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Healthy community-living older men differ from women in associations between myostatin levels and skeletal muscle mass

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Abstract

Background Myostatin is a negative regulator of muscle growth but the relationship between serum myostatin levels and muscle mass is unclear. This study investigated the association between serum myostatin levels and skeletal muscle mass among healthy older community residents in Taiwan, to evaluate the potential of serum myostatin as a biomarker for diagnosing sarcopenia and/or evaluating the effect of its treatment.

Methods Study data were excerpted from a random subsample of the I-Lan Longitudinal Aging Study population. Serum myostatin levels were determined and categorized into tertiles (low, medium, high). Relative appendicular skeletal muscle mass (RASM) was calculated as appendicular lean body mass by dual-energy X-ray absorptiometry divided by height squared (kg/m^2). Low muscle mass was defined as recommended by the Asian Working Group for Sarcopenia.

Results The analytic study sample comprised 463 adults (mean age: 69.1 years; 49.5% men). Compared with subjects with normal RASM, those with lower RASM were older and frailer, with significantly higher prevalence of malnutrition, lower serum dehydroepiandrosterone (DHEA) levels, and were more likely to have low serum myostatin status. Multivariable logistic regression analysis showed that male sex (OR 3.60, 95% CI 1.30–9.92), malnutrition (OR 4.39, 95% CI 1.56–12.36), DHEA (OR 0.99, 95% CI 0.99–1.00), and low myostatin (OR 3.23, 95% CI 1.49–7.01) were all independent risk factors for low RASM (all $P < 0.05$). In men, DHEA (OR 0.99, 95% CI 0.98–1.00) and low myostatin (OR 4.89, 95% CI 1.79–13.37) were significantly associated with low RASM (both $P < 0.05$); however, only malnutrition was associated with low RASM in women (OR 13.59, 95% CI 2.22–83.25, $P < 0.05$).

Conclusions Among healthy community-living older adults, low serum myostatin levels were associated with low skeletal muscle mass in men, but not in women. Our results do not support using serum myostatin levels to diagnose sarcopenia, or to monitor how it responds to treatments. Further research is needed to understand why men apparently differ from women in the interrelationship between their myostatin levels and muscle mass.

Keywords Frailty; Sex; Myostatin; Sarcopenia; Skeletal muscle mass

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Introduction

An important characteristic of aging is temporal changes in body composition between bone, muscle, and fat mass. From age 30, people may lose skeletal muscle mass at 3–8% per decade and at a considerably accelerated rate as they become older.[1,2] Loss of muscle mass may also lead to diminished muscle strength and physical performance, which heighten the risk of gait unsteadiness and falls, functional impairment, disability, and mortality[3–6]; the contemporary

definition of sarcopenia encompasses the aforementioned clinical characteristics. The aetiology and pathophysiology of sarcopenia are complex and multifactorial, involving physical inactivity, nutritional status, loss of muscle fibres and motor units, and hormonal changes, among others.[7] The balance between myostatin, activin, and follistatin in the sarcopenia nexus has garnered extensive research interest, and several potential pharmacotherapeutic agents based on the action of myostatin have been developed.

The growth/differentiation factor 8 gene (*GDF8*) was discovered in 1997[8]; its product, myostatin, is a member of the transforming growth factor-beta superfamily that is secreted primarily by myocytes in skeletal muscle, and was the first negative regulator of skeletal muscle growth to be identified.[8–11] Knockout mice had muscular hypertrophy due to increased numbers of myocytes, myofiber size, and overall muscle size[8] and rats with lower myostatin levels had accelerated myogenesis,[11] whereas transgenic mice with higher serum myostatin had cachexia.[10] Congruently, a child with a *GDF8* loss-of-function mutation had significant muscle hypertrophy.[12]

Given its action on skeletal muscle, serum levels of myostatin have hypothetical potential as a biomarker for sarcopenia.[13] However, the results of studies evaluating the relationship between serum myostatin and skeletal muscle mass are inconsistent, besides mostly deriving from subjects with particular diseases rather than healthy individuals. Inverse correlations between serum myostatin-immunoreactive protein concentration and height-corrected fat-free mass and muscle mass suggest that myostatin may be a biomarker of age-associated sarcopenia.[13] Serum myostatin was also negatively associated with skeletal muscle mass in male patients with chronic obstructive pulmonary disease (COPD).[14] Conversely, decreased plasma myostatin levels in heart-failure patients with cachexia suggest that lower myostatin may prevent loss of skeletal muscle and progression of cachexia.[15] Besides these inconsistencies, little is known about the relationship between serum myostatin and skeletal muscle mass among healthy individuals. Therefore, we investigated the association between serum myostatin and skeletal muscle mass among healthy older community residents.

