



Plasma biomarkers in patients with age-related sarcopenia: a proteomic exploration and experimental validation

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Abstract

Background Various biomarkers associated with sarcopenia have been identified. However, there is a scarcity of studies exploring and validating biomarkers in individuals with age-related sarcopenia.

Aims This study aimed to investigate the proteome and identify potential biomarkers for age-related sarcopenia.

Methods Proteomic analysis and experimental validation were conducted using plasma from hospitalized older adults. Sarcopenia diagnosis was based on the Asian Working Group for Sarcopenia 2019 criteria. Data-independent acquisition-based proteomics was performed on plasma from 60 participants, with 30 diagnosed with sarcopenia and 30 without sarcopenia. Differentially expressed proteins (DEPs) were selected and evaluated by Receiver Operating Characteristic (ROC) analysis. Biomarker candidates were further quantitatively validated by enzyme-linked immunosorbent assay (ELISA) utilizing plasma from 6 participants with sarcopenia and 6 without sarcopenia.

Results A total of 39 DEPs were identified and 12 DEPs were selected for ROC analysis. 8 DEPs were included for ELISA validation based on their predictive performance. Paraoxonase-3 (PON3) consistently showed down-regulation in the sarcopenic group across both methodologies. Insulin-like growth factor-binding protein-2 (IGFBP2) showed inconsistency in the sarcopenic group, with up-regulation observed in proteomic analysis but down-regulation in ELISA.

Discussion Decline in PON3 may result in an overload of oxidative stress in skeletal muscles and contribute to sarcopenia. Protein modifications of IGFBP2 might exhibit during sarcopenia pathogenesis.

Conclusions Plasma proteins are implicated in sarcopenia pathogenesis. PON3 is highlighted as a potential biomarker for patients with age-related sarcopenia. Further studies are imperative to gain an in-depth understanding of PON3 and IGFBP2.

Keywords Sarcopenia · Biomarker · PON3 · Plasma proteomics · ELISA · Older adult

Introduction

Sarcopenia is defined as an age-related pathological loss of skeletal muscle, combined with a decline in muscle strength or physical function [1]. It is prevalent in individuals over

60 years old and is associated with increased adverse health events, poor quality of life, and high economic burden [2, 3]. Common case finding methods include calf circumference or SARC-CalF questionnaires [1]. In Asia, the Asian Working Group for Sarcopenia (AWGS) 2019 criteria is widely

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adopted for sarcopenia diagnosis, with evaluation of muscle strength, physical performance, and muscle mass in clinics [1]. Physical exercise and nutritional support are employed as conventional therapies [4]. In the realm of pharmacotherapy, although numerous interventions are proposed to alleviate muscle aging, none have been successfully translated into definitive treatments for sarcopenia [5]. This underscores the escalating demand for novel intervention targets, especially with the rapid rise in aging population.

As a geriatric syndrome, sarcopenia is associated with multifactorial pathogenesis, including chronic inflammation, oxidative stress, mitochondrial dysfunction, lipotoxicity, and imbalanced protein metabolism [6–8]. However, the molecular mechanisms remain largely unclear. Biomarkers are critical tools for accessing the molecular field, which facilitate disease prediction [9] and diagnosis [10], elucidate underlying mechanisms [11], and pinpoint therapeutic targets [12]. Various biomarkers associated with sarcopenia have been identified, such as the serum creatinine to cystatin C ratio [10], gluconic acid [13], fibroblast growth factor 21 [14], and inflammatory cytokines [15]. Proteomics, a comprehensive profile of expressed proteins, is considered as a robust technique to explore a wider range of potential biomarkers for the complex process of muscle aging [16]. For example, Dlamini et al. found that CD163 was associated with reduced appendicular skeletal muscle mass (ASM) in males but increased ASM in females within a proteomic analysis of a middle-aged South African cohort [17]. Jiang et al. reported druggable proteins, including tumor necrosis factor ligand superfamily member 12 and hepatocyte growth factor, associated with sarcopenia using a Mendelian randomization analysis of proteomic data [18]. Aparicio et al. proposed the diagnostic biomarkers for sarcopenia based on the proteomic profiling of plasma extracellular vesicles [19]. Huemer et al. indicated novel biomarkers associated with the co-occurrence of low muscle mass and high fat mass through plasma proteomics [20]. Furthermore, Ubaida-Mohien et al. revealed altered muscle proteomes associated with disrupted energetic metabolism and pro-inflammatory status in aging muscles [21]. In the proteomic field, data-independent acquisition (DIA) mass spectrometry (MS) is emerging as a widely utilized high-throughput proteomic technology [22] in screening candidate biomarkers of various diseases [23–25]. Since MS identifies proteins by detecting mass spectrum intensities of peptides and is not inherently quantitative [26], quantification strategies are therefore usually carried out for validation of protein biomarkers, among which enzyme-linked immunosorbent assay (ELISA) is one of the most widely utilized methods [27–29]. It should be noted that not all reported sarcopenic biomarkers have been validated. A recent DIA-based proteomic analysis by Wu et al. proposed potential diagnostic proteins for sarcopenia [30], yet these were not further validated. To our knowledge, there

is a scarcity of studies that integrate proteomics exploration with biomarker validation for sarcopenia.

To address this gap, we aim to identify and validate potential plasma biomarkers for age-related sarcopenia by integrating DIA-based proteomic analysis with ELISA, which lays the groundwork for further research into sarcopenia pathogenesis and the discovery of potential therapeutic targets.

Method

Study design

We conducted a cross-sectional study following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [31]. Referring to our previous study [32], this study was approved by the ethics committee of Guangdong Provincial People's Hospital, China (approval no. GDREC20198345H(RI)). All participants provided written informed consent before registration. All procedures followed the institutional guidelines.

To identify potential plasma biomarkers, proteomic exploration and experimental validation were carried out. First, DEPs between participants with and without sarcopenia were identified using DIA-based proteomic analysis. Next, the promising biomarkers were selected based on Receiver Operating Characteristic (ROC) analysis. Subsequently, the selected biomarkers were validated in an independent cohort. The study design flowchart was shown in Supplementary Fig. 1.

Study population

Our study included inpatients in the Department of Geriatric Gastroenterology of Guangdong Provincial People's Hospital from November 2021 to June 2022. All subjects were unrelated to each other. Detailed demographic information, medical history, and prescribed medications were recorded by specialists. The inclusion criteria for the sarcopenic group were as follows: 1) age > 60 years; 2) being diagnosed with sarcopenia according to AWGS 2019 criteria [1]. Individuals older than 60 years old without sarcopenia were enrolled in the non-sarcopenic group. The exclusion criteria for both groups were as follows: (1) being physically inactive caused by other diseases (such as stroke, Parkinson's disease, multiple fractures, and etc.); and (2) being unable to cooperate due to various diseases (such as Alzheimer's disease).

Multimorbidity was evaluated utilizing age-adjusted Charlson comorbidity index (aCCI), which assigns scores for age and 17 comorbid conditions [33]. Polypharmacy was assessed by recording the number of daily medications

taken by each participant for a period of at least three months. Additionally, the duration of hospitalization was recorded.

Plasma collection and biochemical measurements

After fasting for at least 8 h, venous blood was drawn from the participant in the morning and collected in several EDTA tubes. Tubes were inverted 10 times manually to ensure thorough mixing of the blood with the anticoagulant. Plasma was obtained by centrifugation at 2000 rpm for 10 min at 4 °C, then transferred into sterile Eppendorf tubes and stored at –80 °C until protein extraction. Additional blood samples were subsequently sent to the Biochemistry Laboratory at Guangdong Provincial People's Hospital for analysis. Blood lipid profile, comprising triglyceride, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), along with blood inflammatory markers, including interleukin-6 and C-reactive protein, were assessed according to the standard procedures of the laboratory.

