]	+	+	+	+	−	−	+	+	Include
Lim *et al*. (2019)[38]	+	+	+	+	+	+	+	+	Include
Lustosa *et al*. (2017)[39]	+	+	+	+	−	−	+	−	Include
Ma *et al*. (2021)[40]	+	+	+	+	−	−	?	+	Include
Mafi *et al*. (2018)[41]	−	−	+	+	−	−	+	−	Exclude
Nabuco *et al*. (2019)[42]	+	+	+	+	−	−	+	+	Include
Negaresh *et al*. (2019)[43]	+	+	+	+	+	+	+	−	Include
Pietrangelo *et al*. (2009)[44]	?	+	+	+	−	−	+	−	Exclude
Ratkevicius *et al*. (2011)[45]	+	+	+	+	−	−	+	+	Include
Rossi *et al*. (2020)[46]	+	+	+	+	+	+	+	+	Include
Sanada *et al*. (2010)[47]	+	+	+	+	+	+	+	−	Include
Sanada *et al*. (2012)[48]	+	+	?	?	+	+	+	−	Include
Seo *et al*. (2020)[49]	+	+	+	+	−	−	+	+	Include
Tay *et al*. (2018)[50]	+	+	+	+	+	+	+	+	Include
Vezzoli *et al*. (2019)[51]	+	+	+	+	−	−	+	+	Include

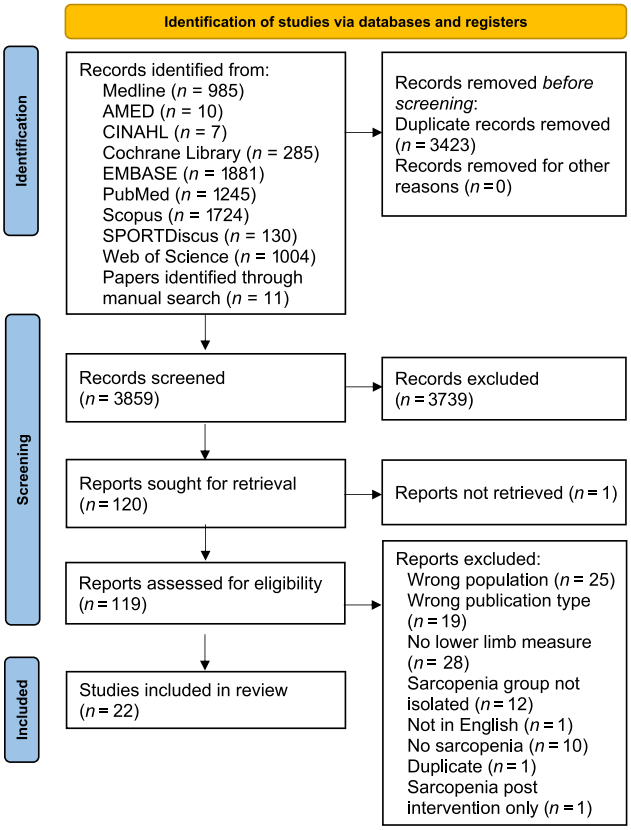

Figure 1 Flow diagram of the study selection process for eligible studies in the systematic review.

inclusion criteria and inappropriate statistical analysis including identifying and dealing with confounders (*Table* 2). Of the remaining studies, the most common risk of bias was the lack of confounders being identified and dealt with and inappropriate statistical analysis. Overall agreement on risk of bias between reviewers was 94%.

Study characteristics

Twenty published studies were included (*Table* 3), of which five were randomized controlled trials (RCT) and 13 were cross-sectional in study design. All RCTs included an exercise intervention, of which two were combined with either nutrition or nutrition and health education. A total of 3306 participants were included [498 males; 2622 females; sex was not reported in one paper (n = 186)], with a mean age ranging from 53 to 92 years. Of these, 36% (1174) were in a distinct subgroup of participants diagnosed with sarcopenia mean age range 55–92 years, of which 73% were female; sex was not reported in 8%. All definitions of sarcopenia were included within this review, with sarcopenia defined using the EWGSOP (n = 4[34,35,39,50]), AWG (n = 3[33,38,40]), and combined EWSOP and International Working Group for Sarcopenia (n = 1[49]). The remaining studies defined sarcopenia using body composition only (n = 7[30,31,42,43,46,47,51]), body composition and handgrip strength (n = 4[32,36,37,48]), or knee extensor strength (n = 1[45]). Within these definitions, sarcopenia was further classified in five studies,[43,45,47,48,51] as 1–2 standard deviation (SD) below young group as mild[43,45] or Class I sarcopenia,[47,48,51] >2 SD below young group as moderate[43] or severe[45] or Class II sarcopenia.[47,51]

A total of 119 biomarkers were reported across the 20 studies; biomarkers were grouped based on their primary characteristics into the following: genetic and microRNAs (n = 64), oxidative stress (n = 10), energy metabolism (n = 18), inflammation (n = 7), enzyme (n = 4), hormone (n = 7), bone (n = 3), vitamin (n = 2), and cytokine (n = 4) markers (*Figure* 2 and *Table S2*). Lower limb muscle function was assessed in a variety of measures, with seven differing outcomes (*Table S3*), the predominant outcomes focused on lower limb strength including power (n = 5), force (n = 3), muscle-specific strength (n = 2), and torque (n = 2).

