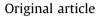
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Cut-off points to identify sarcopenia according to European Working Group on Sarcopenia in Older People (EWGSOP) definition



CLINICAL NUTRITION



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SUMMARY

Background: The reported prevalence of sarcopenia ranges widely depending on its definition criterion. European Working Group on Sarcopenia in Older People (EWGSOP) developed a practical clinical definition and consensus diagnostic criteria. This definition recommends using normative data of the study population rather than other reference populations.

Objective: We aimed to define the reference cut-off values for muscle mass, muscle strength and calf circumference in Turkey in order to improve general applicability of EWGSOP criteria.

Material and methods: Healthy young adults between 18 and 39 years of age with no known chronic disease or chronic drug usage were included in our study to serve as reference population for assessing muscle mass. Community-dwelling older outpatients were prospectively recruited from the geriatrics outpatient clinics of a university hospital for assessing hand grip strength and calf circumference. Body composition was assessed by bioimpedance analysis. Muscle strength was assessed measuring hand grip strength with a Jamar hand dynamometer. The cut-off thresholds for muscle mass were defined as the mean-2SD of the values of the young reference study population; for grip strength were calculated from ROC analyses using cut-off values that predicted gait speed < 0.8 m/s; and for calf circumference were calculated from ROC analyses using cut-off values that predicted low muscle mass.

Results: The young reference group included a total of 301 participants (187 male, 114 female; mean age: 26.5 \pm 4.6 years). The cut-off thresholds for skeletal muscle mass indexes were 9.2 kg/m² and 7.4 kg/m² in males and females, respectively. The older community dwelling group included 406 subjects (123 male, 283 female, mean age: 76.6 \pm 6.7 years). The cut-off thresholds for hand grip strength were 32 kg and 22 kg for males and females. The cut-off threshold for calf circumference was 33 cm for both males and females.

Conclusions: The cut-off thresholds for muscle mass, grip strength and calf circumference were somewhat higher but comparable with other reference populations. Further worldwide studies from different nations and countries are needed to obtain better reference values.

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1. Introduction

Sarcopenia is a well-known adverse consequence of ageing and is recognized as one of the geriatric syndromes [1]. Low muscle mass is prevalent in older populations [2,3] and with subsequent low muscle strength, they represent an impaired health with mobility-disorders, increased fall and fracture risk, impaired functionality, loss of independence and increased death risk [4–8]. Although there is much increase in the number of publications on sarcopenia and its consequences [9], the reported prevalence of sarcopenia obviously varies widely depending on its definition. To address these shortfalls, The European Working Group on Sarcopenia in Older People (EWGSOP) developed a clinical definition and consensus diagnostic criteria for age-related sarcopenia which is easy to implement [1]. EWGSOP suggested an algorithm to detect sarcopenia in older individuals based on gait speed, grip strength

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and muscle mass measurements. The universal use of a sarcopenia definition would aid to correct the confusion in its prevalence, define its consequent course, and enable developing treatment. The cut-off points suggested by EWGSOP depend on the measurement techniques and on the available reference studies. They recommend use of normative data of the study population rather than other predictive reference populations, concluding that more research is urgently needed in order to obtain reference values for populations globally [1]. No are available in our country. However, body composition is affected by ethnicity [10] and environmental factors as industrialization and its consequences for individual lifestyles i.e. nutrition (malnutrition or over-nutrition) [11] and physical activity level [12]. In this study, we aimed to define the reference cut-off values from Turkey in order to improve general applicability of EWGSOP criteria.

2. Material and methods

2.1. Population and setting

Healthy young adults between 18 and 39 years of age with no known chronic disease and chronic drug usage were asked to participate in our study to serve as reference population for assessing muscle mass threshold data. These subjects were recruited among the students and staff of our medical faculty, including doctors, nurses, health aids, technicians, officers, secretaries, cleaners, security members, and accompanying family members of the patients. They constituted the young reference study population. Another group of subjects, the communitydwelling older outpatients, were prospectively recruited from the geriatrics outpatient clinic of our hospital to serve as the older reference population for assessing hand grip strength threshold. This outpatient clinic serves for all older patients (>60 years). They were recruited between December 2012 and April 2014. In this period, 1203 geriatric outpatients were assessed medically. Among them, 446 patients could not be reached by the corresponding staff for test proposal; 324 patients refused to give consent; and 27 had poor general condition due to acute problems and therefore excluded from the study. So, our final study population was composed of 406 patients.