Sarcopenia assessment

Sarcopenia was diagnosed according to the AWGS 2019 criteria [1]. Appendicular skeletal muscle mass (ASM) was measured by bioelectrical impedance analysis (TANITA MC-980MA; Tanitao Corp., Tokyo, Japan). Body composition assessments were uniformly conducted in the morning on fasting participants with empty bladders in a standing position, who were asked to maintain a standing position for 15 min prior to measurement, and were not wearing coats, shoes, or sweaters, in accordance with the instructions of the manufacturer. Appendicular skeletal mass index (ASMI) was derived by dividing ASM by the square of the individual's height. Muscle strength was assessed by grip strength utilizing the electronic grip dynamometer (CAMRY EH101; Zhongshan Camry Electronic Co., Ltd., Zhongshan, China). The maximum reading of the 2 measurements of the dominant hand was considered as the final grip strength. Physical performance was evaluated by the six-meter walk test. The highest walk speed of 2 tests was recorded. Grip strength and walk test were carried out in the inpatient ward in the morning, in compliance with standard operational protocols. Sarcopenia diagnosis was confirmed with low ASMI (Male: < 7.0 kg/m², Female: < 5.7 kg/m²) and low grip strength (Male: < 28 kg, Female: < 18 kg), or with low ASMI and low physical performance (six-meter walk speed: < 1.0 m/s). Participants presenting low ASMI, low grip strength and low physical performance were diagnosed with severe sarcopenia.

DIA proteomic analysis

A total of 60 plasma samples, consisting of 30 from participants with sarcopenia and 30 from participants without sarcopenia, were used for DIA proteomic analysis. Construction of data-dependent acquisition (DDA) spectrum library and identification of DIA mode using UHPLC-MS/MS were conducted at LC-bio Co., Ltd. In Hangzhou, China. The process commenced with extracting proteins from plasma samples, proceeding with a series of quality evaluations and trypsin digestion to prepare the samples for analysis. Fraction separation was conducted using a gradient elution method on a C18 column. EASY-nLC™ 1200 UHPLC system (Thermo Fisher, Germany) coupled with a Q Exactive HF-X mass spectrometer (Thermo Fisher, Germany) was utilized for proteomics analyses operating in both DDA and DIA modes. Spectra acquired from DDA mode were searched against the homo_sapiens_uniprot_2019.01.18.fasta (containing 169,389 sequences) by Spectronaut-Pulsar (Biognosys) for DDA spectrum library construction. Peptide Spectrum Matches (PSMs) with confidence exceeding 99% were identified. PSMs and proteins with a False Discovery Rate (FDR) of less than 1.0% were retained for further analysis. Raw data acquired from DIA mode were subsequently imported into Spectronaut (Biognosys) software for qualitative and quantitative peptide analysis based on DDA spectrum library. The t-test was applied to analyze the protein quantification results. Proteins with significant quantitative differences, defined as either a fold change > 1.5 ($p < 0.05$) or a fold change < 0.667 ($p < 0.05$), were defined as differentially expressed proteins (DEPs).

Functional analysis

InterProScan program was used for Gene Ontology (GO) and InterPro (IPR) functional annotation, including Pfam, PRINTS, ProDom, SMART, ProSite, and PANTHER [34]. Protein families and pathways were examined through Clusters of Orthologous Groups (COG) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. DEPs analysis included volcano map, cluster heatmap, and enrichment analysis of GO, KEGG, and IPR [35]. Predictive potential protein–protein interactions were performed using the STRING website (<https://cn.string-db.org/>).

Enzyme-linked immunosorbent assay

Validation of proteins in 12 plasma samples, with 6 from participants with sarcopenia and 6 from those without sarcopenia, was performed utilizing the double-antibody sandwich enzyme-linked immunosorbent assay (ELISA), based on the manufacturer's suggested protocols. The following ELISA kits were used: IGFBP2, PON3, LRG1,

PRDX6, PTGDS, CD163, PRG4, and TRAJ17 (Camilo, Nanjing, China). Preliminary experiments were conducted to ensure the optimal diluted factor of plasma samples. The 96-well plates were read at 450 nm and 630 nm using a Tecan SPARK microplate reader. All protein assays exhibited intraplate variabilities of less than 10%. CurveExpert version 1.4 was used to draw the standard curve and calculate the protein concentrations. GraphPad Prism 9 was used for plotting.

Statistical analysis

All quantitative data were subjected to the Shapiro–Wilk normality test. Data showing normal distribution were presented as “mean \pm standard deviation” and were analyzed using the two-sided Student’s t test. Data exhibiting skewed distribution were presented as “median (interquartile range)” and were analyzed using the Wilcoxon rank-sum test. Categorical variables were analyzed using the Chi-square test or Fisher’s exact test. Spearman’s correlation analysis was performed for the correlations between DEPs and clinical parameters. Statistical significance was defined as $p < 0.05$. Statistical analysis was conducted using R version 4.2.2 and SPSS version 26, unless otherwise specified.

Result

Baseline characteristics of participants

A total of 72 participants were enrolled in this study, with 30 with sarcopenia and 30 without sarcopenia participating in the DIA-based proteomic analysis. Experimental validation was then conducted on the remaining 12 participants. The baseline characteristics of 60 participants and 12 participants were presented respectively (Table 1).

The baseline characteristics of the participants in the DIA analysis cohort were consistent with our previous research [32]. Participants with sarcopenia were significantly older than those without sarcopenia [sarcopenia, 91.0 (83.5, 92.0) vs. non-sarcopenia, 71.0 (65.2, 76.2) years; $p < 0.001$]. Compared to those without sarcopenia, participants with sarcopenia also had significantly lower BMI (sarcopenia, 21.3 ± 3.5 vs. non-sarcopenia, $24.5 \pm 2.4 \text{ kg/m}^2$; $p < 0.001$), total cholesterol level [sarcopenia, 3.8 (3.0, 4.6) vs. non-sarcopenia, 4.6 (3.5, 5.7) mmol/L; $p = 0.019$], HDL-C (sarcopenia, 1.1 ± 0.3 vs. non-sarcopenia, $1.2 \pm 0.2 \text{ mmol/L}$; $p = 0.032$) and LDL-C level [sarcopenia, 2.1 (1.7, 2.9) vs. non-sarcopenia, 2.9 (2.0, 3.9) mmol/L; $p = 0.044$], with significantly increased CRP level [sarcopenia, 1.6 (0.7, 6.2) vs. non-sarcopenia, 0.8 (0.5, 1.5) mg/L; $p = 0.022$] and IL-6 level [sarcopenia, 5.5 (3.5, 10.5) vs. non-sarcopenia, 2.1 (1.5, 3.7)

Table 1 Baseline characteristics of participants with and without sarcopenia for DIA analysis and ELISA validation [32]