Associations between biomarkers and muscle

Of the 20 studies included, seven reported associations between biomarkers and lower limb muscle measures, of which two reported genetic biomarker associations,[30,33] one reported muscle performance biomarkers,[34] one reported oxidative stress biomarkers,[51] and three reported combined biomarkers[35,38,45] (*Table* 4). Two studies reported sarcopenia only associations,[38,45] whereas four studies combined sarcopenic and non-sarcopenic populations[30,33-35] and another study combined pre and post 12-week intervention in sarcopenia only population.[51]

Two studies examined the relationships between biomarkers and lower limb muscle strength in sarcopenia only individuals. Knee extensor muscle strength was positively associated with free testosterone (r = 0.40; P = 0.01; n = 46)[45]; however, there was no association seen with IGF-1,[45] follistatin,[45] myostatin,[45] follistatin-related gene, and[45] GDF-associated serum protein-1.[45] Inter-muscular adipose tissue ratio was not associated with inflammatory markers interleukin-6 (IL-6) and C-reactive protein in the sarcopenic or sarcopenic obese groups.[38] Although monocyte chemoattractant protein-1 was not associated with inter-muscular adipose tissue ratio for sarcopenic individuals, it was positively associated with inter-muscular adipose tissue ratio in the sarcopenic obese group (r = 0.556, $P < 0.05$; n = 17).[38] In individuals with sarcopenia, associations between biomarkers and lower limb muscle assessed via leg press were positively associated with total antioxidant capacity (R^2 = 0.33; n = 20) and negatively associated with protein carbonyls (R^2 = 0.31; n = 20),[51] when pre-intervention and post-intervention analysis was combined.

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Table 3 Description of study papers

Author/ Country	Study design	Subgroups	Participants (M/F)	Age (years)	Diagnosis criteria	Lower limb muscle measures	Biomarkers
Chen *et al*. (2017)[31] *China*	RCT	Sarcopenic with aerobic training	15 (1/14)	69 ± 3	ASMM (BIA) ≤ 32.5% men; ≤25.7% women	Knee extensor force	IGF-1
		Sarcopenic with resistance training	15 (3/12)	69 ± 4			
		Sarcopenic with combination training	15 (4/11)	69 ± 3			
		Sarcopenic control	15 (2/13)	69 ± 3			
Chen *et al*. (2019)[30] *USA*	Cross-sectional	Sarcopenic	1 (0/1)	87	BMD (DEXA) T-score> −1; SMMI (DEXA) ≤ 5.5 kg/m^2	Jump power	CTX-1; CTX-1/TRAP5b ratio; miR-100-5p; miR-125b-5p; miR-133a-3p; miR-1-3p; miR-20; miR-21–5p; miR-23a-3p; miR-24-3p; TRAP5b
		Sarco-osteopenic	15 (0/15)	69 ± 5	BMD (DEXA) T-score ≤ −1; SMMI (DEXA) ≤ 5.5 kg/m^2		
		Control	13 (0/13)	69 ± 7	BMD (DEXA) T-score ≤ − 1; SMMI (DEXA) > 5.5 kg/m^2		
He *et al*. (2020)[32] *China*	Cross-sectional	Sarcopenic	93	76 ± 1	AWG (BIA) (sarcopenic)	Knee extension torque	Creatinine; HDLc; LDLc; miR-1; miR-126; miR-133a; miR-133b; miR-146a; miR-155; miR-208b; miR-20a; miR-21; miR-210; miR-221; miR-222; miR-328; miR-486; miR-499; total cholesterol; triglycerides; HbA1c
		Control	93	76 ± 1			
He *et al*. (2020)[33] UK	Cross-sectional	Sarcopenic	24 (0/24)	73 ± 4	Both SMMI<6.75 kg/m^2 and HGS < 26 kg	Knee extension torque	Alpha-actinin-3 (ACTN3); angiotensin-converting-enzyme (ACE); ciliary neurotrophic factor (CNTF); fat mass and obesity-associated (FTO); gene-wise methylation score (MSSNP); hypoxia inducible factor 1 subunit alpha (HIF1A); methylation levels of sarcopenia-driven CpG sites (MSSAR); myostatin (MSTN); vitamin D receptor
		Control	24 (0/24)	70 ± 3			
Hinkley *et al*. (2020)[34] *USA*	Cross-sectional	Sarcopenic	7 (2/5)	71 ± 2	EWGSOP (DEXA) (sarcopenic)	Knee extensor power; quadriceps muscle volume	Creatine; glycerol-phosphoethanolamine (GPE); phosphatidylcholine, 108; phosphatidylethanolamine, 79; Phosphatidylglycerol, 157; Phosphocreatine (resting); phosphodiester peak
		Control	17 (10/7)	71 ± 1			
Hofmann *et al*. (2015)[35] *Austria*	Cross-sectional	Sarcopenic	9 (0/9)	65–92	EWGSOP (DEXA) (sarcopenic)	Knee extensor torque	Activin A; follistatin; GDF-15; IGF-1; Myostatin
		Control	70 (0/70)	65–92			
Kim *et al*. (2016)[37] *Japan*	RCT	Sarcopenic obese with exercise and nutrition interventions	36 (0/36)	81 ± 4	Body fat (BIA) ≥ 32% and SMMI (BIA) < 5.67 kg/m2 or HGS < 17 kg or GS < 1 m/s.	Knee extensor force	Albumin; CRP; cystatin C; HDLc; IL-6; leptin; total cholesterol; triglycerides; vitamin D (25OHD -

