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Definitions of sarcopenia: associations with previous falls and fracture in a population sample

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Abstract

Background—Sarcopenia is common in later life and may be associated with adverse health outcomes such as disability, falls and fracture. There is no consensus definition for its diagnosis although diagnostic algorithms have been proposed by the European Working Group for Sarcopenia in Older People (EWGSOP), the International Working Group on Sarcopenia (IWGS) and the Foundation for the National Institutes of Health Sarcopenia Project (FNIH). More recently, Binkley and colleagues devised a score-based system for the diagnosis of “dysmobility syndrome” in an attempt to combine adverse musculoskeletal phenotypes, including sarcopenia and osteoporosis, in order to identify older individuals at particular risk. We applied these criteria to participants from the Hertfordshire Cohort Study (HCS) to define their prevalence in an unselected cohort of UK community dwelling older adults and assess their relationships with previous falls and fracture.

Methods—Body composition and areal bone mineral density (BMD) were measured using dual-energy X-ray absorptiometry (DXA), gait speed was determined by a 3-metre walk test and grip strength was assessed with a Jamar hand-held dynamometer. Researcher-administered questionnaires were completed detailing falls and fracture history.

Conflict of Interest

Cyrus Cooper has received consultancy fees and honoraria from Servier; Eli Lilly; Merck; Amgen; Alliance; Novartis; Medtronic; GSK; and Roche. Bjoern Buehring has received grants from Lilly, Extencicare Foundation and GE Healthcare. Elaine Dennison has received speaking fees from Lilly. Michael Clynes, Mark Edwards and Neil Binkley have no interests to declare.

Declaration

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Findings—The prevalence of sarcopenia in this cohort was 3.3%, 8.3% and 2.0% using the EWGSOP, IWGS and related-definition of FNIH respectively; 24.8% of individuals had dysmobility syndrome. Individuals with dysmobility reported significantly higher number of falls (last year and since the age of 45 years) ($p<0.01$) than those without it, but no increased fracture rate was observed in this group ($p=0.96$). Those with sarcopenia as defined by the IWGS reported significantly higher falls in the last year and prevalent fractures (falls in the last year: OR 2.51 CI 1.09, 5.81 $p=0.03$; fractures OR 2.50 CI 1.05, 5.92 $p=0.04$) but these significant associations were not seen when the EWGSOP definition was applied. The IWGS definition of sarcopenia appears to be an effective means of identifying individuals at risk of prevalent adverse musculoskeletal events.

Keywords

Epidemiology; sarcopenia; dysmobility; falls; fractures

Introduction

Sarcopenia describes the age related loss of skeletal muscle mass and function [1]. It is common with estimated prevalences in older men and women varying from between 1% and 29% in community-dwelling populations aged over 50 years based on the European Working Group on Sarcopenia in Older People (EWGSOP) definition [2]. This value differs for institutionalised and hospitalised groups and may also vary depending on the operational definition implemented. The decline in total muscle mass between the ages of 40 and 80 has been estimated to range from 30% to 60% and this is associated with significant disability, morbidity and mortality [1, 3, 4]. The development of sarcopenia may confer an increased risk of falls and has been associated with osteoporosis, the combination of which leads to a potential increase in fractures [4, 5]. Consequently, it has been suggested that it may be helpful to consider sarcopenia and osteoporosis as components of a ‘geriatric syndrome’ as this would promote their identification and treatment, even when the exact causes are unknown [6].

Despite being a common condition causing a significant healthcare burden, no broadly accepted clinical definition of sarcopenia exists yet. There has, however, recently been substantial progress in the area with convergence of the approaches used. Two principal definitions for sarcopenia and a related definition have been proposed: one from the European Working Group on Sarcopenia in Older People (EWGSOP), another from the International Working Group on Sarcopenia (IWGS) and the most recent from the Foundation for the National Institutes of Health (FNIH) Sarcopenia Project [7-9]. These definitions all recognise that measuring muscle mass in isolation is inadequate and a measure of muscle function is also required. For example, the EWGSOP definition is based on the ascertainment of low lean mass (LM) with low grip strength and/or gait speed [7], whereas the IWGS definition incorporates LM and gait speed only [8]. Due to their differences, these definitions do not identify the same individuals. To date, some studies have shown associations between sarcopenia, using the EWGSOP definition, and history of falls [10, 11]. Similar relationships were shown between previous falls-related injury and sarcopenia defined as appendicular skeletal muscle mass over body weight [12]. Evidence

that sarcopenia is associated with fractures [13] and incident falls [14, 15] is more limited. However, a recent study has demonstrated the validity of the EWGSOP definition of sarcopenia for predicting the rate of falls [16].