Items	DIA analysis cohort			ELISA validation cohort		
	Sarcopenia (n=30)	Non-sarcopenia (n=30)	p-value	Sarcopenia (n=6)	Non-sarcopenia (n=6)	p-value
Age, years	91.0 (83.5, 92.0)	71.0 (65.2, 76.2)	<0.001 ^a	78.7 \pm 10.8	70 \pm 5.4	0.109 ^b
Male, n (%)	18 (60)	21 (70)	0.417 ^c	4 (67)	4 (67)	1 ^d
BMI, kg/m^2	21.3 \pm 3.5	24.5 \pm 2.4	<0.001 ^b	21.2 \pm 1.4	25.2 \pm 3.1	0.017 ^b
ASMI, kg/m^2	5.5 \pm 0.9	7.3 \pm 0.9	<0.001 ^b	5.8 \pm 0.9	7.9 \pm 1.3	0.01 ^b
Handgrip strength, kg	15.2 (0.0, 21.4)	28.2 (25.2, 35.1)	<0.001 ^a	18.7 \pm 10.5	37.4 \pm 5.5	0.003 ^b
Six-meter Walk speed, m/s	0.4 (0.0, 0.7)	1.2 (1.0, 1.3)	<0.001 ^a	0.9 (0.3, 1.1)	1.2 (1.0, 1.4)	0.037 ^a
Triglyceride, mmol/L	1.1 (0.8, 1.3)	1.0 (0.8, 1.7)	0.631 ^a	0.9 (0.8, 1.6)	1.2 (1.0, 2.0)	0.2 ^a
Total cholesterol, mmol/L	3.8 (3.0, 4.6)	4.6 (3.5, 5.7)	0.019 ^a	4.0 (3.3, 4.3)	4.7 (4.4, 5.9)	0.01 ^a
HDL-C, mmol/L	1.1 \pm 0.3	1.2 \pm 0.2	0.032 ^b	1.3 \pm 0.4	1.1 \pm 0.2	0.392 ^b
LDL-C, mmol/L	2.1 (1.7, 2.9)	2.9 (2.0, 3.9)	0.044 ^a	2.1 \pm 0.5	3.2 \pm 0.7	0.011 ^b
CRP, mg/L	1.6 (0.7, 6.2)	0.8 (0.5, 1.5)	0.022 ^a	1.6 \pm 1.5	1.2 \pm 0.7	0.625 ^b
IL-6, pg/mL	5.5 (3.5, 10.5)	2.1 (1.5, 3.7)	<0.001 ^a	3.4 (1.5, 6.0)	3.2 (1.9, 3.7)	0.628 ^a
aCCI	8.0 (6.2, 9.0)	6.0 (5.0, 8.0)	0.004 ^a	6.0 (5.2, 8.2)	6.5 (3.7, 7.0)	0.746 ^a
Number of drugs	9.5 (5.0, 20.7)	4.5 (2.0, 7.0)	<0.001 ^a	10.0 (6.5, 13.5)	6.0 (5.2, 6.7)	0.258 ^a
Duration of hospitalization, days	10.0 (6.0, 15.0)	7.5 (6.2, 14.0)	0.582 ^a	9.0 (6.5, 11.5)	3.0 (2.2, 3.7)	0.006 ^a

The p -value was obtained for comparison of sarcopenic and non-sarcopenic groups by following tests: ^aRank sum test, ^bStudent’s t-test, ^cChi-square test, ^dFisher’s exact test

BMI body mass index, ASMI appendicular skeletal muscle index, HDL-C high density lipoprotein cholesterol, LDL-C low density lipoprotein cholesterol, CRP C-reactive protein, IL-6 interleukin-6, aCCI age-adjusted Charlson comorbidity index

pg/mL; $p < 0.001$]. Also, individuals with sarcopenia had higher aCCI [sarcopenia, 8.0 (6.2, 9.0) vs. non-sarcopenia, 6.0 (5.0, 8.0); $p = 0.004$] and number of drugs [sarcopenia, 9.5 (5.0, 20.7) vs. non-sarcopenia, 4.5 (2.0, 7.0); $p < 0.001$] compared with those without sarcopenia.

In the ELISA validation cohort, the age, IL-6 level, CRP level, aCCI, and number of drugs showed no significant differences between the sarcopenic group and non-sarcopenic group. Compared with individuals in the non-sarcopenic group, individuals in the sarcopenic group had lower BMI (sarcopenia, 21.2 ± 1.4 vs. non-sarcopenia, 25.2 ± 3.1 kg/m 2 ; $p = 0.017$), total cholesterol level [sarcopenia, 4.0 (3.3, 4.3) vs. non-sarcopenia, 4.7 (4.4, 5.9) mmol/L; $p = 0.01$] and LDL-C level (sarcopenia, 2.1 ± 0.5 vs. non-sarcopenia, 3.2 ± 0.7 mmol/L; $p = 0.011$).

Overview of DIA-based proteomic analysis

DDA mode identified a total of 18,816 peptides and 2832 proteins. DIA-based analysis recognized 9207 peptides and 1127 proteins, with a false discovery rate (FDR) of less than 1%. Ultimately, 1,018 proteins were annotated and quantified (Supplementary Table 1). Partial Least-Squares regression analysis was performed on both sarcopenic and non-sarcopenic groups to examine within-group reproducibility and between-group differences (Fig. 1A for PLS-DA score plot, Supplementary Fig. 2 for validation model). The results indicated consistency within each group and a clear distinction between them.

Identification of differentially expressed proteins

Compared with the non-sarcopenic group, a total of 39 DEPs were identified in the sarcopenic group, with a cutoff value of fold change > 1.5 (or < 0.667) and $p < 0.05$, including 17 up-regulated proteins and 22 down-regulated proteins (Fig. 1B, Supplementary Table 2). Following the exclusion of proteins: a) without a defined gene ($n = 7$), b) with an uncharacterized protein description ($n = 1$), and c) related to hemoglobin ($n = 9$) or immunoglobulin ($n = 10$), 12 proteins were selected for further analysis (Supplementary Table 3). Hierarchical clustering analysis of these 12 DEPs revealed different protein expression patterns between the sarcopenic and non-sarcopenic groups, with proteins of each group generally clustering together (Fig. 1C).

ROC analysis and random forest test of DEPs

Receiver Operating Characteristic (ROC) analysis was performed to evaluate the potential diagnostic value of the 12 selected DEPs (Fig. 1D). Applying a threshold of AUC > 0.7 , a total of 8 proteins, namely insulin-like growth factor-binding protein-2 (IGFBP2), paraoxonase-3 (PON3),

leucine rich alpha-2-glycoprotein 1 (LRG1), peroxiredoxin 6 (PRDX6), prostaglandin D2 synthase (PTGDS), scavenger receptor cysteine-rich type 1 protein M130 (CD163), proteoglycan 4 (PRG4), and T cell receptor alpha joining 17 (TRAJ17), were selected for further investigation (Supplementary Table 4). IGFBP2 (AUC 0.8756) and PON3 (AUC 0.8344) demonstrated the top 2 AUC values, indicating their decent ability to differentiate individuals with sarcopenia from those without sarcopenia.

ROC model including these 8 proteins achieved an AUC value of 0.9533, with specificity of 0.933 and sensitivity of 0.833 (Supplementary Fig. 3A). ROC model enrolling IGFBP2 and PON3, the top 2 proteins, displayed an AUC value of 0.9022 (specificity 0.867, sensitivity 0.867) (Supplementary Fig. 3B). Random forest test was performed to evaluate variable importance of the selected proteins in sarcopenia prediction. Of them, IGFBP2 and PON3 were determined to be the top 2 crucial proteins, showing remarkably higher mean decrease accuracy (MDA) and mean decrease Gini (MDG) index (Fig. 1E and F, Supplementary Table 5).

Associations between DEPs and clinical parameters

To investigate the associations between selected DEPs and clinical indices, Spearman's correlation analysis was conducted, with results displayed in a heatmap (Fig. 2A, Supplementary Table 6). The significant associations were presented in a co-occurrence network (Fig. 2B). ASMI, grip strength, and six-meter walk speed were positively correlated with PRDX6, PON3, and TRAJ17, and inversely correlated with PTGDS, CD163, LRG1, and IGFBP2. aCCI was positively correlated with IGFBP2 and PTGDS, and negatively correlated with PON3 and PRG4. The number of drugs showed positive correlations with IGFBP2, LRG1, CD163, and PTGDS, with negative correlations with PRG4, PON3, and PRDX6. IL-6 demonstrated positive correlations with IGFBP2, LRG1, CD163, and PTGDS, and negative correlations with TRAJ17, PRG4, PON3, and PRDX6. HDL-C level exhibited positive correlations with PON3 and PRG4. Triglyceride level was positively correlated with PRG4, and negatively correlated with IGFBP2 and LRG1. LDL-C and total cholesterol both displayed positive correlations with PRDX6 and PRG4, and negative correlations with IGFBP2, LRG1, and PTGDS.