(*Continues*)

Table 3 (continued)

Author/ Country	Study design	Subgroups	Participants (M/F)	Age (years)	Diagnosis criteria	Lower limb muscle measures	Biomarkers
		Sarcopenic obese with exercise intervention	35 (0/35)	81 ± 4			serum 25-hydroxyvitamin D); HbA1c
		Sarcopenic obese with nutrition intervention	34 (0/34)	81 ± 5			
		Sarcopenic obese with health education intervention	34 (0/34)	81 ± 5			
Khanal *et al.* (2021)[36] England/UK	Cross-sectional	Sarcopenic obese	77 (0/77)	73	SMI (BIA) $<$ 6.76 kg/m^2, HGS $<$ 28.5 kg, body fat $>$38%	Knee extensor torque; muscle-specific strength	Activin A receptor Type 1B (ACVR1B) rs2854464, rs10783485; alpha-actinin-3 (ACTN3) rs1815739; angiotensin-converting-enzyme (ACE) rs4341 (I/D); ciliary neurotrophic factor (CNTF) rs1800169; ciliary neurotrophic factor (CNTF)R rs2070802; collagen Type I alpha 1 chain (COL1A1) rs1800012; erythrocyte sedimentation rate 1 rs4870044; fat mass and obesity-associated (FTO) rs9939609; hypoxia inducible factor 1 subunit alpha (HIF1A) rs11549465; ID3 rs11574; IGF-1 rs35767; IL-6 rs1800795; methylenetetrahydrofolate reductase (MTHFR) rs1801131, rs1537516, rs17421511; NOS 3 rs1800000; protein tyrosine kinase 2 (PTK2) rs7843014, rs7460; the titin gene (TTN) rs10497520; thyrotropin-releasing hormone receptor (TRHR) rs7832552; vitamin D receptor rs2228570
		Control obese	176 (0/176)	71	SMI (BIA) $\geq$ 6.76 kg/m^2, HGS $\geq$ 28.5 kg, body fat $>$38%		
		Sarcopenic non obese	7 (0/7)	69	SMI (BIA) $<$ 6.76 kg/m^2, HGS $<$ 28.5 kg, body fat $\leq$38%		
		Control (non-Sarcopenic, non-obese)	47 (0/47)	68	SMI (BIA) $\geq$ 6.76 kg/m^2, HGS $\geq$ 28.5 kg, body fat $\leq$38%		
Lim *et al.* (2019)[38] *Singapore*	Cross-sectional	Sarcopenic	26 (10/16)	70 ± 7	AWG (DEXA) (sarcopenic)	Knee extensor force	CRP; IL-6; monocyte chemoattractant protein-1; vitamin D (25OHD - serum 25-hydroxvitamin D)
		Control non-obese	42 (19/23)	67 ± 7			
		Sarcopenic obese	17 (2/15)	75 ± 10			
		Obese control	102 (25/77)	67 ± 7			
Lustosa *et al.* (2017)[39] *Brazil*	Cross-sectional	Sarcopenia	31 (0/31)	77 ± 5	EWGSOP (DEXA) (sarcopenic)	Knee extensor power/torque	Soluble TNF-alpha receptor-1 (sTNFR1); IL-6; TNF-α
		Control	32 (0/32)	77 ± 7			
Ma *et al.* (2021)[40] China	Intervention	Waitlist control	12 (6/6)	70 ± 4	AWG (sarcopenic)	Knee extensor force	Annexin A6 (ANXA6); bridging integrator 1 (BIN1); eukaryotic translation initiation factor 3
		Exercise intervention	11 (5/6)	76 ± 7			
			23 (11/12)	74 ± 6			

(*Continues*)

Table 3 (continued)

Author/ Country	Study design	Subgroups	Participants (M/F)	Age (years)	Diagnosis criteria	Lower limb muscle measures	Biomarkers
		Exercise and nutritional intervention					subunit E (EIF3E); histidine triad nucleotide-binding protein 1 (HINT1); interleukin 7 (IL-7)R; lactate dehydrogenase B (LDHB); leucine-rich repeat protein 3 (LRRN3); lymphoid enhancer binding factor 1 (LEF1); musculoaponeurotic fibrosarcoma (MAF); protein kinase C theta (PRKCQ); RAS guanyl nucleotide-releasing protein (RASGRP1); superoxide dismutase (SOD1); translocase of outer mitochondrial membrane 7 (TOMM7)
Nabuco *et al.* (2019)[42] *Brazil*	RCT	Sarcopenic obese with exercise and protein intervention	13 (0/13)	68 ± 4	Body fat (DEXA) ≥ 35% appendicular l ean soft tissue	Knee extensor force; lower soft tissue mass	Advanced oxidation protein products; albumin; CRP; creatine; glucose (fasted); HDLc; homeostasis model assessment insulin resistance (HOMA-IR); insulin; IL-6; LDLc; total cholesterol; total radical-trapping antioxidant parameter; triglycerides; TNF-α
		Sarcopenic obese with exercise and placebo	13 (0/13)	70 ± 4	(DEXA) ≤ 15.02 kg		
Negaresh *et al.* (2019)[43] Iran	RCT	Sarcopenic with resistance training	16 (16/0)	55–70	1–2 SD (mild) or >2 SD (severe) muscle mass below mean of healthy young group (skinfold)	Squat press force	Follistatin; IGF-1; myostatin; testosterone
		Control with resistance training	15 (15/0)	55–70			
Ratkevicius *et al.* (2011)[45] *Scotland/UK*	Cross-sectional	Mildly sarcopenic	20 (20/0)	69 ± 3	Maximal knee extension torque 1–2 SD below young men	Knee extensor torque; knee extensor voluntary activation	Follistatin; follistatin-related gene; GDF-associated serum protein-1; IGF-1; IL-6; myostatin; testosterone; TNF-α
		Severely sarcopenic	26 (26/0)	76 ± 6	Maximal knee extension torque 2 SD below young below young men		
Rossi *et al.* (2020)[46] Italy	Longitudinal	Healthy	116 (43/73)	72 ± 2	ASMM (DEXA) > 7 kg/m^2 men, >5.18 kg·m^2 women, body fat (DEXA) < 31.17% men, <44.01% women	Knee extensor torque	Albumin; fibrinogen; glucose; HDLc; total cholesterol; triglycerides; Vitamin D3
		Sarcopenia	68 (24/44)	72 ± 2	ASMM (DEXA) < 7 kg/m^2 men, <5.18 kg·m^2 women, body fat (DEXA) < 31.17% men, <44.01% women		
		Obese	68 (24/44)	72 ± 3	ASMM (DEXA) > 7 kg/m^2 men, >5.18 kg·m^2 women, body fat		

