Research Article

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The Possible Sarcopenia Associated with Independent Walking Older Adults

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Abstract

The amount of skeletal muscle mass and mobility function increase during the childhood, peak at young adult, and then decline after middle age. Older adults with low skeletal mass and mobility function are associated with reduced physical performance. The older adult with a low level of mobility function, regardless of the amount of muscle mass, is defined as "possible sarcopenia". This study aims 1) to characterize the current state of the physical and mobility function at the peak levels of these parameters among young adults 2) to characterize their deterioration rates during middle-age and older-age years and older adults; and 3) to study the prevalence of possible sarcopenia among older adults. The cross-sectional study of anthropometric parameters, body compositions, and mobility assessments was conducted from independent walking young, middle, and older age adults in the community. Young adult male had higher levels of physical and mobility function than female. The changes in physical and mobility function during middle age were subtle. Older age male (female) lost skeletal muscle mass at -0.125 (-0.0190) kg/year, grip strength at -0.495 (-0.307) kg per year, and gait speed at -0.007 (-0.013) m/sec per year, respectively. The prevalence of possible sarcopenia in older age independent walking adults, determined by low grip strength, was 19.4%. The prevalence increased with advancing age.

Keywords: Possible sarcopenia, Grip strength, Gait speed, Skeletal muscle mass, Aging

What was Known

- An older adult with sarcopenia has low grip strength and/or low physical performance and low appendicular skeletal muscle mass

- Possible sarcopenia adult has low grip strength and/or low physical performance, regardless of appendicular skeletal muscle mass

What's New and Next

- The decline in grip strength is observed as of middle age in female. Older age male lost skeletal muscle mass and grip strength at faster rate than older age female.
- Early intervention of the sarcopenia adult is feasible by identification of a possible sarcopenic adult, and the prevalence of possible sarcopenia is high among older adults.

Introduction

Skeletal muscles, a component of the musculoskeletal system, play an important role in supporting the body and initiating body movement[1]. The amount of skeletal muscle mass and its movement function change over the course of life. During childhood growth, the muscle mass and its function increase until reaching their peak in young adult then start to decline after middle age[2]. The ability to maximize the peak levels of both skeletal muscle mass and its function during young adulthood, maintain the peak levels and minimize the deterioration is crucial and related to the health status of older individuals[3]. The deterioration rates vary among individuals depending on several factors, including age, race, gender, nutritional status, physical activity level and chronic illness[4,5]. Older adults with decreased skeletal mass and function below the threshold levels are associated with limited body movement, reduced physical performance, inability to perform daily activities, or disability. Older adults with reduced muscle function and physical performance may be at higher risks of falling, difficulty walking, difficulty rising from a chair, weakness, weight loss, wasting, hospitalization, or loss of independence[6,7]. As the world's older population is increasing, early detection and early intervention to delay or avoid disability and dependency in older adults are the main strategies for healthy aging[8].

Clinical conditions, including sarcopenia, frailty, cachexia, and malnutrition are associated with reduced skeletal muscle mass or muscle wasting and declined muscle function in older adults[9]. Sarcopenia is defined as generalized, progressive and age-related loss of skeletal muscle mass. The amount of skeletal muscle mass in sarcopenia must be below a certain level that results in declined muscle function and/or impaired physical performance in severe cases[10]. Several guidelines have been developed for the diagnosis of sarcopenia. In general, the diagnosis of sarcopenia must establish the low amount of skeletal muscle mass and the low level of muscle function in an older adult[11,12] However, the assessment of muscle mass requires a special tool that is not commonly available at community setting.

The concept of “probable sarcopenia” or “possible sarcopenia” has been developed to identify sarcopenia in older adults. This concept focuses primarily on the low level of mobility: muscle function or low physical performance, regardless of the determination of low skeletal muscle mass. The mobility impairment is evaluated through the assessments of muscle function and physical performance. These assessments are simpler and more accessible in the community or primary care facilities. An older person with low handgrip strength is defined as “probable sarcopenia”, according to the European Working Group on Sarcopenia in Older People-2 (EWGSOP2)[11]. “Possible sarcopenia” is defined as an older adult with either low muscle strength or reduced physical performance, according to the Asian Working Group for Sarcopenia (AWGS) 2019[12].

Because the amount of skeletal muscle mass and its function peaks during young adulthood and deteriorates after middle age, and even more during older age. The objectives of this study were 1) to characterize the current state of the physical (anthropometric parameters, body composition) and mobility function at the peak levels of these parameters among young independent walking adults (19-39 years old); 2) to characterize their deterioration rates during middle-age (40-59 years) and older-age (60 years and older) independent walking adults; and 3) to study the prevalence of possible sarcopenia among independent walking older adults.

Materials and Methods

1. Study design

A cross-sectional study of physical (anthropometric measurements, body composition) and mobility assessments were conducted in three age groups of participants: young adult (19-39 years), middle age (40-59 years), and older age (60 years and older) who lived in Lat-Krabang district, an eastern suburban area of Bangkok, Thailand.