DEPs functional enrichment and PPI network analysis

For further understanding of the mechanisms of plasma proteins in sarcopenia development, gene ontology (GO) enrichment, Kyoto encyclopedia of genes and genomes (KEGG) enrichment and Interpro (IPR) domains analysis were performed on these 8 proteins. GO analysis revealed

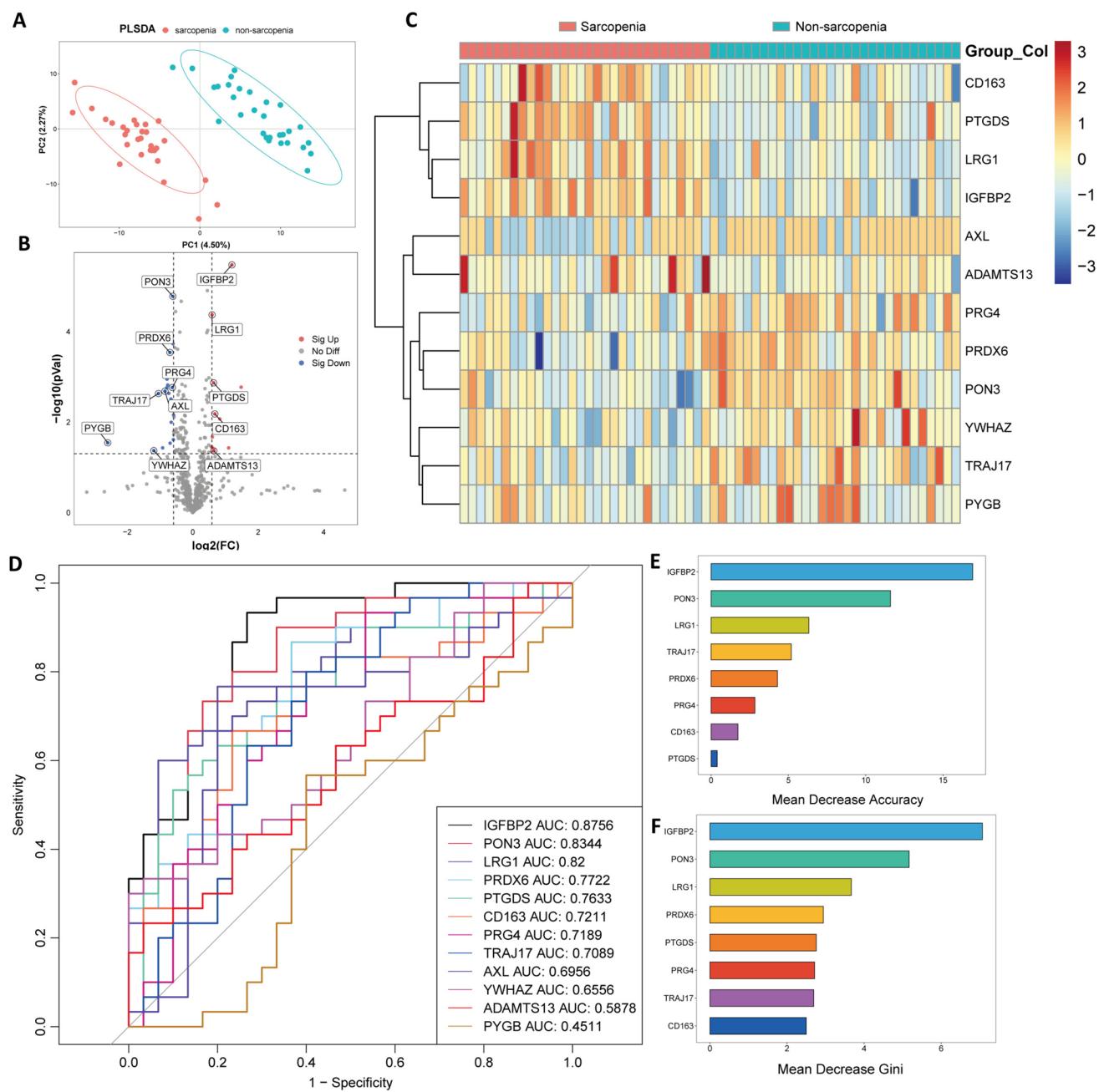


Fig. 1 DIA analysis and DEPs selection. **A** Partial Least-Squares analysis of proteomic data. **B** Volcano plot of DEPs. Blue dots represented significantly down-regulated ($FC < 0.667$ and $p < 0.05$) proteins in the sarcopenic group; Red dots represented significantly up-regulated ($FC > 1.5$ and $p < 0.05$) proteins in the sarcopenic group; Grey dots represented non-significant proteins. **C** Hierarchical clustering heatmap of 12 DEPs. Proteins were shown in rows. Samples were shown in columns. Colour represented the expression levels between two groups. **D** ROC analysis of 12 DEPs to differentiate individuals with sarcopenia from those without sarcopenia, with AUC values listed in lower right. Eight selected DEPs were ranked in descending order of importance to the prediction accuracy based on mean decrease accuracy (**E**) and Gini index (**F**). *ROC* Receiver Operating Characteristic, *AUC* area under the ROC curve

significant enrichment in scavenger receptor activity, anti-oxidant activity, peroxiredoxin activity, arylesterase activity, and polysaccharide binding (Supplementary Fig. 4A and 4B, Supplementary Table 7). KEGG pathway analysis illustrated that the DEPs were mainly annotated with terms

were shown in columns. Colour represented the expression levels between two groups. **D** ROC analysis of 12 DEPs to differentiate individuals with sarcopenia from those without sarcopenia, with AUC values listed in lower right. Eight selected DEPs were ranked in descending order of importance to the prediction accuracy based on mean decrease accuracy (**E**) and Gini index (**F**). *ROC* Receiver Operating Characteristic, *AUC* area under the ROC curve

about Metabolic pathways and Arachidonic acid metabolism (Supplementary Fig. 4C and 4D, Supplementary Table 8). IPR enrichment analysis identified the enriched functional domains of DEPs, including Alkyl hydroperoxide reductase subunit C/Thiol specific antioxidant, Peroxiredoxin

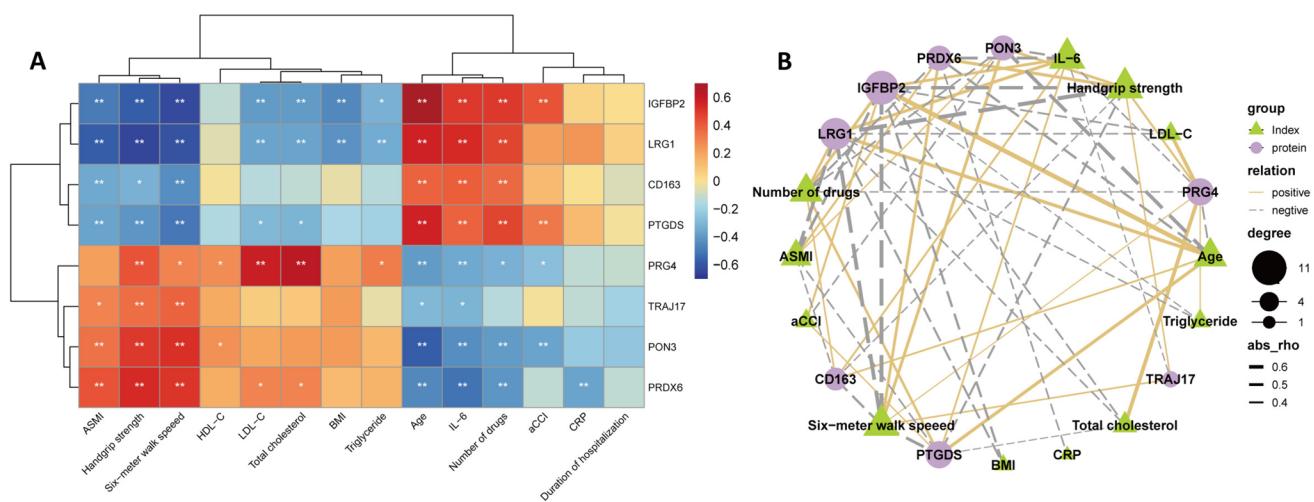


Fig. 2 Correlation analysis. **A** Heatmap of correlations between DEPs and clinical parameters, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$. **B** Correlation network of significant correlations ($p < 0.05$). Degree represented the number of the nodes connected to the exact node, demonstrated by the size of the node. Purple circles represented DEPs.