If relationships with adverse health consequences were the main focus of such definitions, it could be argued that including osteoporosis with sarcopenia as part of a 'geriatric syndrome' could enhance the identification of those most at risk. This was recently proposed by Binkley and colleagues who devised a score-based system for the diagnosis of "dysmobility syndrome" [17]. An excess of adipose tissue in combination with a low muscle mass has been termed 'sarcopenic obesity' and has been shown to be associated with impaired function and increased disability risk [18, 19]. Consequently, this factor, amongst others, was also included in dysmobility syndrome which has specifically been defined as 3 or more of a low skeletal mass index; low grip strength; low gait speed; low leg lean mass : fat mass ratio; a T-score in the osteoporotic range; and fall in the last year [17]. Dysmobility syndrome has recently been shown to be associated with increased mortality rate in a cohort of individuals above the age of 50 years in the US [20]. However, the definition of this condition is still relatively early in its development and the factors selected are arbitrary. The proponents of the definition suggest that the approach is evaluated in epidemiological studies with multiple outcomes to identify the combination of factors that are best able to predict adverse musculoskeletal outcomes [17].

In this study we aim to explore whether the EWGSOP, IWGS and FNIH definitions and the criteria for dysmobility syndrome are associated with prevalent adverse musculoskeletal health outcomes in a cohort of community-dwelling older adults from the Hertfordshire Cohort Study (HCS). We hypothesized that all of these definitions may be associated with falls and fracture.

Methods

This study used data from men and women of the HCS, a population-based sample of men and women born in Hertfordshire between 1931-9 who still lived there in late adult life. The recruitment process has been described previously [21] but, in brief, from 1911 all births which occurred in the county of Hertfordshire were notified by the attending midwife. Upon notification the following information was recorded in ledgers: the name and address of the baby; the date of birth; and the birth weight. The baby was also followed up periodically in the first year by a health visitor who documented whether the baby was breast fed or not, and its weight at 1 year of age. With the assistance of the National Health Service Central Registry at Southport and Hertfordshire Family Health Service Association, men and women from the ledgers who were born between 1931-9 and still living in Hertfordshire were traced and recruited between 1998-2003.

Between 2010-12, those men and women living in East Hertfordshire were approached again (n=570). A total of 444 individuals were visited at home where a researcher-administered questionnaire was carried out detailing self-reported falls and fracture history. Falls were defined as an event which results in a person coming to rest inadvertently on the ground or floor or other lower level. Questions were asked to ascertain falls in the last year,

falls since the age of 45 years, and fractures since the age of 45 years. Fractures from any skeletal site were included and not stratified by force of injury.

Gait speed was determined by 3m walk test and grip strength was measured three times in each hand using a Jamar hand-held isokinetic dynamometer using a standardised protocol which has been described previously [22]. The maximum value was used in analyses. Three hundred and seventy six attended a clinic for imaging by dual-energy X-ray absorptiometry (DXA) using a Lunar Prodigy Advance densitometer (GE Medical Systems Lunar) when body composition and femoral neck BMD were both measured. Height was assessed at the same visit using a wall-mounted SECA stadiometer (SECA Ltd, Hamburg, Germany). Complete data were available on a total of 298 individuals (156 men and 142 women) who were all included in these analyses.

Participants were deemed to have sarcopenia as defined by the IWGS if they had a gait speed of less than 1.0 ms^{-1} and a skeletal mass index (appendicular mass relative to ht^2) of 7.23 kg/m^2 in men and 5.67 kg/m^2 in women. To fulfil the diagnostic criteria for sarcopenia using the EWGSOP definition participants required a skeletal mass index 7.26 kg/m^2 in men and 5.5 kg/m^2 in women and either a grip strength $< 30 \text{ kg}$ in men and $< 20 \text{ kg}$ in women and/or a gait speed of less than 0.8 ms^{-1} (figure 1). The FNIH definition was grip strength $< 26 \text{ kg}$ in men and 16 kg in women and ALM_{BMI} (ratio of appendicular lean mass over body mass index) of < 0.789 in men and < 0.512 in women. Dysmobility syndrome was defined as 3 or more of: skeletal mass index of 7.26 kg/m^2 in men and 5.45 kg/m^2 in women; grip strength of $< 30 \text{ kg}$ in men and $< 20 \text{ kg}$ in women; gait speed less than 1.0 ms^{-1} ; leg lean mass: fat mass ratio > 0.39 in men and > 0.67 in women; T-score < -2.5 ; and fall in the last year.