(*Continues*)

Table 3 (continued)

Author/ Country	Study design	Subgroups	Participants (M/F)	Age (years)	Diagnosis criteria	Lower limb muscle measures	Biomarkers
					(DEXA) > 31.17% men, >44.01% women		
		Sarcopenic obese	22 (8/14)	71 ± 2	ASMM (DEXA) < 7 kg/m^2 men, <5.18 kg.m^2 women, body fat (DEXA) > 31.17% men, >44.01% women		
Sanada *et al.* (2010)[47] *Japan*	Cross-sectional	Mildly sarcopenic	219 (63/156)	M: 65 ± 14 F: 61 ± 11	Class 1: SMMI 1SD below sex-specific reference value (DEXA)	Leg extension power; lean soft tissue mass	Glucose; HDLc; LDLc; total cholesterol; total cholesterol/ HDLc ratio; triglyceride/HDL ratio; triglycerides; HbA1c
		Severely sarcopenic	27 (5/22)	M: 67 ± 17 F: 63 ± 11	Class 2: SMMI 2SD below sex-specific reference value (DEXA)		
		Control	713 (100/613)	M: 65 ± 8 F: 60 ± 9			
Sanada *et al.* (2012)[48] *Japan*	Cross-sectional	Sarcopenic	129 (0/129)	62 ± 1	Class 1: SMMI 6.7 kg/m^2 and HGS 1SD below young mean (DEXA)	Leg extension power	Glucose (fasted); HDLc; triglyceride/HDLc ratio; triglycerides; HbA1c
		Control	404 (0/404)	53 ± 1			
Seo *et al.* (2020)[49] Korea	Cross-sectional	Sarcopenic	27 (0/27)	72 ± 4	Based on EWGSOP and IWGS (DEXA) - GS < 1.0 m·s^{-1} and ASMI < 5.67 kg·m^{-2}, or GS > 1.0 m·s^{-1}, HGS < 20 kg and ASM I < 5.67 kg·m^2	Knee extension torque; relative knee extensor torque; thigh inter-muscular adipose tissue volume; total thigh volume	Activin A; follistatin; GDF-15; myostatin
		Non-Sarcopenic	32 (0/32)	71 ± 5	Non-sarcopenic (DEXA), body fat <35%, and lumbar or femur BMD T-score <−2.5		
Tay *et al.* (2018)[50] *Singapore*	Cross-sectional	Pre-sarcopenic	14 (9/5)	72 ± 8	EWGSOP Asian gender-specific cut-off. Pre-sarcopenia: Low muscle mass without impact on muscle strength or gait speed	Knee extensor force; muscle-specific strength	APOE genotyping into APOEε2; 3; 4 isoforms; CRP; dehydroepiandrosterone sulphate (DHEAS); IGF-1; IL-6; soluble TNF-alpha receptor-1 (sTNFR1); TNF-α; vitamin D (25OHD - serum 25-hydroxvitamin D)
		Sarcopenic	53 (18/35)	78 ± 7	EWGSOP Asian gender-specific cut-off (DEXA). Low muscle mass and weak HGS and/or slow GS		
		Control	108 (11/97)	76 ± 6	EWGSOP Asian gender-specific cut-off (DEXA). Normal muscle mass		
Vezzoli *et al.* (2019)[51] *Italy*	RCT	Sarcopenic with training	15 (6/9)	71 ± 3	Class 1: SMMI 1SD below young mean (BIA)	Stair-climbing power; leg press force	8-Isoprostane (8-iso-PGF2-α); 8-OH-2-deoxyguanosine (8-OH0dG); creatinine; protein carbonyls; reactive oxygen species production rate; thiobarbituric acid-reactive substances; total antioxidant capacity
		Sarcopenic non-training	20 (10/10)	73 ± 6	Class 2: SMMI 2SD below young mean (BIA)		