Methods

Study design and subjects

Study subjects were a random subsample of the I-Lan Longitudinal Aging Study (ILAS), which is a population-based research cohort of 1839 adults aged 53–92 years, residing in I-Lan (Yilan) County, Taiwan, who were randomly sampled through household registration records; individuals were invited to participate via mail or telephone and those who accepted were enrolled after giving written informed consent. ILAS excluded subjects who (i) were unable to cooperate or communicate with study investigators; (ii) declined or were unable to grant consent; (iii) were currently institutionalized; (iv) were known to have active diseases (cancer, sepsis, heart failure, COPD, etc.) or functional dependence, or had life-expectancy <6 months; and (v) were planning to leave Yilan County.

This study analysed serum myostatin, activin, and follistatin measurements taken from a random subsample of 463 ILAS subjects. The Institutional Review Boards of National Yang Ming University and Taipei Veterans General Hospital approved the study protocol.

Demographic characteristics and laboratory data

Research nurses collected participants' demographic and medical details and took anthropometric measurements. Alcohol consumption was categorized as drinking or non-drinking, and tobacco usage as currently smoking or non-smoking.

All participants gave peripheral blood samples at 7–9 AM, after a 10 h overnight fast. Biochemistry measurements included serum albumin, alanine aminotransferase, uric acid, total cholesterol, serum creatinine, high-sensitivity C-reactive protein, homocysteine, testosterone, insulin-like growth factor-1, dehydroepiandrosterone (DHEA), and vitamin D3. The homeostasis model assessment of insulin resistance index was calculated for insulin resistance. Serum levels of myostatin, activin, and follistatin were measured by sandwich enzyme immunoassay kits (R&D systems, Inc., Minneapolis, USA). Low serum myostatin was defined as the lower tertile of subjects' collective serum myostatin levels.

Functional assessment and physical performance

Research nurses performed comprehensive functional assessments for all participants. Nutrition status was evaluated by Mini Nutritional Assessment,[16] and cognitive impairment defined by a Mini-Mental State Examination score <24.[17] Mood was evaluated by the five-item geriatric depression scale, with the five-item geriatric depression scale ≥ 2 denoting depressive symptoms.[18] Physical activity was assessed by International Physical Activity Questionnaire,[19] the severity of underlying medical conditions by the Charlson Comorbidity Index,[20] and frailty status was determined according to the Fried criteria, which comprise weight loss, physical inactivity, weakness, slowness, and exhaustion[21]; frailty was defined as having ≥ 3 items and pre-frailty was defined as 1 or 2 items—those who met no Fried criteria were considered robust. Gait speed was measured by a timed 6 m walk at the participant's usual pace, and handgrip strength of the dominant hand in an

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upright position was measured with a digital dynamometer (Smedlay's Dynamo Meter; TTM, Tokyo, Japan).

Body composition

Total fat mass, fat-free lean body mass, and bone mineral density were calculated from whole-body dual-energy X-ray absorptiometry scan data. Appendicular skeletal mass was defined as the total fat-free lean body mass from four limbs, and the relative appendicular skeletal muscle mass index (RASM) was derived from appendicular skeletal mass divided by height squared (kg/m^2). Total body fat percentage was calculated as total fat mass divided by total body mass times 100.

Diagnosis of sarcopenia and related measurements

This study defined sarcopenia, according to recommendations by the Asian Working Group for Sarcopenia (AWGS),[22] as low muscle mass plus low muscle strength and/or low physical performance; the respective cut-off values were RASM: 7.0 kg/m^2 for men and 5.4 kg/m^2 for women based on dual-energy X-ray absorptiometry; handgrip strength: $<$ 26 kg for men and $<$18 kg for women; gait speed: less than 0.8 m/s.