Statistical analysis

Stata version 12.1 was used for all analyses. Study participants' characteristics were summarised using means and standard deviations (SD) for continuous variables and counts and percentages for binary and categorical variables. Logistic regression was used to model the associations between the IWGS definition of sarcopenia, EWGSOP definition of sarcopenia, the FNIH definition and dysmobility syndrome with i) fall(s) in the last year, ii) fall(s) since age 45 and iii) fractures since the age of 45. These analyses were completed with and without adjustment for sex and utilisation of psychoactive medications (hypnotics, tricyclic antidepressants or anti-psychotics). Other covariates were assessed including age, number of hours of physical activity, smoking status, alcohol consumption, social class, number of co-morbidities, and mini mental state examination score. However, these were not found to be significantly associated ($p < 0.10$) with any of the outcomes of interest and therefore not included in the adjusted models. Due to the low prevalence in the FNIH group the logistic regression model was found to be very unstable and consequently results are not presented.

Results

Table 1 shows the summary characteristics of the study participants. The mean (SD) age of participants was 76.0 (2.54) years for men and 76.2 (2.60) for women with a mean (SD) BMI of 27.2 (3.56) kg/m² for men and 27.6 kg/m² for women. In terms of smoking behaviour there was no significant difference between the proportion of men and women who were current smokers (6.5% and 9.8% respectively, $p=0.480$). This did not hold true for alcohol consumption, however, with a higher proportion of men than women reporting that they currently consumed alcohol (95.5% and 72.5% respectively, $p<0.001$). There was no significant difference between the number of men and women with two or more co-morbidities (10.9% and 9.4% respectively, $p=0.740$), in social class IIIN or above (56.0% and 59.2% respectively, $p=0.586$), MMSE score (27.44 and 27.26 respectively, $p=0.556$), physical activity time in the last two weeks (210 min/day and 223 min/day respectively, $p=0.352$) or use of one or more psychoactive drugs (2.6% and 5.6% respectively, $p=0.178$). The mean (SD) femoral neck BMD of male participants was higher than female 0.93 (0.73) g/cm² for men and 0.84 (0.12) g/cm² for women, $p<0.001$). Furthermore, men also had a higher skeletal mass index than women (8.04 (0.73) kg/m² and 6.43 (0.75) kg/m² respectively, $p<0.001$). Unsurprisingly, mean (SD) leg:fat mass ratio was lower in men than in women (0.39 (0.12) and 0.85 (0.24) respectively, $p<0.001$). Grip strength was higher in men than women (37.20 (7.19) kg and 21.91 (6.15) kg respectively, $p<0.001$) as was gait speed (0.97 (SD 0.21) m/s for men and 0.91 (SD 0.22) m/s for women). The proportion of men and women who reported a fall in the last year was not significantly different (24.4% in men and 26.8% in women). Interestingly, a higher proportion of women than men reported having a fall since the age of 45 (58.1% in men and 70.4% in women, $p=0.027$). Approximately a quarter of both men and women reported having a fracture since the age of 45 years (21.1% of men and 26.8% of women) with no statistically significant difference between the two.

Table 2 shows the prevalence of dysmobility, sarcopenia as defined by the IWGS and EWGSOP, and the related FNIH definition within the study population. The prevalence of dysmobility was higher in older adults of both sexes and is overall more common in women than men in all age groups. The prevalence of sarcopenia as defined by the EWGSOP is highest in the 78-82 year group in both sexes and is more common in women in the 70-74 year age group. Sarcopenia as defined by the IWGS increases in prevalence with age in both sexes but is more prevalent in women in all but the 70-74 year age group. The prevalence as defined by the FNIH definition increases with age in men and is highest in the 74-78 year group in women.