2.2. Sample size estimation

We determined our sample size assuming a type 1 error of 0.05, a type 2 error of 0.2, and a power of 80%. A sample size of 106 was calculated to detect a mean difference of 5.5% and a standard deviation of 0.2 for gait speed between elderly and younger subjects; 100 subjects to detect a mean difference of 2% and a standard deviation of 7 for grip strength; and 75 subjects to detect a mean difference of 3% and a standard deviation of 3 for muscle mass. Therefore, minimal reference sample size was regarded as n = 106.

2.3. Measurements

Height and weight were measured using a regularly standardized stadiometer with subjects wearing light clothes without shoes. Body weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. Body mass index (BMI) was calculated as weight (kg) divided by height (m) [2].

Body composition was assessed with bioimpedance analysis (BIA) using a Tanita BC 532 model body analysis monitor. Fat free mass was measured by BIA and skeletal muscle mass (SMM) was calculated by the following equation: SMM (kg) = 0.566 * FFM (fat free mass). This formula [SMM (kg) = 0.566 * FFM] was validated on individual and group data and compared with SMM data calculated

from 24 h creatinine excretion in a group of healthy subjects as well [13]. Skeletal muscle mass index (SMMI) was calculated as skeletal muscle mass (kg)/height squared (m^2) [2].

Muscle strength was assessed by measuring hand grip strength with a Jamar hydraulic hand dynamometer using a validated protocol [14.15]. Grip strength was measured in sitting position, elbow in 90° flexion and wrist in neutral position. Participants were asked to apply the maximum grip strength for 3 times with both left and right hands. Between the each measurement at least 30 s resting intervals were allowed. The maximal measured grip strength was regarded as the grip strength. For usual gait speed test, participants were informed to walk 4 m with their usual speed. Calf circumference (CC) was measured at the widest circumference of the calf while the subjects were standing. Middle arm circumference was measured at the middle of humerus while the arm was mildly elevated and internally rotated without contracting the biceps muscle. All of the measurements were made by the same health profession - a geriatric physiotherapist qualified on these measurements previously.

The study was conducted according to the guidelines in the Declaration of Helsinki. Informed consent was obtained for all participants. The study was approved from the local ethic committee.

2.4. Statistical analysis

All data entered into the database were verified by a second independent person. Descriptive statistics, as mean and standard deviation for normally distributed continuous variables and relative frequencies for categorical (qualitative) variables, were generated for all variables. The cut-off thresholds for muscle mass were defined as the mean-2SD of the values of the young reference study population; for grip strength were calculated from receiver operator characteristics (ROC) curve analyses using cutoff values that predicted gait speed < 0.8 m/s; and for calf circumference were calculated from ROC curve analyses using cutoff values that predicted low muscle mass. p < 0.05 was considered statistically significant. The statistical analysis was carried out with the statistical package SPSS Version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Young reference group

The young reference group included a total of 301 participants (187 male, 114 female). Mean age was 26.5 ± 4.6 years and SMMIs were 11 ± 0.9 , 9 ± 0.8 kg/m², respectively. The summary including the data on hand grip strength, BMI, usual gait speed, calf circumference and middle arm circumference is given in Table 1. The cut-off thresholds for skeletal muscle mass indexes were designated as 9.2 kg/m^2 and 7.4 kg/m^2 , in males and females, respectively. The comparative data of muscle mass measurements of young reference populations from different nations – reported so far – are given in Table 2 [3,16,17].

3.2. Older reference group

Group of older subjects from the community included a total of 406 community-dwelling outpatients between 65 and 99 years of age. 123 (30.3%) were male and 283 (69.7%) were female. Mean age was 76.6 \pm 6.7. The summary including the data on hand grip strength, body mass index, usual gait speed and CC is given in Table 3 and corresponding percentiles of muscle mass, grip strength and CC are given in Table 4. For males, the grip strength value that best predicts the usual gait speed (UGS) < 0.8 m/s was

Table 1

The study parameters across the genders of the healthy young adults reference population (n = 301).