Green triangles represented the clinical parameters. The thickness of the lines represented the rho value. Yellow solid line represented a positive association. Grey dashed line represented a negative association

C-terminal, and Arylesterase (Supplementary Fig. 4E and 4F, Supplementary Table 9). Furthermore, a protein–protein interaction (PPI) network analysis was conducted to investigate the interactions between these proteins in sarcopenia (Supplementary Fig. 4G).

Experimental validation of potential protein biomarker for sarcopenia

ELISA validation was conducted for the 8 selected proteins. Plasma samples from 6 participants with sarcopenia and 6 without sarcopenia were utilized to quantify the concentration of these proteins, as detailed in Table 2 and depicted in Fig. 3. Notably, PON3 was significantly down-regulated in the sarcopenic group, consistent with the DIA analysis, indicating its potential as a biomarker for sarcopenia development. Although IGFBP2 showed significant differences between the two groups in ELISA validation, its regulation

pattern was opposite to that observed in the DIA analysis. LRG1, PRDX6, PTGDS, CD163, PRG4, and TRAJ17 showed no significant differences between the sarcopenic and non-sarcopenic group in ELISA validation.

Discussion

Our study investigated the plasma proteome of individuals with age-related sarcopenia and identified 8 DEPs that showed promising predictive performance, including IGFBP2, PON3, LRG1, PRDX6, PTGDS, CD163, PRG4, and TRAJ17. Functional analysis revealed significant enrichment of scavenger receptor activity, arylesterase activity, and antioxidant activity, along with notable alterations in metabolic pathways. Subsequent ELISA validation in an independent cohort confirmed significantly different expression levels of PON3 and IGFBP2 between individuals with and

Table 2 Plasma concentration validation of selected proteins in participants with and without sarcopenia by ELISA

Protein (ng/mL)	Sarcopenia (n=6)	Non-sarcopenia (n=6)	Z-value	p-value
IGFBP2	0.4432 (0.4282, 0.4484)	0.5174 (0.4952, 0.5629)	2.882	0.004*
PON3	2.7285 (2.4897, 2.9605)	3.21561 (3.0542, 3.4118)	2.242	0.025*
LRG1	308.1870 (163.9352, 356.2098)	337.5315 (254.4637, 602.1790)	0.961	0.337
PRDX6	120.6250 (104.0819, 129.5493)	132.3493 (87.8002, 147.4762)	0.48	0.631
PTGDS	23.7686 (12.0484, 28.8587)	28.1447 (19.9521, 45.6905)	1.281	0.200
CD163	0.3446 (0.2277, 0.5318)	0.3928 (0.3752, 0.4721)	0.962	0.336
PRG4	358.7390 (290.6268, 611.8025)	420.3340 (315.7745, 523.6555)	0.32	0.749
TRAJ17	72.2865 (45.1982, 135.6611)	85.8205 (62.5182, 123.5002)	0.32	0.749

Rank sum test was performed (* $p < 0.05$)

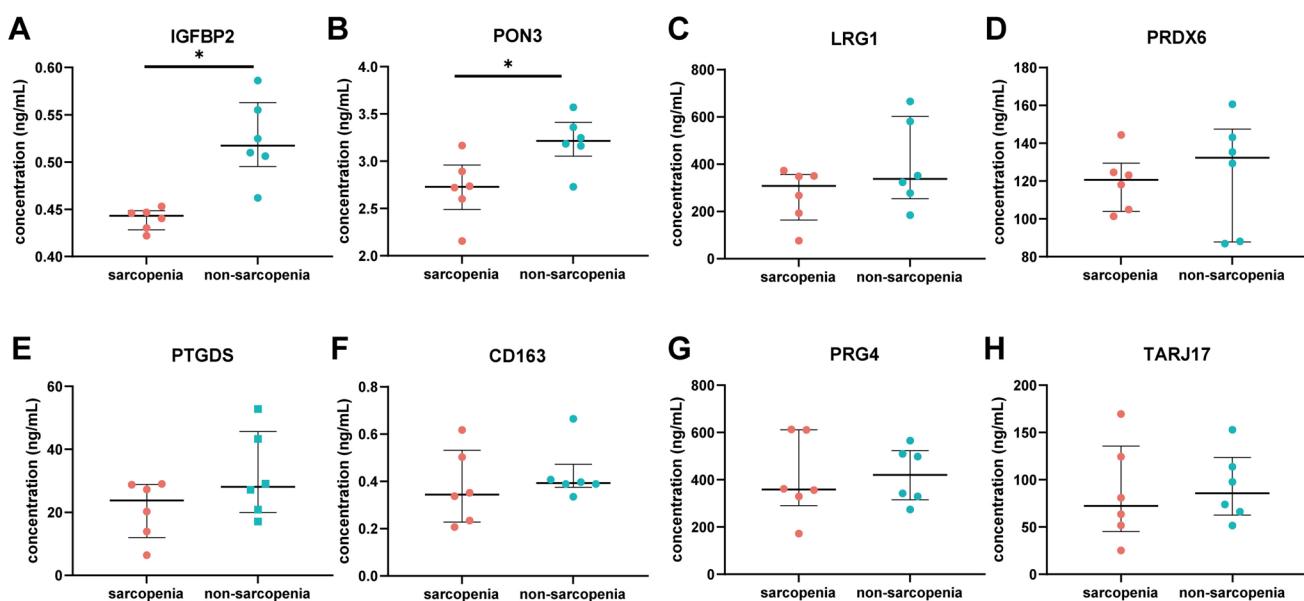


Fig. 3 ELISA validation of selected DEPs in plasma samples of participants with sarcopenia ($n=6$) and those without sarcopenia ($n=6$). Protein concentrations determined by ELISA for **A** IGFBP2,

B PON3, **C** LRG1, **D** PRDX6, **E** PTGDS, **F** CD163, **G** PRG4, and **H** TRAJ17. Results were expressed as “median (interquartile range)”. Rank sum test was performed ($*p < 0.05$)

without sarcopenia. PON3 was consistently down-regulated in the sarcopenic group across both proteomic analysis and validation, while IGFBP2 showed inconsistency, being up-regulated in the sarcopenic group in proteomics but down-regulated in validation. Consistent with prior research, we found distinct plasma proteomic profiles between participants with and without sarcopenia. We identified sarcopenia-associated proteins that were not reported in the literature and observed proteins with differences from existing research findings, which may be related to various factors such as the geographic and ethnic backgrounds of the participants. Ultimately, we propose PON3 as a potential biomarker for age-related sarcopenia.