Statistical analysis

Categorical variables are expressed by percentage, and continuous data as mean plus/minus standard deviation. Categorical variables were compared by Chi-square test, and continuous variables by Student's *t*-test, as appropriate. Multivariate logistic regression was used to determine independent risk factors of RASM below the AWGS lower reference limit compared with normal RASM, by entering all variables with $P < 0.1$ in univariate analysis as covariates. All statistical analyses were performed using SPSS Statistics Version 18.0 for Microsoft Windows XP (SPSS Inc., Chicago, IL, USA). A two-tailed *P*-value of $<$0.05 was considered statistically significant.

Results

The 463 enrolled subjects had mean age of 69.1 ± 9.2 years and 49.5% were men. Based on the AWGS cut-offs, 54 subjects (11.7%) had low RASM; however, the overall prevalence of sarcopenia was low, only 4.1% overall. Table 1 summarizes the functional, cognitive and clinical characteristics, and health behaviour of groups with low RASM and normal RASM. Low RASM was significantly more prevalent in men than in women, and men with low RASM had a significantly lower total body fat percentage than those with normal RASM. Compared with participants with normal RASM, the group with low RASM had lower mean body mass index, and had a higher proportion who were current smokers, and a lower proportion who consumed alcohol. Among the functional domains, a significantly higher proportion of subjects with low RASM were malnourished, but there were no significant differences in cognitive function, depressive symptoms, or walking speed.

Among known risk factors for sarcopenia—specifically, the inflammatory marker C-reactive protein and hormonal profiles—only serum DHEA levels were significantly lower among subjects with low vs. normal RASM. Although there were no significant between-group differences in serum activin or follistatin levels, the low RASM group serum tended to have lower myostatin levels, and subjects with low RASM were significantly more likely to have serum myostatin in the lowest tertile.

Table 2 summarizes the logistic regression results. In Model 4 (adjusted for age, sex, smoking status, alcohol consumption, Charlson Comorbidity Index score, total body fat percentage, malnutrition, DHEA, and myostatin status), male sex, malnutrition, DHEA, and low myostatin status were all independent risk factors for low RASM.

Due to different muscle mass between the sexes, we performed separate logistic regression analyses in men and women (Table 3); lower DHEA level and low myostatin status were significantly associated with low RASM in men, whereas only malnutrition was directly related to low RASM in women.

In post hoc analyses, there was no significant difference in serum myostatin levels between subjects aged 53–70 vs. $\geq$70 years ($P = 0.085$) or significant associations in either sex between serum myostatin and follistatin (men: $P = 0.921$; women $P = 0.410$); however, an association between myostatin and total body fat percentage was evident in men (men: $P = 0.041$; women: $P = 0.704$).

Discussion

To the best of our knowledge, this is the first report of the association between serum myostatin and lower skeletal muscle mass among healthy community-living older adults; lower serum DHEA levels and low serum myostatin status were independent risk factors of low RASM in men. Although the muscle-wasting effect of myostatin is well-established,[13,14] the relationship between serum myostatin and skeletal muscle mass in humans is complex and remains controversial; moreover, reported associations were observed in patients with COPD or heart failure.[13,15,23,24] Age-related loss of muscle mass was associated inversely with serum myostatin among frail older adults,[13] and circulating myostatin in male patients with COPD also negatively

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Table 1 Demographic characteristics of subjects with low vs. normal relative appendicular skeletal muscle mass