Parameter	Males ($n = 187$) Mean \pm SD (range)	Females $(n = 114)$ Mean ± SD (range)
Age (years)	26.8 ± 4.5 (19–39)	25.9 ± 4.7 (18–39)
Height (cm)	175 ± 6 (157–191)	161 ± 6 (147–178)
Weight (kg)	78 ± 12 (48–126)	$58 \pm 8.5 (42 - 81)$
BMI (kg/m ²)	25.5 ± 3.6 (16.4–35.8)	$22.4 \pm 3.4 (16.2 - 31.2)$
Usual gait speed (m/s)	$1.4 \pm 0.2 (1-2)$	$1.4 \pm 0.2 (1 - 1.7)$
Hand grip strength (kg)	53.4 ± 7.2 (38-80)	33.1 ± 5.3 (20-46)
Skeletal muscle mass (kg)	33.6 ± 3.6 (24.1-44.7)	23.5 ± 1.7 (19.7-29.5)
Skeletal muscle mass index (kg/m ²)	$11 \pm 0.9/(9.2) (8.4-13.5)$	9 ± 0.8 (7.4–11.6)
Calf circumference (cm)	37.4 ± 3 (28–46)	34.7 ± 2.7 (28-43)
Middle arm circumference (cm)	29.1 ± 2.8 (22-39)	24.2 ± 2.3 (20-31)

SD: standard deviation, BMI: body mass index.

Table 2

Comparative data for muscle mass in young reference populations from different using bioimpedance analysis.

	Turkey		Taiwan ^a [3]		France ^b [16]		Spain ^c [17]	
	М	F	М	F	М	F	М	F
n	187	114	100	100	394	388	110	120
Age (mean)	26.8	25.9	26.7	27.6	30.2	29.2	28.6	28.2
(SD)	4.5	4.7	5.7	5.9	6.1	6.3	5	6
BMI (mean)	25.5	22.4	23.2	20.6	23.9	22.5	24.6	21.9
(SD)	3.6	3.4	3.5	2.5	3	3.4	2.6	2.2
SMM (mean)	33.6	23.5	32.6	20	32.2	21	29.9	20.5
(SD)	3.6	1.7	3.5	2.2	3.3	2.5	3	1.5
SMMI (mean)	11	9	10.9	7.9	10.4	7.8	9.6	7.6
(SD)	0.9	0.8	1	0.7	0.9	0.8	0.7	0.5
SMMI cut-off value	9.2	7.4	8.9	6.5	8.6	6.2	8.3	6.7

Age (years), BMI (kg/m²), SSM (kg), SSMI (kg/m²).

M: male, F: female, BMI: body mass index, SMM: skeletal muscle mass, SMMI: skeletal muscle mass index.

SMMI cut-off: cut-off for total skeletal muscle mass for defining sarcopenia.

^a Bioelectrical impedance analysis (BIA): performed with a Maltron system (Maltron BioScan 920, Rayleigh, UK).

^b Bioelectrical impedance analysis (BIA): performed with Impedimed multifrequency analyser (Impedimed, Brisbane, Australia).

^c Bioelectrical impedance analysis (BIA): performed with a RJL Systems BIA 101 device.

Table 3

The study parameters across the genders of the older reference population (n = 406).

Parameter	Male $(n = 123)$ (30.3%)	Female (n = 283) (69.7%)	Total $(n = 406) (100\%)$	р
Age (years)	$[77.5 \pm 6.2](66-92)$	$[76.2 \pm 6.8](65-99)$	$[76.6 \pm 6.7]$	0.06
Heights (cm)	$[165 \pm 7](148 - 187)$	$[150.9 \pm 5.9](135 - 167)$	$[155.2 \pm 9]$	< 0.001
Weights (kg)	$[75.2 \pm 13](43 - 105)$	$[69.9 \pm 13.5](27 - 111)$	$[71.5 \pm 13.6]$	< 0.001
$BMI (kg/m^2)$	$[27.7 \pm 4.5](18.2 - 41.4)$	$[30.6 \pm 5.5](11.7 - 52.1)$	$[29.7 \pm 5.4]$	0.001
Usual gait speeds (m/s)	$[0.95 \pm 0.3] (0.2 - 1.5)$	$[0.84 \pm 0.3](0.14 - 1.9)$	$[0.87 \pm 0.3]$	0.001
Hand grip strength (kg)	$[34.5 \pm 8.4](16-58)$	$[21.9 \pm 5.4](2-38)$	$[25.7 \pm 8.7]$	< 0.001
Skeletal muscle indexes (kg/m ²)	$[10.8 \pm 1.1](7.8 - 14.1)$	$[10 \pm 1.2](6.2-17.5)$	$[10.3 \pm 1.2]$	< 0.001
Calf circumferences (cm)	$[35.3 \pm 3.1](28-42)$	$[35.4 \pm 3.9](21-48)$	$[35.4 \pm 3.7]$	0.80

Data are given as [Mean ± SD] (min-max).