Table 3 shows that 20 (74.1%) and 39 (83.0%) of men and women respectively with dysmobility reported a fall since the age of 45 years. These proportions were similar or lower in the other groups. The prevalence of reported fractures was highest in both men and women in the IWGS group. Only 3 men and 3 women met the FNIH definition and, of these, only 1 reported a fracture since the age of 45 years.

Table 4 details the overall prevalence of dysmobility and sarcopenia as defined by EWGSOP and IWGS. It also shows the odds ratios for musculoskeletal outcomes (fall in the last year,

fall since the age of 45 and fracture since the age of 45) in participants who fit these clinical criteria compared to those that do not. The overall prevalence of dysmobility syndrome (24.8%) was higher than both EWGSOP and IWGS defined sarcopenia (8.4% and 3.3% respectively). A diagnosis of dysmobility syndrome was associated with significantly higher rates of self-reported falls since age 45 (OR 2.76 CI 1.48, 5.17 $p=0.001$) and falls in the last year (OR 5.17 CI 2.91, 9.17 $p<0.001$) but not fractures (OR 0.986 CI 0.53, 1.84 $p=0.96$). All relationships were maintained after adjustment for sex and use of psychoactive medications. There was no significant association observed between sarcopenia as defined by EWGSOP and falls since age 45, falls in the last year, or fractures since the age of 45. A diagnosis of sarcopenia using the IWGS definition was associated with both significantly higher rates of falls in the last year (OR 2.51 CI 1.09, 5.81 $p=0.03$) and fractures since the age of 45 (OR 2.50 CI 1.05, 5.92 $p=0.04$) but not falls since the age of 45 (OR 1.870 CI 0.72, 4.84 $p=0.20$). Again relationships remained robust following adjustment.

Discussion

The prevalence of sarcopenia in this cohort was 3.3% and 8.3% using the EWGSOP and IWGS definitions respectively and 2.0% using the related FNIH definition. 24.8% of individuals had dysmobility syndrome. As expected, individuals with dysmobility syndrome reported significantly higher number of falls (last year and since the age of 45 years) ($p<0.01$) than those without it, but no increased fracture rate was observed in the dysmobility group ($p=0.96$). Those with sarcopenia as defined by the IWGS reported significantly higher falls in the last year and fractures since the age of 45 (OR 2.51 (CI 1.09, 5.81) $p=0.03$; OR 2.50 (CI 1.05, 5.92) $p=0.04$ respectively) but these significant associations were not seen when the EWGSOP definition was applied.

The prevalence of sarcopenia, as defined by the EWGSOP and IWGS, and dysmobility syndrome all increased with age. Given the wider diagnostic criteria it is unsurprising that the number of participants that met the clinical criteria for dysmobility syndrome (24.8%) was considerably higher than that for both EWGSOP and IWGS defined sarcopenia (3.3% and 8.4% respectively) and the related FNIH definition (2.0%). Although there are some methodological differences between studies our findings are also broadly comparable with other reports from the literature, which have estimated prevalence of sarcopenia in other populations of between 6.0% and 6.8% [23, 24]. It has been shown, however, that the prevalence of sarcopenia is highly dependent on the nature of the assessment tools used and the cut-offs employed [25, 26].

The algorithm for dysmobility syndrome has only been applied in two previous cohorts prior to the current study. Binkley and colleagues found the prevalence of dysmobility syndrome to be 34.0% in a population of 97 Caucasian adults aged above 70 years in the USA [17]. This is slightly higher than in the Hertfordshire cohort (24.8%) but is consistent with the higher mean age of the US cohort compared with the current participants (80.7 and 76.1 years respectively). A more recent study by Looker in 2014 applied the algorithm in a relatively younger cohort (2975 individuals of mixed race over the age of 50 years in the US) and found a similar prevalence to that in the HCS at 22% [20].

In this study, we have shown that using the IWGS, but not the EWGSOP, algorithm to define sarcopenia provides a group with a greater rate of prevalent adverse musculoskeletal outcomes. There were significant associations between sarcopenia diagnosed using the IWGS algorithm and both falls in the last year and fractures since the age of 45. However, this certainly does not exclude the possibility of identifying associations between the EWGSOP definition of sarcopenia and falls or fractures, particularly if a larger sample was used, as confidence intervals reported here are relatively large presumably in part due to the lower prevalence of this condition. Furthermore, such an association has recently been shown with rate of falls in a prospective analysis [16].