	Relative appendicular skeletal muscle mass		
Data show number (%) or mean ± standard deviation	Low (n = 54)	Normal (n = 409)	P-value
Demographic & health-related lifestyle factors			
Age (years)	73.0 ± 9.6	68.6 ± 9.0	<0.001
Sex			<0.001
Male	38 (13.0)	191 (86.9)	
Female	16 (6.8)	218 (93.2)	
Education (>6 years)	11 (20.4)	76 (18.6)	0.75
Body mass index (kg/m^2)			
Male	22.3 ± 5.2	25.3 ± 3.1	<0.001
Female	21.2 ± 2.8	24.9 ± 3.5	<0.001
Total body fat (%)	27.3 ± 9.6	31.6 ± 8.1	<0.001
Male	23.4 ± 7.6	26.0 ± 6.1	0.021
Female	36.5 ± 7.5	36.4 ± 6.3	0.991
Smoking	30 (55.6)	140 (34.2)	<0.001
Alcohol drinking	165 (40.3)	29 (53.7)	0.040
International physical activity questionnaire (Kcal/week)	10432 ± 4426	11902 ± 6459	0.11
Medical history			
Hypertension	29 (53.7)	203 (49.6)	0.57
Diabetes mellitus	8 (14.8)	79 (19.3)	0.43
Dyslipidemia	5 (9.3)	47 (11.5)	0.64
Coronary artery disease	1 (1.9)	18 (4.4)	0.38
Charlson Comorbidity Index	1.9 ± 1.5	1.4 ± 1.5	0.013
Functional performance			
Frail	11 (20.4)	31 (7.6)	0.005
Walking speed	1.3 ± 0.4	1.4 ± 0.4	0.063
Male	1.4 ± 0.5	1.5 ± 0.5	0.23
Female	1.3 ± 0.3	1.3 ± 0.4	0.99
Cognitive impairment	19 (35.2)	138 (33.7)	0.88
Depressive symptoms	1 (1.9)	15 (3.7)	0.71
Malnutrition	12 (22.2)	17 (4.2)	<0.001
Laboratory test results			
Fasting blood glucose (mg/dL)	96.1 ± 27.3	103.2 ± 30.0	0.11
Uric acid (mg/dL)	6.1 ± 1.5	5.9 ± 1.4	0.44
Serum creatinine (mg/dL)	0.9 ± 0.3	0.8 ± 0.3	0.35
Alanine aminotransferase (IU/mL)	27.1 ± 23.8	28.7 ± 21.9	0.62
Albumin (mg/dL)	4.4 ± 0.2	4.4 ± 0.3	0.64
Free androgen index	20.4 ± 14.9	17.1 ± 18.3	0.15
Homeostasis model assessment of insulin resistance	1.5 ± 0.9	1.7 ± 1.1	0.26
High-sensitivity C-reactive protein (mg/dL)	0.2 ± 0.4	0.2 ± 0.4	0.62
Homocysteine (μmol/L)	14.8 ± 5.5	14.3 ± 7.0	0.65
Insulin-like growth factor-1 (ng/mL)	116.2 ± 50.5	121.2 ± 52.5	0.52
Dehydroepiandrosterone (μg/dL)	74.8 ± 42.8	94.1 ± 68.6	<0.005
Vitamin D3 (ng/mL)	25.2 ± 6.1	25.1 ± 8.0	0.87
Serum myostatin (pg/mL)	4390 ± 2336	4978 ± 2313	0.080
Myostatin status (tertiles)			0.043
Low	26 (48.2)	136 (33.3)	
Medium	14 (25.9)	137 (33.4)	
High	14 (25.9)	136 (33.3)	
Activin A (pg/mL)	521.2 ± 161.1	532.1 ± 177.0	0.66
Follistatin (pg/mL)	1688 ± 525	1577 ± 631	0.22

correlated with skeletal mass calculated using a validated formula.[14] Conversely, others have reported skeletal muscle wasting associated with lower myostatin levels, especially in heart failure patients with compensatory status, cachexia, or undertaking exercise training.[15,23,24] This inconsistent evidence makes it difficult to assess the potential of serum myostatin as a biomarker for sarcopenia.

Our finding that low serum myostatin was an independent risk factor for lower RASM in healthy older adults appears contradictory to current understanding. One explanation may relate to the myostatin splice variant protein, which binds to myostatin and antagonizes canonical signalling[25]; Jeanplong et al. reported that an energy-restricted diet reduced the semitendinosus muscle mass of young ewes and that myostatin activity was inhibited by increased myostatin splice variant expression, implying an important influence of nutritional status on the action of myostatin in skeletal muscle development.[25] Another reason could be differences in the age distributions of study cohorts. Yarasheski et al. observed that serum myostatin was higher among women aged