SD: standard deviation.

BMI: body mass index.

ADL: activities of daily living.

IADL: instrumental activities of daily living.

<32 kg (sensitivity: 80.5%, 95%CI: 65.1–91.2; specificity: 76.2%, 95% CI: 65.4–85.1) (Fig. 1a). For females, the grip strength value that best predicts the usual gait speed (UGS) < 0.8 m/s was <22 kg (sensitivity: 76.9%, 95%CI: 68.2–84.2%; specificity: 62.5%, 95%CI: 54.3–70.2) (Fig. 1b). The comparative thresholds proposed for hand grip strength are given in Table 5 [5,18,19]. By this study-derived Turkish normative reference cut-off values, the prevalence of low muscle mass was 2% [n = 5 (4.1%) in males; n = 3 (1%) in females] and low muscle strength was 42.9% [n = 43 (35%) in males; n = 131 (46.3%) in females].

We derived the calf circumference threshold by ROC analysis –representing cutoff value for identifying participants with

SMMI < 9.2 kg/m² for males and <7.4 kg/m² for females. For both males and females, the calf circumference value that best predicts the low SSMI (<9.2 kg/m² and <7.4 kg/m², respectively) was <33 cm (sensitivity: 100, 95%CI: 47.8–100; specificity: 73.7, 95%CI: 64.8–81.4 and sensitivity: 100, 95%CI: 29.2–100; specificity: 68.6, 95%CI: 62.8–74.0; respectively) (Fig. 2a and b).

4. Discussion

We have defined the cut-off thresholds for skeletal muscle mass indexes as 9.2 kg/m² and 7.4 kg/m² in males and females, respectively. The measurement of cut-off thresholds for skeletal

Table 4

Percentiles for muscle mass, h	hand grip stren	gth and calf circumference	parameters of the older reference	e population ($n = 406$).

Percentiles	Males (<i>n</i> = 123)			Females ($n = 283$)		
	SSMI (kg/m ²)	HGS (kg)	CC (cm)	SSMI (kg/m ²)	HGS (kg)	CC (cm)
5	9.3	20	30.2	8.3	12.4	29
10	9.4	24	31	8.8	16	31
15	9.6	26	31	8.9	16	32
20	9.9	28	32	9.1	18	32
25	10.1	28	33	9.2	18	33
30	10.3	30	34	9.4	18	33
35	10.4	30.8	34	9.5	20	34
40	10.5	32	35	9.6	20	34
45	10.7	34	35	9.7	20	35
50	10.9	34	35	9.8	22	35
55	10.9	36	36	9.9	22	36
60	11.1	36	36	10.1	24	36
65	11.2	38	37	10.2	24	37
70	11.3	38	37	10.5	24	37
75	11.6	40	38	10.7	26	38
80	11.7	40.4	38	10.9	26	39
85	11.8	42	39	11.2	26	40
90	12.1	46	39	11.5	30	40
95	12.7	49.6	40	11.8	32	41.9

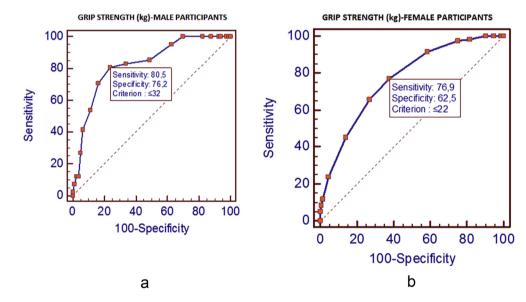


Fig. 1. Figure of ROC analysis results of grip strength for predicting usual gait speed (UGS) < 0.8 m/s indicating the grip strength value of ideal highest sensitivity and specificity in (a) males and (b) females.