Paraoxonase-3 (PON3), the most recently discovered and the least studied protein of the paraoxonase family (PON1-3) [36], is mainly located in the mitochondria, endoplasmic reticulum and circulating HDL particles [37]. Current evidence suggests that PON3 participates in the pathogenesis of various diseases, primarily through its significant antioxidant properties [36, 38, 39]. HDL-associated PON3 also helps prevent LDL oxidation and inactivate oxidized LDL [40]. In the field of aging, PON family is involved in senescence and age-related diseases, with a focus on their antioxidative potential [41]. Oxidative stress is one of the key mechanisms in aging and sarcopenia [42–44]. Researchers observed a decline in PON1 level with increasing age [45]. Additionally, the rs662 polymorphism in the PON1 gene demonstrated a significant association with longevity, potentially attributed to its ability to enhance the anti-oxidant capacity of PON1 [46]. However, research on the

role of PON3 in muscle aging is limited. Our study is the first to report the role of PON3 as a biomarker for sarcopenia in aged population. The correlation analysis revealed that PON3 was positively correlated with HDL-C level and muscle parameters. It could suggest that a decline in PON3 may lead to an overload of oxidative stress in skeletal muscles, which eventually contributes to sarcopenia onset and progression. Restoring PON3 level in aged individuals may reinstate the antioxidant activity and alleviate oxidative stress in skeletal muscles, eventually improving muscle mass and function.

Insulin-like growth factor-binding protein-2 (IGFBP2) is a member of the insulin-like growth factor-binding protein family (IGFBP1-6) [47]. Recent studies have established links between IGFBP2 and muscle cachexia [48], low skeletal muscle mass [17, 49] and decline in muscle strength and function [50]. In the proteomic analysis, IGFBP2 was found to be significantly up-regulated in individuals with sarcopenia. However, subsequent ELISA validation showed a low concentration of IGFBP2 in the plasma from the sarcopenic group. Given the demographics of our study cohort and the characteristics of IGFBP2, we propose that this finding may be attributed to two potential factors.

First, discrepancies were observed between the two cohorts. According to previous research, IGFBP2 was significantly associated with age [51], senescence [52], inflammation status [53–55], and various diseases [56–58]. In the cohort for proteomic analysis, compared with those without sarcopenia, individuals with sarcopenia exhibited older age, higher inflammatory levels, higher aCCI, and higher number

of drugs, which could influence IGFBP2 expression level. Conversely, the cohort for ELISA validation exhibited balanced baseline characteristics. These discrepancies could account for the inconsistent results of IGFBP2. It is unrigorous to conclude a definitive association between IGFBP2 and sarcopenia based on a limited sample size and a single set of results. Studies with larger sample sizes and different validation methods are necessary.

Second, the possible existence of protein modifications of IGFBP2 and the antigen–antibody binding characteristics of sandwich ELISA should be considered. A targeted analysis approach may fail to replicate proteins identified by an untargeted method due to different detection mechanisms [59]. While DIA-based proteomics is an untargeted technique that identifies proteins by analyzing fragmented peptides, ELISA is a targeted technique that quantifies the specific epitope recognized by the antibody [59]. It has been noted that ELISA is not sensitive enough to quantify modified proteins, including post-translational modifications (PTMs) [60], isoforms, and complexes with other circulating plasma proteins [61, 62]. Since modified epitopes may affect the success of recognition or the effectiveness of binding of antibodies to antigen, ELISA quantification result depends on the antibody affinity of specific protein forms rather than its total amount. Researchers found age-specific protein modifications in dysfunctional aged skeletal muscles [63, 64]. Furthermore, IGFBP2 has been observed in phosphorylated [65], complexed [66–68], or fragmented forms [68] during pathophysiological processes. Thus, we speculate that IGFBP2 might undergo various modifications during sarcopenia pathogenesis, which may alter the targeted antigenic epitope for recognition and affect the affinity of antigen binding, ultimately resulting in detection discrepancies.

This is the first study revealing plasma biomarkers in Chinese hospitalized older adults with sarcopenia, with two independent cohorts for exploration and validation. PON3 has been identified as a potential novel biomarker for age-related sarcopenia. However, there are several limitations to consider. First, this cross-sectional study lacks longitudinal data on biomarker levels, emphasizing the need for longitudinal studies to elucidate dynamic changes of biomarkers throughout the pathogenetic process of sarcopenia. Second, the imbalanced baseline characteristics of the participants in the DIA analysis suggested the presence of potential confounding factors that could influence the outcomes. Despite experimental validation, the results, particularly regarding IGFBP2, should be interpreted cautiously and warrant further validation. Third, recruiting hospitalized individuals with sarcopenia limits the generalizability of our findings to community-dwelling and nursing home residents. Multi-center and multi-regional research are necessary to identify specific biomarkers for individuals with sarcopenia with various characteristics. Fourth, this study provided preliminary

validation of sarcopenia biomarkers but lacked insight into the underlying mechanisms. Considering the perspectives of criteria for aging biomarkers [69], functional validation and mechanism elucidation through cellular experiments, animal models, and clinical trials should be carried out. Additionally, beyond protein abundance, the specific protein modification forms, such as phosphorylation, ubiquitination, and methylation, should be detected and validated using updated technologies.

Conclusion

In conclusion, this cross-sectional study applied proteomic analysis and experimental validation to explore the sarcopenic proteome and to identify candidate biomarkers for age-related sarcopenia. Our findings indicated a distinguishable proteome between individuals with and without sarcopenia. PON3 was confirmed to be down-regulated in older adults with sarcopenia, which may lead to sarcopenia due to impaired antioxidant defenses. For IGFBP2, the discrepant result suggested a possible modification of IGFBP2, and further studies are needed to confirm this hypothesis. We propose that PON3 could be considered as a potential biomarker for age-related sarcopenia and a promising target for intervention. Further cellular, animal and clinical studies of PON3 are needed for an in-depth understanding of the pathogenesis and mechanism of sarcopenia.

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Author contributions Qin Qing Lin, Jing Zhou and Lishu Xu contributed to the research conception and design. Kang Yong Li, Li Wei Li, Lichang Guan and Ying Tong Zeng were responsible for participant enrollment and data collection. Qin Qing Lin performed the experiment and statistical analysis. Qin Qing Lin wrote the draft of the manuscript and all authors reviewed the draft. Dake Cai, Jing Zhou and Lishu Xu edited and revised this manuscript. All authors read and approved the submitted version.

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Data availability The proteomics data have been deposited to the ProteomeXchange Consortium (<https://proteomecentral.proteomexchange.org>) via the iProX (<https://www.iprox.cn/>) partner repository with the dataset identifier PXD053735.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was performed in line with the principles of the 1964 Helsinki Declaration and its later amendments. This study was approved by the Ethics Committee of Guangdong Provincial People's Hospital in Guangzhou, China (approval no. GDREC20198345H(RI)). All procedures followed the local legislation and institutional requirements. All participants were informed and provided written informed consents.

Consent to participate Informed consents were obtained from all participants included in this study.