ASMI, appendicular skeletal muscle index; ASMM, appendicular skeletal muscle mass; AWG, Asian Working Group Criteria; BIA, bioelectrical impedance analysis; BMD, bone mineral density; CRP, C-reactive protein; CTX-1 C-terminal cross-linking telopeptide of Type 1 collagen; DEXA, dual-energy X-ray absorptiometry; EWGSOP, European Working Group on Sarcopenia in Older People; GDF, growth/differentiation factor; GS, gait speed; HbA1c, whole-blood glycated haemoglobin A1c; HDLc, high-density lipoprotein cholesterol; HGS, handgrip strength; IGF-1, insulin-like growth factor-1; IL-6, interleukin 6; IWGS, International Working Group on Sarcopenia; LDLc, low-density lipoprotein cholesterol; NOS, nitric oxide synthase; RCT, randomized controlled trial; SMMI, skeletal muscle mass index = (skeletal muscle mass/body mass) × 100; TNF-α, tumour necrosis factor-alpha; TRAP5b, tartrate-resistant acid phosphatase 5b. Data presented as mean ± SD.

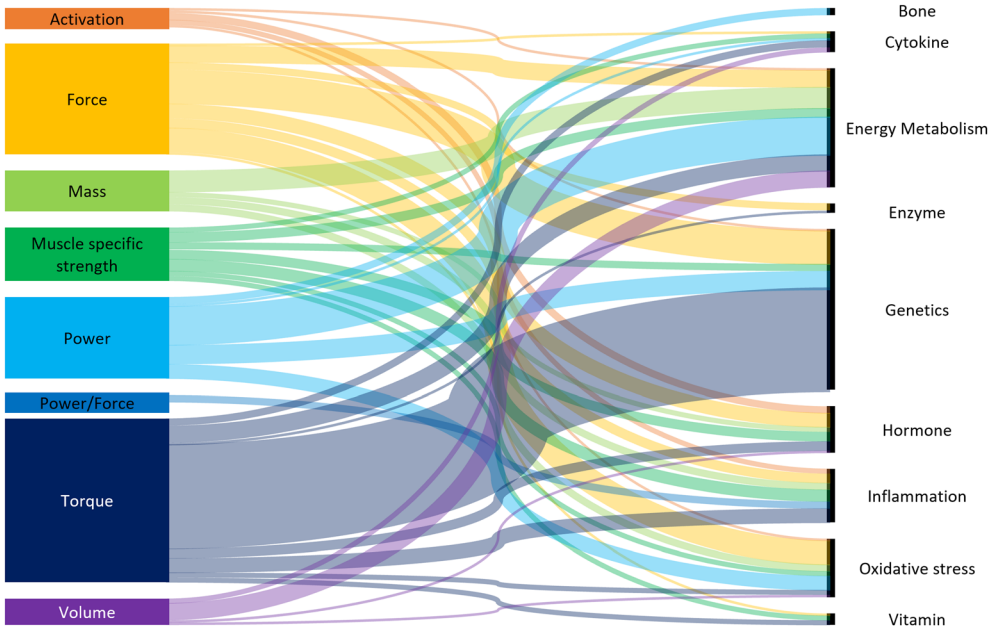

Figure 2 Sankey diagram of lower limb muscle measures and biomarkers (based on the primary role).

Four studies combined sarcopenic and non-sarcopenic populations, of which two studies examined the relationship between biomarkers and lower limb muscle power and assessed via jump power[30] and knee extensor peak power.[34] Muscle power was positively associated with MicroRNA miR-125b-5p (r = 0.294, P = 0.028; n = 56)[30] and negatively associated with phosphatidylethanolamine (r = −0.433, P = 0.044; n = 23).[34] Yet, there were no associations between muscle power and miR-1-3p, miR-133a-3p,[30] phosphocreatine,[34] phosphodiester,[34] phosphatidylcholine,[34] or phosphatidylglycerol.[34]

Two studies with combined sarcopenic and non-sarcopenic populations assessed the association between knee extension torque and biomarkers. Knee extensor torque was negatively associated with sarcopenia-driven methylation score (partial R^2 = 0.401, P = <0.01; n = 48) adjusted for age and BMI (R^2 = 0.406; n = 48)[33] and positively associated with combined genetic and methylation score (partial R^2 = 0.122, P = 0.03; n = 48) adjusted for age and BMI (R^2 = 0.112; n = 48).[33] That said, knee extensor torque was not associated with single nucleotide polymorphism-driven methylation score,[33] muscle-driven genetic predisposition score,[33] IGF-1,[35] GDF-15,[35] follistatin,[35] activin A,[35] or myostatin.[35]

Associations between biomarkers and lower limb muscle volume was reported on only one occasion using combined sarcopenic and non-sarcopenic populations. Negative associations were found between muscle volume and muscle performance biomarkers of phosphodiester (r = −0.625, P = 0.001; n = 21), phosphatidylcholine (r = −0.529, P = 0.011; n = 23), phosphatidylethanolamine (r = −0.522, P = 0.008; n = 23), and phosphatidylglycerol (r = −0.435, P = 0.043; n = 23) and no association with phosphocreatine was found in sarcopenia and non-sarcopenic adults.[34]

Discussion

The current study has summarized current literature focusing on biological markers and lower limb muscle dysfunction in adults with sarcopenia. The review's main finding was that although biomarkers and lower limb muscle measures were frequently reported, there was a lack of consistency in both biomarkers and lower limb muscle measures reported across the 20 studies included, with only seven studies including associations. Lower limb muscle strength (e.g. power, force, and torque) was significantly associated with several biomarkers whose primary role is related to genetics, muscle performance, hormones, and oxidative stress. However, numerous biomarkers also related to these roles did not report significant associations with lower limb muscle strength. Furthermore, current research is strongly weighted towards assessment of lower limb muscle strength, with research sparse in drawing associations between biomarkers and other contributors to sarcopenia. Based on the current literature, it remains difficult to understand the relationship between biomarkers and lower limb muscle dysfunction in adults with sarcopenia.