muscle mass has been performed in few populations worldwide [3,16,17,20–25]. However, there are some different methods used to define cut-off thresholds for muscle mass. Some used young adults' data (-as mean-2standard deviation) [3,16,17,21] while some others used the older study participants [19,21–25]. As an example Janssen et al. used ROC curves to develop the skeletal muscle cut-off points associated with high physical disability probability. The relative frequencies of subjects with and without physical disability were determined at SSMI intervals of 0.25 kg/m² separately for the genders. Skeletal muscle cut-points of 5.76-6.75 and $=<5.75 \text{ kg/m}^2$ were denoted as moderate and high physical disability risk thresholds in women. Corresponding values in the men were 8.51–10.75 and $=<8.50 \text{ kg/m}^2$ [22]. Some used the sex specific lowest 20% of the older study participants [24,25]. On the other hand, some used "fat free mass" [26,27] and some others used skeletal muscle mass [3,16,17,20,21]. Also among the studies using SMM as the parameter for muscle mass, some used appendicular SMM [20,21,28] while some others used total SMM [3,16–29]. Accordingly, the reported prevalences of sarcopenia range widely depending on its adopted definition which makes the subsequent comparisons non-practical. To address this shortfall, EWGSOP has already recommended use of normative healthy young adults' data of the corresponding population with cut-off points at two standard deviations below the mean. Our study represents one of the few data specific to a nation which would aid to speak the same language with our colleagues within our country and internationally. The similar data for young reference population of different nations and their consequently calculated thresholds for muscle mass are given in Table 2 for such comparison. The Turkish skeletal muscle indices were somewhat higher but comparable with the French, Spanish and Taiwanese counterparts.

We have found the cut-off thresholds for hand grip-strengths as 32 kg and 22 kg to be used in sarcopenia assessment. Similar to the muscle mass indices, grip-strength cut points are proposed in few studies so far from different nations [5,18]. Again, there are

Table 5

Thresholds for low hand grip strength in different studies.

	Turkey M/F	USA ^a [18] (Cardiovascular Health Study) M/F	Italy ^b (InCHIANTI) [5] M/F	Japan ^c (Obu) [19] M/F
n	123/283	2240/3077	469/561	1848/1962
Age (years)	$77.5 \pm 6.2/76.2 \pm 6.8$	Range: 65–101	Range: 20-102	71.2 ± 4.9
HGS (kg)	32/22	29-32*/17-21**	30/20	28.8/18.2

M: male, F: female.

HGS: hand grip strength.

USA [18]: Grip strength threshold was measured from the population of 65–101 years age range as the grip strength in the lowest 20% at baseline adjusted for gender and body mass index (from non-young reference group).

Italy [5]: Grip strength threshold was measured from the population of 20–102 years age range by ROC analysis representing cutoff value for identifying participants walking slower than 0.8 m/s and unable to walk for 1 km without difficulty.

[apan [19]: Grip strength threshold was measured from the healthy older adult population aged over 65 years, with mean age of 71.2 ± 4.9 years, as the grip strength in the lowest 20% adjusted for gender.

Maximal grip strength (kg) in the dominant hand via a Jamar hand-held dynamometer (3 measures averaged).

Maximal grip strength was measured for each hand alternately. Three trials were allowed for each hand. The best result was chosen for analyses.

Maximal voluntary isometric strength of hand grip was measured using a hand dynamometer. The measurement was taken with the dominant hand in a standing position. The muscle strength test was carried out once only.

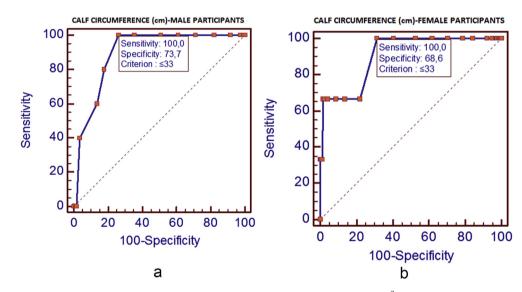


Fig. 2. (a) Figure of ROC analysis results of calf circumference for predicting the skeletal muscle mass index < 9.2 kg/m² indicating the calf circumference value of ideal highest sensitivity and specificity for males. (b) Figure of ROC analysis results of calf circumference for predicting the skeletal muscle mass index < 7.4 kg/m² indicating the calf circumference value of ideal highest sensitivity and specificity for females.

different methods used to define cut-off thresholds for grip strength. Fried et al. proposed the grip strength threshold in USA - measured from the population of 65-101 years age in Cardiovascular Health Study – as the lowest 20% at baseline adjusted for gender and BMI [18]. They suggested different cut points according to body mass indices and gender. The corresponding thresholds were in between 29 and 32 kg for males and 17-21 kg for females and were increasing in relation to body mass index (Table 5). On the other hand, Lauretani et al. [5] proposed grip strength cut point – measured from the population of 20–102 years range aged participants of inCHIANTI study - by ROC analysis representing cutoff value for identifying participants walking slower than 0.8 m/s and unable to walk for 1 km without difficulty. The corresponding thresholds were 30 kg and 20 kg in males and females, respectively. To derive grip strength threshold, we used the elderly data and similar to the later study, we derived the grip strength threshold by ROC analysis representing cutoff value for identifying participants walking slower than 0.8 m/s. Our data indicate that the grip strength cut point thresholds are also a bit higher in Turkish population but comparable with the other reference population data (Table 5).