In its current format the diagnostic criteria for dysmobility syndrome was associated with an increased risk of falls (in the last year and since age 45 years) but not fractures since the age of 45 in this cohort. As mentioned previously, falls are one of the diagnostic criteria for dysmobility syndrome and this finding is therefore not surprising. It would, however, be interesting to determine in future studies whether dysmobility syndrome is also associated with prospective fall risk. Furthermore, currently the criteria included in the dysmobility syndrome definition are arbitrary and its development is still in its infancy. It would therefore be important to determine whether further modifications to these diagnostic criteria, perhaps by incorporating components for neurological disease, vascular disease and joint disease, would increase its ability to identify those at risk of fracture.

This study does have limitations. There were only a moderate number of participants (156 men and 142 women), which might limit our ability to detect statistically significant relationships, particularly if the disease groups were small. The results may not be entirely representative of the wider UK population since all recruited participants were born in the county of Hertfordshire and had continued to reside there until they were 75 [27]. It has, however, been previously demonstrated that this cohort are similar to the general population with regard to body build and lifestyle factors, such as smoking and alcohol intake [28]. Furthermore, all the comparisons undertaken were internal. The study is cross-sectional and not longitudinal leading to potential difficulties in attributing causality. Gait speed was assessed using a 3 metre walk test whereas it is usually assessed using a 4 or 6 metre walk test. Although the speeds obtained are likely to be highly correlated, there is some evidence that longer distances may be more appropriate for single assessments[29]. Several cut offs for skeletal mass index were proposed in the EWGSOP definition and so the values used in this study were chosen arbitrarily. It has however been shown that this does not affect prevalence in men and is less important than grip strength in women [26]. The data on falls and fractures were obtained retrospectively and may therefore be limited by the participants' ability to remember such events and is also prone to recall bias. Furthermore, fractures were not validated by review of radiology reports or radiographs which may also affect the accuracy of this outcome.

In conclusion, we have shown that employing the IWGS diagnostic criteria for identifying sarcopenia appears to be an effective means of identifying those at risk of prevalent adverse musculoskeletal outcomes in older community-dwelling adults. However, the EWGSOP may also be shown to be similarly effective in a larger study sample. Future work is required to apply these diagnostic criteria in other ageing cohorts in a prospective manner to assess

their predictive capacity before the clinical implications can be fully appreciated. Further research is also necessary to assess and refine the diagnostic criteria for dysmobility syndrome.

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Figure 1. EWGSOP algorithm for defining sarcopenia in older populations

Key: ¹ Grip strength of < 30 kg in men and < 20 kg in women (in current study); ² Skeletal mass index of 7.26 kg/m² in men and 5.45 kg/m² in women (in current study)

Table 1
Summary of participant characteristics

	Mean (SD)	Mean (SD)	
Demographics and lifestyle factors			
	Men (n=156)	Women (n=142)	p value
Age (years)	76.0 (2.54)	76.2 (2.60)	0.441
Height (metres)	1.74 (0.06)	1.60 (0.06)	<0.001
Weight (kg)	82.2 (11.50)	70.8 (12.40)	<0.001
BMI (kg/m ²)	27.2 (3.56)	27.6 (4.58)	0.376
Smoking (current) ^I	6 (6.5)	5 (9.8)	0.480
Alcohol consumption (current) ^I	149 (95.5)	103 (72.5)	<0.001
Two or more co-morbidities ^I	16 (10.9)	13 (9.4)	0.740
Social class IIIN and above ^I	84 (56.0)	84 (59.2)	0.586
MMSE score	27.44 (2.66)	27.26 (2.45)	0.556
Activity time last 2 weeks (min/day)	210 (118)	223 (120)	0.352
Psychoactive medication use ^I	4 (2.6)	8 (5.6)	0.178