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Table 2 Independent predictors of low muscle mass among otherwise healthy community-living older adults

	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
(n = 463)	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Age (years)	1.05 (1.01–1.08)	0.005	1.04 (1.00–1.08)	0.071	1.01 (0.97–1.06)	0.597	1.02 (0.97–1.07)	0.423
Male[e]	2.14 (1.29–4.51)	0.006	2.39 (1.15–4.96)	0.02	3.28 (1.47–7.29)	0.004	3.60 (1.30–9.92)	0.013
Smoking			0.99 (0.74–1.32)	0.934	0.97 (0.71–1.32)	0.841	1.00 (0.73–1.36)	0.976
Alcohol consumption			1.07 (0.79–1.45)	0.672	1.12 (0.82–1.55)	0.476	1.12 (0.81–1.55)	0.507
Charlson Comorbidity Index			1.12 (0.90–1.41)	0.31	1.03 (0.81–1.31)	0.837	1.04 (0.81–1.33)	0.755
Frailty					1.58 (0.64–3.89)	0.323	1.57 (0.61–4.00)	0.35
Malnutrition					5.16 (2.11–12.61)	<0.001	4.39 (1.56–12.36)	0.005
Dehydroepiandrosterone					0.99 (0.99–1.00)	0.034	0.99 (0.99–1.00)	0.019
Total body fat percentage							0.99 (0.94–1.05)	0.786
Myostatin								
Low vs. high							3.23 (1.49–7.01)	0.003
Medium vs. high							1.17 (0.51–2.71)	0.713

[a]Adjusted for age and sex.
[b]Adjusted for age, sex, smoking, alcohol consumption, and Charlson Comorbidity Index.
[c]Adjusted for age, sex, smoking, alcohol consumption, Charlson Comorbidity Index, malnutrition, and dehydroepiandrosterone.
[d]Adjusted age, sex, smoking, alcohol consumption, Charlson Comorbidity Index, malnutrition, dehydroepiandrosterone, and myostatin tertile.
[e]Female and male were assigned values of 0 and 1, respectively.

Table 3 Independent predictors of low muscle mass in men and women aged ≥53 years

	Men (n = 229)		Women (n = 234)	
	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
Age	1.03 (0.98–1.09)	0.249	1.02 (0.94–1.10)	0.703
Smoking	1.02 (0.72–1.45)	0.917	1.34 (0.57–3.12)	0.502
Alcohol consumption	1.07 (0.74–1.56)	0.719	1.58 (0.79–3.15)	0.197
Charlson Comorbidity Index	0.89 (0.64–1.23)	0.474	1.18 (0.80–1.73)	0.398
Frailty	2.20 (0.69–6.98)	0.183	0.37 (0.05–3.00)	0.353
Malnutrition	2.59 (0.65–10.31)	0.178	13.59 (2.22–83.25)	0.005
Dehydroepiandrosterone	0.99 (0.98–1.00)	0.009	1.00 (0.99–1.01)	0.76
Total body fat percentage	0.96 (0.90–1.04)	0.31	1.06 (0.97–1.16)	0.236
Myostatin				
Low vs. High	4.89 (1.79–13.37)	0.002	1.47 (0.40–5.49)	0.563
Medium vs. High	1.55 (0.56–4.31)	0.40	0.38 (0.07–2.18)	0.277

76–92 years old than younger ones,[13] indicating that age might influence myostatin expression; however, we found no significant difference in serum myostatin between subjects aged 53–70 vs. ≥70 years.

Follistatin is a myostatin antagonist that maintains tissue homeostasis. Interestingly, we found no significant association between serum follistatin and myostatin in either sex, perhaps because the liver usually secretes follistatin immediately after exercise and our participants' blood samples were all taken early in the morning, before daily activities. Myostatin also inhibits or promotes adipogenesis, depending on the circumstances[26]; although its action is chiefly inhibitory, myostatin may also have an adipogenic effect, by promoting mesenchymal stem cell differentiation.[27] In our study, we found an association between myostatin and adipose tissue in men, but not in women. The pathophysiology of myostatin in adipose tissue is very complex and incompletely understood; it is thought to be influenced by insulin resistance,[28] insulin-like growth factor-1,[29] and the sex steroid precursor DHEA,[30,31] which was associated with low muscle mass among male participants in our study. Given these complicated relationships, a longitudinal study is needed to clarify whether low serum myostatin is a cause or a consequence of low muscle mass.