We have found the calf circumference threshold representing cutoff value for identifying participants with low skeletal muscle mass indexes as <33 cm for both males and females. This finding is important and in accordance with our previous data of sarcopenia assessment project in Turkish nursing homes [30]. In that study, we aimed to investigate the prevalence of sarcopenia in nursing homes in Turkey and included 711 patients in 14 nursing homes crosssectionally. We determined the optimal CC value for predicting low hand grip strength by also using ROC curve analysis and suggested the optimum CC cutoff as 35 cm (sensitivity: 46.7%, specificity: 64.8%) which is also higher than the standard 31 cm threshold value. However, the sensitivity and specificity of CC cutoff 35 cm were not good enough. Our current study suggests CC cutoff value of 33 cm with significantly better sensitivity and specificity values. These two studies from Turkish population suggest that calf circumference threshold shall be somewhat higher in the Turkish population as shall the muscle mass and muscle strength thresholds.

Our study has some strengths and drawbacks. First, muscle strength was assessed by using a very valid protocol – separately for each hand with three trials – and the best result was chosen for analyses. This was only provided in InCHIANTI study so far [5]. All the measurements were made by the same qualified geriatric physiotherapist making the inter-assessor variability zero. Our young reference population has sample sizes either higher than or comparable with the most of the other reference population data (data from Taiwan and Spain) in the literature being only lower than the French data (Table 2). On the other hand, the older reference population used to derive hand grip strength threshold has sample size lower than the others (Table 5). The young reference population was not randomly selected from the general population but recruited from the healthy subjects more readily accessible for assessments. However, they were amongst the students of the faculty and workers from diverse jobs. The study location - Istanbul – is a metropolitan city receiving high rates of migration from diverse cities of Turkey both as students and workers. Accordingly, the study participants may also be from diverse cities of Turkey. Older reference population was not also randomly assigned community dwelling elderly but recruited from the patients admitting to our geriatric outpatient clinic. However, as our outpatient clinic serves for all older patients (>60 years), they were not very ill or fragile patients, representing the general community-dwelling population to some extent. Lastly, we assessed body composition with BIA. BIA is sensitive to hydration status, temperature, the timing of measurement, body symmetry, and position [31] and it may overestimate muscle mass [32]. However, while CT and MRI are the precise imaging modalities -being the gold standards for muscle mass estimation-, related cost, limited access and radiation exposure limit their use [1,3]. Dual energy X-ray absorptiometry (DXA) exposes to minimal radiation but is not portable having physical limitations restricting its application. On the other hand, assessment of body composition by using BIA has been validated by underwater weighing, magnetic resonance imaging and DXA [33,34]. BIA is a preferred method of muscle mass estimation to be used in cut-off determinations in a number of studies [3,16,17] and is considered as a good portable alternative to DXA by the EWGSOP consensus [1]. It is widely available, rapid, non-invasive, inexpensive, readily reproducible and operator friendly not requiring highlevel training [35] and appropriate for both ambulatory and bedridden patients [1]. Another concern related to BIA may be that very recently, it is suggested that the adaptation of BIA cut-offs shall be device dependant since all types of BIA devices are probably not equal to each other [32]. In this study, the Tanita BC 532 was preferred which is specifically reported accurate in the estimation of body composition, especially FFM, against the DXA and MR [34]. As a whole, our findings should be considered in view of these strengths and limitations.

In conclusion, our study suggests cut-off thresholds for skeletal muscle index, hand grip strength, and calf circumference in a Turkish population. Further worldwide studies from different nations and countries are needed to obtain reference values for populations enabling the researchers for comparison and also more valid reports on sarcopenia prevalence.

Conflict of interest

None of the authors of this manuscript have any financial or personal relationships with other people or organizations that could inappropriately influence (bias) their work. None of the coauthors have direct or indirect conflicts of interest, financial or otherwise, relating to the subject of our report. There is role of no sponsorship and funding.

Author contributions

GB has participated in the design of the study, carried out the studies, data analyses and wrote the manuscript. AT, FT, CK, TSA,

MK, NE, MAK have helped to collect the data and data analyses. AJC participated in the design of the study, data analyses and helped to draft the manuscript. All of the authors have made substantial contributions to interpretation of the data and revision of the article critically for important intellectual content. All authors have read and approved the final manuscript.

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