Components and outcomes of sarcopenia and dysmobility			
	Men (n=156)	Women (n=142)	
Femoral neck BMD (g/cm ²)	0.93 (0.13)	0.84 (0.12)	<0.001
Appendicular Lean mass (kg/m ²)	8.04 (0.73)	6.43 (0.75)	<0.001
Leg mass:fat ratio	0.39 (0.12)	0.85 (0.24)	<0.001
Grip strength (kg)	37.20 (7.19)	21.91 (6.15)	<0.001
Gait speed (m/s)	0.97 (0.21)	0.91 (0.22)	0.020
Falls in last year ^I	38 (24.4)	38 (26.8)	0.635
Falls since 45yrs ^I	90 (58.1)	100 (70.4)	0.027
Fractures since 45yrs ^I	32 (21.1)	38 (26.8)	0.251

Key:

^IN (%)

Table 2
Prevalence of dysmobility and sarcopenia by age and sex

Age (years)	70-74 (n=78)		74-78 (n=139)		78-82 (n=81)	
	Men (n=41) Count (%)	Women (n=37) Count (%)	Men (n=75) Count (%)	Women (n=64) Count (%)	Men (n=40) Count (%)	Women (n=41) Count (%)
Dysmobility	4 (9.8)	10 (27.0)	14 (18.7)	20 (31.3)	9 (22.5)	17 (41.5)
EWGSOP ¹	0 (0.0)	1 (2.7)	3 (4.0)	1 (1.6)	4 (10.0)	1 (6.2)
IWGS ²	2 (4.9)	0 (0.0)	7 (9.3)	6 (9.4)	4 (10.0)	6 (14.6)
FNIH ³	0 (0.0)	0 (0.0)	1 (1.3)	2 (3.1)	2 (5.0)	1 (2.4)

Key:

¹European Working Group on Sarcopenia in Older People;

²International Working Group on Sarcopenia;

³Foundation for the National Institutes of Health Sarcopenia Project.

Table 3
Prevalence of falls and fractures in participants with dysmobility syndrome and definitions of sarcopenia by sex

	Dysmobility		EWGSOP ¹		IWGS ²		FNIH ³	
	Men (n=27) Count (%)	Women (n=47) Count (%)	Men (n=7) Count (%)	Women (n=3) Count (%)	Men (n=13) Count (%)	Women (n=12) Count (%)	Men (n=3) Count (%)	Women (n=3) Count (%)
Falls in last year	16 (59.3)	22 (46.8)	3 (42.9)	1 (33.3)	5 (38.5)	6 (50.0)	1 (33.3)	0 (0.0)
Falls since age 45	20 (74.1)	39 (83.0)	5 (71.4)	1 (33.3)	10 (76.9)	9 (75.0)	2 (66.7)	2 (66.7)
Fracture since age 45	5 (18.5)	12 (25.5)	2 (28.6)	0 (0.0%)	4 (30.8)	6 (50.0)	0 (0.0)	1 (33.3)

Key:

¹European Working Group on Sarcopenia in Older People;

²International Working Group on Sarcopenia;

³Foundation for the National Institutes of Health Sarcopenia Project.

Table 4
Odds ratios for falls and fractures in dysmobility and sarcopenia with and without adjustment for sex and use of psychoactive drugs

	Prevalence (%)	Falls since age 45 (OR (CI))		Falls in last year (OR (CI))		Fractures since age 45 (OR (CI))	
		Unadjusted	Adjusted [#]	Unadjusted	Adjusted [#]	Unadjusted	Adjusted [#]
Dysmobility	24.8	2.76^{**} (1.48, 5.17)	2.54^{**} (1.34, 4.81)	5.17^{***} (2.91, 9.17)	5.53^{***} (3.03, 10.1)	0.99 (0.53, 1.84)	0.92 (0.48, 1.73)
EWGSOP ¹	3.3	0.84 (0.23, 3.04)	0.73 (0.19, 2.86)	1.32 (0.55, 7.29)	1.62 (0.41, 6.31)	0.91 (0.19, 4.49)	0.99 (0.20, 4.95)
IWGS ²	8.4	1.87 (0.72, 4.84)	1.80 (0.69, 4.73)	2.51[*] (1.09, 5.81)	2.41[*] (1.03, 5.65)	2.50[*] (1.05, 5.92)	2.51[*] (1.06, 5.95)

Key:.

¹European Working Group for Sarcopenia in Older People;

²International Working Group on Sarcopenia;

[#] Adjusted for sex and use of psychoactive drugs.

^{*} p<0.05,

^{**} p<0.01,

^{***} p<0.001.