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References

- Chen LK, Woo J, Assantachai P et al (2020) Asian working group for sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *J Am Med Dir Assoc.* <https://doi.org/10.1016/j.jamda.2019.12.012>
- Beaudart C, Demonceau C, Reginster JY et al (2023) Sarcopenia and health-related quality of life: a systematic review and meta-analysis. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.13243>
- Ye C, Zheng X, Aihemaitijiang S et al (2022) Sarcopenia and catastrophic health expenditure by socio-economic groups in China: an analysis of household-based panel data. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.12997>
- Liu D, Wang S, Liu S et al (2024) Frontiers in sarcopenia: Advancements in diagnostics, molecular mechanisms, and therapeutic strategies. *Mol Aspects Med.* <https://doi.org/10.1016/j.mam.2024.101270>
- Tsai SY (2024) Lost in translation: challenges of current pharmacotherapy for sarcopenia. *Trends Mol Med.* <https://doi.org/10.1016/j.molmed.2024.05.016>
- Cruz-Jentoft AJ, Sayer AA (2019) Sarcopenia. *Lancet.* [https://doi.org/10.1016/s0140-6736\(19\)31138-9](https://doi.org/10.1016/s0140-6736(19)31138-9)
- Larsson L, Degens H, Li M et al (2019) Sarcopenia: aging-related loss of muscle mass and function. *Physiol Rev.* <https://doi.org/10.1152/physrev.00061.2017>
- Li CW, Yu K, Shyh-Chang N et al (2022) Pathogenesis of sarcopenia and the relationship with fat mass: descriptive review. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.12901>
- Shin HE, Won CW, Kim M (2023) Development of multiple biomarker panels for prediction of sarcopenia in community-dwelling older adults. *Arch Gerontol Geriatr.* <https://doi.org/10.1016/j.archger.2023.105115>
- Lian R, Liu Q, Jiang G et al (2024) Blood biomarkers for sarcopenia: a systematic review and meta-analysis of diagnostic test accuracy studies. *Ageing Res Rev.* <https://doi.org/10.1016/j.arr.2023.102148>
- Hsu WH, Wang SY, Chao YM et al (2024) Novel metabolic and lipidomic biomarkers of sarcopenia. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.13567>
- Zou Y, Tang X, Yang S et al (2024) New insights into the function of the NLRP3 inflamasome in sarcopenia: mechanism and therapeutic strategies. *Metabolism.* <https://doi.org/10.1016/j.metabol.2024.155972>
- Shin HE, Won CW, Kim M (2022) Metabolomic profiles to explore biomarkers of severe sarcopenia in older men: a pilot study. *Exp Gerontol.* <https://doi.org/10.1016/j.exger.2022.111924>
- Jung HW, Park JH, Kim DA et al (2021) Association between serum FGF21 level and sarcopenia in older adults. *Bone.* <https://doi.org/10.1016/j.bone.2021.115877>
- Li CW, Yu K, Shyh-Chang N et al (2019) Circulating factors associated with sarcopenia during ageing and after intensive lifestyle intervention. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.12417>
- Liu JC, Dong SS, Shen H et al (2022) Multi-omics research in sarcopenia: current progress and future prospects. *Ageing Res Rev.* <https://doi.org/10.1016/j.arr.2022.101576>
- Dlamini SN, Norris SA, Mendham AE et al (2022) Targeted proteomics of appendicular skeletal muscle mass and handgrip strength in black South Africans: a cross-sectional study. *Sci Rep.* <https://doi.org/10.1038/s41598-022-13548-9>
- Jiang W, Zhan W, Zhou L et al (2023) Potential therapeutic targets for sarcopenia identified by Mendelian randomisation. *Age Ageing.* <https://doi.org/10.1093/ageing/afad024>
- Aparicio P, Navarrete-Villanueva D, Gómez-Cabello A et al (2024) Proteomic profiling of human plasma extracellular vesicles identifies PF4 and C1R as novel biomarker in sarcopenia. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.13539>
- Huemer MT, Bauer A, Petreira A et al (2021) Proteomic profiling of low muscle and high fat mass: a machine learning approach in the KORA S4/FF4 study. *J Cachexia Sarcopenia Muscle.* <https://doi.org/10.1002/jcsm.12733>
- Ubaida-Mohien C, Lyashkov A, Gonzalez-Freire M et al (2019) Discovery proteomics in aging human skeletal muscle finds change in spliceosome, immunity, proteostasis and mitochondria. *Elife.* <https://doi.org/10.7554/elife.49874>
- Lou R, Shui W (2024) Acquisition and analysis of DIA-based proteomic data: a comprehensive survey in 2023. *Mol Cell Proteomics.* <https://doi.org/10.1016/j.mcpro.2024.100712>
- Karayel O, Virreira Winter S, Padmanabhan S et al (2022) Proteome profiling of cerebrospinal fluid reveals biomarker candidates for Parkinson's disease. *Cell Rep Med.* <https://doi.org/10.1016/j.xcrm.2022.100661>
- Li H, Xu Y, Zhou X et al (2022) DIA-based proteomic analysis of plasma protein profiles in patients with severe acute pancreatitis. *Molecules.* <https://doi.org/10.3390/molecules27123880>
- Wang C, Liu G, Liu Y et al (2022) Novel serum proteomic biomarkers for early diagnosis and aggressive grade identification of prostate cancer. *Front Oncol.* <https://doi.org/10.3389/fonc.2022.1004015>
- Ankney JA, Muneer A, Chen X (2018) Relative and absolute quantitation in mass spectrometry-based proteomics. *Annu Rev Anal Chem (Palo Alto Calif).* <https://doi.org/10.1146/annurev-anchem-061516-045357>
- Che YQ, Zhang Y, Li HB et al (2021) Serum KLKB1 as a potential prognostic biomarker for hepatocellular carcinoma based on data-independent acquisition and parallel reaction monitoring. *J Hepatocell Carcinoma.* <https://doi.org/10.2147/jhc.S325629>
- Ji S, Liu Y, Yan L et al (2023) DIA-based analysis of the menstrual blood proteome identifies association between CXCL5 and IL1RN and endometriosis. *J Proteomics.* <https://doi.org/10.1016/j.jprot.2023.104995>
- Cao Q, Zhu H, Xu W et al (2023) Predicting the efficacy of glucocorticoids in pediatric primary immune thrombocytopenia using plasma proteomics. *Front Immunol.* <https://doi.org/10.3389/fimmu.2023.1301227>