The research within this area is limited, with identification of biomarkers associated with muscle dysfunction vital given

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Table 4 Association data between biomarkers and lower limb muscle measures

Author	Analysis type	Outcome/associations
Chen *et al*. (2019)[30]	Spearman correlation (combined sarcopenic and non-sarcopenic)	• MicroRNA-125b-5p associated with jump power (r = 0.294, $P = 0.028$; $n = 56$) and velocity ($r = 0.263$, $P = 0.05$; $n = 56$) • No other microRNA's (miR-1-3p, miR-133a-3p) were correlated with jump power or velocity
He *et al*. (2020)[33]	Multiple linear regression (combined sarcopenic and non-sarcopenic)	• Model 1: Sarcopenia-driven methylation score (partial $R^2 = 0.394$, $P = <0.01$; $n = 48$) but not muscle-driven genetic predisposition score (partial $R^2 = 0.001$, $P = 0.82$; $n = 48$) was associated with knee extension torque when adjusted for age and BMI (adjusted $R^2 = 0.392$) • Model 2: SNP-driven methylation score (partial $R^2 = 0.003$, $P = 0.74$; $n = 48$) and muscle-driven genetic predisposition score (partial $R^2 = 0.014$, $P = 0.45$; $n = 48$) were not associated with knee extension torque when adjusted for age and BMI (adjusted $R^2 = <0.001$) • Model 3: Combined genetic and methylation score (partial $R^2 = 0.122$, $P = 0.03$; $n = 48$) was associated with knee extension torque age and BMI (adjusted $R^2 = 0.112$) • Model 4: Sarcopenia-driven methylation score (partial $R^2 = 0.401$, $P = <0.01$; $n = 48$) was associated with knee extension torque age and BMI (adjusted $R^2 = 0.406$). • Model 5: SNP-driven methylation score (partial $R^2 = 0.044$, $P = 0.18$; $n = 48$) was not associated with knee extension torque age and BMI (adjusted $R^2 = 0.010$) • Model 6: Muscle-driven genetic predisposition score (partial $R^2 = 0.013$, $P = 0.47$; $n = 48$) was not associated with knee extension torque age and BMI (adjusted $R^2 = 0.021$)
Hinkley *et al*. (2020)[34]	Pearson's correlation (combined sarcopenic and non-sarcopenic)	• Phosphocreatine was not associated with peak power ($r = 0.355$, $P = 0.097$; $n = 21$) • Phosphodiester peak was not associated with peak power ($r = 0.344$, $P = 0.12$; $n = 21$) • Phosphatidylcholine (residual value) was not peak power ($r = -0.394$, $P = 0.070$; $n = 23$) • Phosphatidylethanolamine (residual value) was associated peak power (r = −0.433, $P = 0.044$; $n = 23$) in sarcopenia and non-sarcopenic adults • Phosphatidylglycerol (residual value) was not peak power ($r = -0.091$, $P = 0.686$; $n = 23$)
Hofmann *et al*. (2015)[35]	Spearman's Rho, multi-linear regression (combined sarcopenic and non-sarcopenic)	• Knee extensor peak torque was not associated with IGF-1 ($\rho = 0.191$, $P > 0.05$; $n \geq 77$), GDF-15 ($\rho = -0.193$, $P > 0.05$; $n \geq 78$), follistatin ($\rho = 0.061$, $P > 0.05$; $n \geq 79$), activin A ($\rho = -0.205$, $P > 0.05$; $n \geq 80$) or myostatin ($\rho = 0.052$, $P > 0.05$; $n \geq 81$) • Multiple regression forced entry of five biomarkers (IGF-1, GDF-15, follistatin, myostatin, activin A) did not add to prediction model of age and fat mass for peak torque (−2.8% adjusted R^2 22.0; $n \geq 82$)
Lim *et al*. (2019)[38]	Pearson's correlation, Spearman's Rho, multiple linear regression (sarcopenic and sarcopenic obesity only)	• Inter-muscular adipose tissue ratio was not associated with knee extensor force ($r = 0.118$, $P > 0.05$; $n = 26$), monocyte chemoattractant protein-1 ($r = 0.296$, $P > 0.05$; $n = 26$), IL-6 ($r = 0.089$, $P > 0.05$; $n = 26$), or C-reactive protein ($r = -0.207$, $P > 0.05$; $n = 26$) in the sarcopenic group • Inter-muscular adipose tissue ratio was associated with monocyte chemoattractant protein-1 (r = 0.556, $P < 0.05$; n = 17) but not knee extensor strength ($r = 0.147$, $P > 0.05$; $n = 17$), IL-6 ($r = -0.016$, $P > 0.05$; $n = 17$), or C-reactive protein ($r = 0.235$, $P > 0.05$; $n = 17$) in the sarcopenic obese group
Ratkevicius *et al*. (2011)[45]	Pearson's product–moment correlation coefficient (combined mildly and severely sarcopenic only)	• Myostatin did not correlate with either knee extensor torque (r = 0.01; $P = 0.97$; $n = 46$), and there was also no correlation between follistatin, follistatin-related gene, and GDF-associated serum protein-1 and knee extensor torque • IGF-1 did not correlate with isometric knee extension torque. In contrast, free testosterone correlated with isometric knee extension torque (r = 0.40; $P = 0.01$; $n = 46$)
Vezzoli *et al*. (2009)[51]	Pearson's product of moment (combined pre and post intervention in combined type 1 and type 2 sarcopenic only)	• Total antioxidant capacity was associated with muscle force (leg press $R^2 = 0.33$; $n = 20$) • Protein carbonyls were associated with muscle force (leg press $R^2 = 0.31$; $n = 20$)