The risk of becoming malnourished increases with advancing age, due to decreased appetite, poor digestion, polypharmacy, cognitive impairment, or depressed mood, as well as acute or chronic medical conditions and socioeconomic factors. Although only 6.3% of ILAS subjects were malnourished, malnutrition and lower skeletal muscle mass were strongly interrelated, consistent with other studies in older men or women.[32–35] Among people with heart failure, malnutrition plays a prominent role in muscle loss and sarcopenia, as well as disease severity,[36] and in a prospective cohort, lower Mini Nutritional Assessment score independently predicted muscle wasting and mortality.[34] Among

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postmenopausal women receiving weight-loss intervention, higher protein intake prevented loss of lean body mass.[33] Hence, nutritional factors should be taken into account when evaluating serum myostatin as a sarcopenia biomarker. Among ILAS subjects, female sex was not a risk factor for low RASM or the association between serum myostatin and low muscle mass. Sex is an important determinant of reduced muscle strength and age-related diminution of muscle mass.[37] Men generally start losing muscle mass when serum testosterone drops after age 40.[38] Women may gradually lose 10–15% of their muscle mass between age 25 and menopause onset, rising to 2% annually thereafter.[39]

The prevalence of sarcopenia among community-dwelling adults in Minnesota, USA, as determined by dual-energy X-ray absorptiometry, was 10% for men and 8% for women aged 60–69 years, and 40% for men and 18% for women aged over 80 years,[2] and whole body magnetic resonance imaging has shown that men lose more skeletal muscle mass with advancing age than do women.[40] Men and women in a longitudinal study all lost significant total and leg lean muscle mass over 3 years, with greater proportional and absolute lean mass diminution in men.[41] This evidence suggests that male sex is a potential risk factor for low muscle mass, possibly through mechanisms associated with age-related decline of endocrine factors, such as androgens, growth hormone, and insulin-like growth factor-1.[40]

Serum DHEA peaks during puberty declines to 20% of the maximal value in later life, and falls to only 5% after age 85.[42–44] The waning DHEA level parallels other age-related changes, such as muscle wasting.[40] Consistent with other studies,[30,46,47] we found serum DHEA to be significantly associated with lower RASM, with a stronger association in older men than among women. Balagopa et al. reported that myosin heavy-chain synthesis declined progressively with age, and that serum DHEA was significantly associated with the fractional synthesis rate.[45] Others found that DHEA was positively correlated with lean body mass in men aged over 60 years,[30] and have reported that serum DHEA level was an independent associated factor for calf-muscle area.[47] However, data supporting an effect of DHEA supplementation in preventing sarcopenia and frailty are equivocal.[48–50]

Study strengths and limitations

As the first investigation on this specific question, our results have important implications for further research. Moreover, we have made comprehensive adjustments for major factors related to skeletal muscle mass (high-sensitivity C-reactive protein, hormone profiles, vitamin D, nutritional status, cognitive function, physical activity), which previous studies did not take into account. Nevertheless, there are several noteworthy limitations. First, due to the cross-sectional design, the causality of relationships between the biomarkers assayed and muscle mass could not be established; low muscle mass may either cause or result from low serum myostatin. Second, the dynamic effect of myostatin-activin-follistatin interaction on skeletal muscle mass is uncertain. Third, low serum myostatin might result from low muscle mass among otherwise healthy older adults not currently experiencing rapid muscle loss.

Conclusions

Male sex, malnutrition, lower serum DHEA, and low serum myostatin status were significantly associated with low muscle mass among otherwise healthy community-living older adults in Taiwan; however, the associations with low serum myostatin and low skeletal muscle mass were observed only in men, not women.

Although myostatin signalling is a potential pharmaceutical target for sarcopenia, our results do not support using serum myostatin levels to diagnose sarcopenia nor to monitor its response to treatments. Further research to clarify the potential of myostatin as a biomarker is needed.

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Conflict of interest

All authors declare that they have no conflict of interest.

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