30. Wu J, Cao L, Wang J et al (2022) Characterization of serum protein expression profiles in the early sarcopenia older adults with low grip strength: a cross-sectional study. *BMC Musculoskeletal Disorders*. <https://doi.org/10.1186/s12891-022-05844-2>
31. Vandenbroucke JP, von Elm E, Altman DG et al (2014) Strengthening the reporting of observational studies in epidemiology (STROBE): explanation and elaboration. *Int J Surg*. <https://doi.org/10.1016/j.ijsu.2014.07.014>
32. Zhou J, Liu J, Lin Q et al (2023) Characteristics of the gut microbiome and metabolic profile in elderly patients with sarcopenia. *Front Pharmacol*. <https://doi.org/10.3389/fphar.2023.1279448>
33. Pan ZM, Zeng J, Li T et al (2024) Age-adjusted Charlson comorbidity index is associated with the risk of osteoporosis in older fall-prone men: a retrospective cohort study. *BMC Geriatr*. <https://doi.org/10.1186/s12877-024-05015-z>
34. Jones P, Binns D, Chang HY et al (2014) InterProScan 5: genome-scale protein function classification. *Bioinformatics*. <https://doi.org/10.1093/bioinformatics/btu031>
35. da Huang W, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. <https://doi.org/10.1093/nar/gkn923>
36. Mohammed CJ, Lamichhane S, Connolly JA et al (2022) A PON for all seasons: comparing paraoxonase enzyme substrates, activity and action including the role of PON3 in health and disease. *Antioxidants (Basel)*. <https://doi.org/10.3390/antiox111030590>
37. Furlong CE, Marsillach J, Jarvik GP et al (2016) Paraoxonases-1, -2 and -3: What are their functions? *Chem Biol Interact*. <https://doi.org/10.1016/j.cbi.2016.05.036>
38. Bacchetti T, Ferretti G, Sahebkar A (2019) The role of paraoxonase in cancer. *Semin Cancer Biol*. <https://doi.org/10.1016/j.semcancer.2017.11.013>
39. Khalaf FK, Connolly J, Khatib-Shahidi B et al (2023) Paraoxonases at the heart of neurological disorders. *Int J Mol Sci*. <https://doi.org/10.3390/ijms24086881>
40. Reddy ST, Wadleigh DJ, Grijalva V et al (2001) Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. *Arterioscler Thromb Vasc Biol*. <https://doi.org/10.1161/01.atv.21.4.542>
41. Levy D, Reichert CO, Bydlowski SP (2019) Paraoxonases activities and polymorphisms in elderly and old-age diseases: an overview. *Antioxidants (Basel)*. <https://doi.org/10.3390/antiox8050118>
42. Luo J, Mills K, le Cessie S et al (2020) Ageing, age-related diseases and oxidative stress: what to do next? *Ageing Res Rev*. <https://doi.org/10.1016/j.arr.2019.100982>
43. Hajam YA, Rani R, Ganey SY et al (2022) Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells*. <https://doi.org/10.3390/cells11030552>
44. Qaisar R, Bhaskaran S, Premkumar P et al (2018) Oxidative stress-induced dysregulation of excitation-contraction coupling contributes to muscle weakness. *J Cachexia Sarcopenia Muscle*. <https://doi.org/10.1002/jcsm.12339>
45. Li Y, Liang G, Shi L et al (2016) Paraoxonase-1 (PON1) rs662 polymorphism and its association with serum lipid levels and longevity in the Bama Zhuang population. *Med Sci Monit*. <https://doi.org/10.12659/msm.898231>
46. Erdman V, Tuktarova I, Nasibullin T et al (2024) Polygenic markers of survival and longevity in the antioxidant genes PON1, PON2, MTHFR, MSRA, SOD1, NQO1, and CAT in a 20-year follow-up study in the population from the Volga-Ural region. *Gene*. <https://doi.org/10.1016/j.gene.2024.148510>
47. Haywood NJ, Slater TA, Matthews CJ et al (2019) The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes. *Mol Metab*. <https://doi.org/10.1016/j.molmet.2018.10.008>
48. Dong J, Yu J, Li Z et al (2021) Serum insulin-like growth factor binding protein 2 levels as biomarker for pancreatic ductal adenocarcinoma-associated malnutrition and muscle wasting. *J Cachexia Sarcopenia Muscle*. <https://doi.org/10.1002/jcsm.12692>
49. Lebrasseur NK, Achenbach SJ, Melton LJ 3rd et al (2012) Skeletal muscle mass is associated with bone geometry and microstructure and serum insulin-like growth factor binding protein-2 levels in adult women and men. *J Bone Miner Res*. <https://doi.org/10.1002/jbm.1666>
50. van den Beld AW, Blum WF, Pols HA et al (2003) Serum insulin-like growth factor binding protein-2 levels as an indicator of functional ability in elderly men. *Eur J Endocrinol*. <https://doi.org/10.1530/eje.0.1480627>
51. van den Beld AW, Carlson OD, Doyle ME et al (2019) IGFBP-2 and aging: a 20-year longitudinal study on IGFBP-2, IGF-I, BMI, insulin sensitivity and mortality in an aging population. *Eur J Endocrinol*. <https://doi.org/10.1530/eje-18-0422>
52. Evans DS, Young D, Tanaka T et al (2024) Proteomic analysis of the senescence-associated secretory phenotype: GDF-15, IGFBP-2, and Cystatin-C are associated with multiple aging traits. *J Gerontol A Biol Sci Med Sci*. <https://doi.org/10.1093/gerona/glad265>
53. Wang Y, Huang J, Zhang F et al (2023) Knock-down of IGFBP2 ameliorates lung fibrosis and inflammation in rats with severe pneumonia through STAT3 pathway. *Growth Factors*. <https://doi.org/10.1080/08977194.2023.2259497>
54. Wang W, Ye J, Xu L et al (2024) The effects of IGF-1 and IGFBP-2 treatments on the atherosclerosis in the aorta and the coronary arteries of the high cholesterol diet-fed rabbits. *Int Immunopharmacol*. <https://doi.org/10.1016/j.intimp.2023.111409>
55. Zhang J, Jin L, Hua X et al (2023) SARM1 promotes the neuro-inflammation and demyelination through IGFBP2/NF- κ B pathway in experimental autoimmune encephalomyelitis mice. *Acta Physiol (Oxf)*. <https://doi.org/10.1111/apha.13974>
56. Boughanem H, Yubero-Serrano EM, López-Miranda J et al (2021) Potential role of insulin growth-factor-binding protein 2 as therapeutic target for obesity-related insulin resistance. *Int J Mol Sci*. <https://doi.org/10.3390/ijms22031133>
57. Li T, Forbes ME, Fuller GN et al (2020) IGFBP2: integrative hub of developmental and oncogenic signaling network. *Oncogene*. <https://doi.org/10.1038/s41388-020-1154-2>
58. Wang W, Yu K, Zhao SY et al (2023) The impact of circulating IGF-1 and IGFBP-2 on cardiovascular prognosis in patients with acute coronary syndrome. *Front Cardiovasc Med*. <https://doi.org/10.3389/fcvm.2023.1126093>
59. Ashton NJ, Nevado-Holgado AJ, Barber IS et al (2019) A plasma protein classifier for predicting amyloid burden for preclinical Alzheimer's disease. *Sci Adv*. <https://doi.org/10.1126/sciadv.aau7220>
60. Tong QH, Tao T, Xie LQ et al (2016) ELISA-PLA: a novel hybrid platform for the rapid, highly sensitive and specific quantification of proteins and post-translational modifications. *Biosens Bioelectron*. <https://doi.org/10.1016/j.bios.2016.02.006>
61. Fortin T, Salvador A, Charrier JP et al (2009) Clinical quantitation of prostate-specific antigen biomarker in the low nanogram/milliliter range by conventional bore liquid chromatography-tandem mass spectrometry (multiple reaction monitoring) coupling and correlation with ELISA tests. *Mol Cell Proteomics*. <https://doi.org/10.1074/mcp.M800238-MCP200>
62. Shi T, Fillmore TL, Sun X et al (2012) Antibody-free, targeted mass-spectrometric approach for quantification of proteins at low picogram per milliliter levels in human plasma/serum. *Proc Natl Acad Sci U S A*. <https://doi.org/10.1073/pnas.1204366109>
63. Neal CL, Kronert WA, Camillo JRT et al (2024) Aging-affiliated post-translational modifications of skeletal muscle myosin affect biochemical properties, myofibril structure, muscle function, and proteostasis. *Aging Cell*. <https://doi.org/10.1111/acel.14134>

64. Li M, Ogilvie H, Ochala J et al (2015) Aberrant post-translational modifications compromise human myosin motor function in old age. *Aging Cell*. <https://doi.org/10.1111/acel.12307>
65. Graham ME, Kilby DM, Firth SM et al (2007) The in vivo phosphorylation and glycosylation of human insulin-like growth factor-binding protein-5. *Mol Cell Proteomics*. <https://doi.org/10.1074/mcp.M700027-MCP200>
66. Šunderić M, Đukanović B, Malenković V et al (2014) Molecular forms of the insulin-like growth factor-binding protein-2 in patients with colorectal cancer. *Exp Mol Pathol*. <https://doi.org/10.1016/j.yexmp.2013.11.006>
67. Šunderić M, Malenković V, Nedić O (2015) Complexes between insulin-like growth factor binding proteins and alpha-2-macroglobulin in patients with tumor. *Exp Mol Pathol*. <https://doi.org/10.1016/j.yexmp.2015.03.003>
68. Šunderić M, Mihailović N, Nedić O (2014) Protein molecular forms of insulin-like growth factor binding protein-2 change with aging. *Exp Gerontol*. <https://doi.org/10.1016/j.exger.2014.08.002>
69. Moqri M, Herzog C, Poganik JR et al (2023) Biomarkers of aging for the identification and evaluation of longevity interventions. *Cell*. <https://doi.org/10.1016/j.cell.2023.08.003>

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