BMI, body mass index; GDF, growth/differentiation factor; IGF-1, insulin-like growth factor-1; IL-6, interleukin 6; *P*, *P*-value; n, sample size; r, Pearson's correlation; SNP, single nucleotide polymorphisms; ρ, spearman's rho.

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the potential to be novel therapeutic targets and aid with early diagnosis for the prevention and treatment of sarcopenia and disability. Within the current systematic review, 119 individual biomarkers were reported, ranging from genetic markers such as microRNAs to inflammatory markers such as IL-6. This breadth of biomarkers indicates interest in this research area, yet most of the biomarkers included within this review were reported on a singular basis demonstrating a breadth of potential targets. It is vital that future researchers consider strengthening the depths of the current body of literature through validation and confirmation of biomarkers and their association with a range of muscle dysfunction measures, thus ensuring recommendation are based on robust data. Furthermore, to identify biomarkers as potential therapeutic targets, it is also important to determine if the biomarkers associated with lower limb muscle dysfunction are state (changeable) or trait (static) characteristics. Although there is limited evidence available, based on the current review, future research should consider examining the relationship between MicroRNA miR-125b-5p, phosphatidylethanolamine, sarcopenia-driven methylation score, combined genetic and methylation score, free testosterone, total antioxidant capacity, protein carbonyls, monocyte chemoattractant protein-1, and lower limb muscle measures associated with sarcopenia given the reported associations with measures of lower limb muscle strength. In support of the suggestion by the International Working Group on Sarcopenia,[53,54] it is vital to consider the multifactorial nature of sarcopenia, and although single markers are of interest, it may be the case that a composition of markers from a variety of mechanistic pathways provides a greater insight into the understanding of sarcopenia. However, a study reporting a composition of multiple with varying primary roles including protein, oxidative stress, muscle performance, and hormone found no association with lower limb muscle torque.[35] In the current systematic review, a variety of muscle strength measures (e.g. force, torque, and power; muscle-specific strength) and other muscle function measures (e.g. volume and mass) were reported (*Table* S3). Whereas muscle strength plays a large role in mobility limitations, other muscle dysfunction measures such as muscle activation and tissue attenuation[55] warrant further investigation given their association with both mobility limitations and sarcopenia. Currently, the EWGSOP does not provide recommendations regarding methods of assessing muscle mass and other parameters of sarcopenia. That said, DEXA and bioelectrical impedance (BIA) are suggested in the evaluation of whole-body muscle mass[14] including in the AWG criteria.[16] There needs to be consideration regarding the sensitivity of these methods and their clinical application when providing recommendations, for example, when assessing muscle mass by BIA, the prevalence of sarcopenia was higher compared with DEXA assessment in both males [BIA: 13% (95% CI: 7–19%); DEXA: 8% (95% CI 7–9%)] and females [BIA: 13% (95% CI 9–19%); DEXA: 8% (95%CI 6–11%)].[56] Although prevalence is higher using BIA, it is less time consuming, quicker, and easier to perform in a clinical setting. Although it would be beneficial to provide recommendations regarding sarcopenia diagnostic assessment methods, real-world considerations including access, ease of use, and cost effectiveness need to be considered. The same could be said for examination of biological biomarkers whereby certain markers, such as ESR and CRP, are routinely reported clinical practice settings such as rheumatology, whereas other markers, for example, microRNAs, are not routinely performed clinically. Additionally, although long-term tracking of biological markers associated with sarcopenia could be a beneficial process, especially markers such as the inflammatory marker IL-6, which has a pleiotropic nature,[57] the cost effectiveness of these recommendations would need to be evaluated. These recommendations do however support the increased efforts by researchers and clinical practitioners to define blood-based biomarkers, providing a quick and cost-effective practice that could aid in the facilitation of sarcopenia diagnosis, tracking changes over time, and aiding clinical and therapeutic decision processes.[53]

The prevalence of sarcopenia is widely reported,[13] yet these data could be significantly impacted by the diagnostic criteria being implemented.[13,58] Of the eligible studies, four[34,35,39,50] defined sarcopenia based on the EWGSOP,[15] three studies[33,38,40] used the AWG criteria,[16] one study[49] combined the EWGSOP with the International Working Group on Sarcopenia, and the remaining studies aligned to population and sex-specific cut-offs for knee extensor strength[45] and body composition.[30–32,36,37,42,43,46–48,51] The inconsistencies in criteria make it difficult to accurately compare results across studies, even when similar outcomes are reported. This is even more difficult to evaluate with studies that have limited their sarcopenia diagnosis to one muscle assessment criterion, for example, body composition only, body composition and handgrip strength, or knee extensor strength. To add to the difficulty of evaluating data between studies, recently, the definition of sarcopenia according to the EWGSOP has been updated (EWGSOP2). The latest definition is based on low skeletal muscle mass and low muscle strength, whereas low physical performance is used to determine sarcopenia severity, rather than a diagnostic criterion.[7] Sarcopenia defined using EWGSOP2 is reportedly better than EWGSOP-defined sarcopenia for predicting the 1-year incidence of falls or hospitalization, especially when using the modified cut-offs.[59] Yet, there has already been reported of discrepancies between the prevalence of sarcopenia when applying the EWGSOP and EWGSOP2 definitions[60–62] with the suggestion that cut-off points for some of the measures might not be comparable and may lead to differing groups being identified as sarcopenic between different trials.[13] To aid with consistency, understanding, and impact of future research, it is proposed that sarcopenia should be diagnosed

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using a diagnostic criterion such as the EWGSOP2 consisting of all areas of sarcopenia (muscle strength, mass, and performance).

Sarcopenia is prevalent in both sexes, albeit to a greater degree in males compared with females.[63,64] It is therefore surprising that of the 3306 participants included within this systematic review, the majority were female (79%) and of the sarcopenic population included (n = 1220), 70% were female. Eleven of the eligible studies were single sex groups, with two males[43,45] and nine females only and two studies recruited mixed groups, then split data by sex.[32,40,51] The remaining five studies reported uneven sex ratio within groups.[31,38,46,47,50] Although there is limited evidence, one study reported differences between males and females in the associations between biological markers, muscle strength, and body composition. That said, individual factors contributing to sarcopenia have been suggested to differ between males and females, with the catabolic influence of myostatin in men potentially contributing to sarcopenia, whereas in women was due to anabolic decline represented by reduced IGF-1.[64] Furthermore, there is an abundance of literature supporting differences in muscle function including, strength, wasting,[65] muscle morphology,[66] and mobility[67] measures between males and females. To support the understanding of this potential sex-specific pathophysiological mechanism for sarcopenia, future work needs to look at sex-specific associations and/or control for sex through normalization of data or the inclusion of sex as a cofounder factor in regression models. These processes should aid in more targeted clinical interventions, with potentially differing guidelines and recommendations based on sex-specific data.

The current systematic review evaluated the quality of the research using Joanna Briggs Institute checklist for analytical cross-sectional studies; several studies lacked the appropriate statistical information, thereby in some instances impacting the quality of analysis and data provided. Based on quality assessment, two papers were excluded from the review; these studies lacked in areas such as robust criteria for inclusion in the study and implementation and consideration of confounding factors and appropriate statistical measures (Table 2). Sarcopenia may be a composition of markers from a variety of mechanistic pathways; therefore, a strong statistical framework to examine these avenues is required.[53,54] Future research should consider the inclusion of robust reliability data, examination of cofounding variables, assessment of multiple relationships within set models, the inclusion of confidence intervals, and following reporting guidelines such as EQUATOR reporting guidelines. These recommendations would aid in improving the quality of published data within the field and thereby more robust recommendations for therapeutic interventions.

During the current review, studies were excluded if groups contained individuals with co-morbidities, including hypertension, Type I and II diabetes, cancer, and osteoarthritis. This stringent criterion was designed to provide a greater understanding of impact of sarcopenia on the relationship between biological markers and skeletal muscle function, without the influence of diseases status, especially given the influence disease status can have on both biological markers and skeletal muscle function. That said, this approach does mean the exclusion of a wealth of data that may change the narrative of this relationship, especially given that sarcopenia is highly prevalent in individuals with cardiovascular disease, dementia, diabetes mellitus, and respiratory disease.[68] Additionally, given the requirement for real-world knowledge and applicable recommendations to aid the early diagnosis, treatment, and prevention of -sarcopenia, and given the potential worthwhile impact of this research area, and the demand for greater understanding regarding diagnosis, treatment, and prevention of sarcopenia, future research should confirm and validate the following biomarkers and their association with muscle dysfunction: microRNA 125b-5p, sarcopenia-driven methylation score, combined genetic and methylation score, total oxidant capacity, protein carbonyls, phosphodiester, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and free testosterone. Moreover, confirmation of associations of these biomarkers across different lower limb muscle measures, and in individuals with and without co-morbidities especially given the large number of factors such as diagnosis time, severity, and treatment practices, which would significantly influence the assessment of sarcopenia-related outcomes, important for identifying biomarkers to for diagnosis, treatment, and prevention of sarcopenia.

In conclusion, 119 different biomarkers and seven assessment methods of lower limb muscle function were identified, with association reported in seven out of the 20 studies included. Associations between biomarkers and lower limb muscle function are limited due to a lack of repetition of biomarkers and lower limb muscle measures. A lack of depth of biomarkers and heterogeneity of biomarkers and lower limb muscle measures make comparisons difficult. International classification of sarcopenia and a set of core standardized outcome measures should be adopted to aid future investigations. Future work needs to also include sex-specific associations to understand the underlying sex-specific pathophysiological mechanisms for sarcopenia, which may also confound associations when mixed-sex groups are assessed.

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Conflict of interest

Rebecca Louise Jones, Lorna Paul, Martijn Steultjens, and Stephanie Louise Smith declare that they have no conflicts of interest.

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Online supplementary material

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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