2. Participants and setting

From December 2019 to February 2021, solicited volunteers were recruited at community centers, community fairs and health fairs in the district. Posters and flyers were distributed about the study in advance and during the events. Inclusion criteria were adults, age 19 years and older, and able to walk independently. Exclusion criteria were adult who walking with any kind of assistive device, unable to maintain a steady upright position due to any medical or surgical condition, and having increased total body water that interfered with bioelectrical impedance analysis (BIA) due to underlying medical illnesses, such as congestive heart disease, chronic kidney disease, or pregnancy. The current state of peak physical and mobility function would be represented by the anthropometric, body composition, and mobility data from young independent walking adults (19-39 years old). The change or deterioration of the anthropometric, body composition, and mobility data would be conducted among middle-age (40-59 years), and older-age (60 years and older). To exclude overnutrition, malnutrition, frailty and cachexic syndrome, the study would be conducted in normal BMI participant which normal BMI was defined as more than 18.5 and less than 24.9 kg/m^2. The prevalence of possible sarcopnia would be studied in independent walking older age adult (60 years and older). "Possible sarcopenia" is defined as an older adult with either low muscle strength, less than 28 kg in male, 18 kg in female or reduced physical performance, gait speed less than 1 m/sec in both male and female[12].

3. Data collection

Anthropometric and body composition measurements

Anthropometric parameters included weight (kg), height (cm), and body mass index (BMI, kg/m2). Body composition parameters included muscle mass (kg) and the percentage of skeletal muscle mass per body weight (%). Height was measured by a stadiometer, Tanita WB-380[13]. Weight, BMI and body composition were evaluated using a dual frequency BIA machine, Tanita DC 360P[14]. A dual frequency 4 electrode BIA machine was a foot to foot BIA device. The machine applies weak alternative current at 6.25 and 50 kHz throgh each foot to meaure body impedance during measurment. Each participant was asked to stand, kept both feet touch to foot electrode, and remained still on the BIA machine for 10 seconds. The weight (kg), BMI (kg/m^2), and muscle mass (kg) were recorded and reported. The percentage of skeletal muscle mass per body weight (%) was calculated by the following formula:

$$\%muscle\ (\%) = muscle\ mass(kg) / weight\ (kg) * 100$$

Mobility assessments

Mobility assessments included evaluations of muscle strength and physical performance. Muscle strength was assessed using grip strength. Physical performance was assessed by gait speed. Grip strength (kg) of the dominant hand was measured by using a T.K.K.5001 GRIP-D handgrip dynamometer[15]. Gait Speed (m/sec) was calculated from the time (sec) that the participant took to walk a distance of six meters (20 feet) on a flat pavement or hallway[16]. Both grip strength and gait speed measurements were repeated twice.

Prevalence of possible sarcopenia

Prevalence of "possible sarcopenia" was studied in the older-age participants which were classified by age into three subgroups: 60 – 69 years, 70–79 years, and older than 79 years. The prevalence of "possible sarcopenia" in each subgroup was calculated as the percent of participants who had low grip strength with or without low gait speed in each subgroup.

4. Statistical Analysis

According to the Bangkok Metropolitan Administration 2016 data, the estimated adult population of Lat-Krabang district is 178,604, of which 35,400 (19.8%) were older than 60 years[17]. Sample size was calculated using the level of confidence: 0.95, expected standard deviation: 4, and a margin of error: 0.5. A sample size of 249 for each age group was required to ensure the expected means of anthropometric, body composition, and mobility parameters of the population. The prevalence of possible sarcopenia was reported as % cases with 95% confidence interval (CI). The 95% CI of the prevalence of possible sarcopenia from the population of 35,400 older adults was analyzed by the epiR package[18].

Data analysis was performed by using the Rstudio software[18]. The peak level of each parameter was represented by the value of the young adult group. The anthropometric, body composition, and mobility parameters of the young adult group were analyzed and presented as mean and standard deviation (SD). In the middle-age and older-age groups, the deterioration (Δ) in each parameter was estimated by the slope of the linear regression model between each parameter and age. The difference between genders were analyzed by using the Student's t-test for continuous parameters and ANOVA for the slope of the residual analysis. A p <0.05 was considered as significant.

Results

A total of 3,737 adults were enrolled in the study. At the peak physical and mobility function, the young adult male had higher weight, height, BMI, muscle mass, %muscle, grip strength than female. After then, middle and older age subtlety lost their weight, height, muscle mass, grip strength, and gait speed. These changes were more prominent during older age, especially in muscle functions, grip strength and gait speed. (Table 1).

Middle-aged male lost their weight at a rate of -0.139 (female =-0.085) kg per year and older-age male lost at -0.389 (female =-0.328) kg per year. The rate of height loss in the middle-aged male was -0.115 (female = -0.158) cm per year and -0.189 for the older-age male (female = -0.214) cm per year. Middle-aged male gained BMI at +0.022 kg/m^2 per year, but older-age male lost BMI at -0.019 kg/m^2 per year. Female gained BMI in both age groups, at +0.034 kg/m^2 in the middle-age group and +0.018 kg/m^2 per year in the older-age group (Table 1).

Body composition studies: muscle mass and percentage of muscle mass for body weight (%muscle) were conducted in 2,092 independent walking adults with normal BMI. Young adult male had higher skeletal muscle mass (50.5±5.1 kg vs. 35.6±3.4 kg, $p < 0.05$), and %muscle mass (77.8±4.3% vs 67.0±4.5%, respectively, $p < 0.05$) than female. The rate of muscle mass loss in the middle-aged male was -0.025 kg per year and the rate increased to -0.125 kg per year in the older male, while the rate in the middle-aged female was -0.053 kg per year and -0.019 kg per year in the older female. During middle age, body adiposity increased as reflected in a decrease in %muscle at the rate of -0.020% in male and -0.087 % in female per year. Although older adults gained %muscle at a rate of +0.030% in male, +0.076% in female per year.

The mobility assessments: grip strength and gait speed were performed in 1,139 adults with normal BMI. Young male had a higher mean grip strength (42.5±7.0 kg) than young female (26.5±5.2 kg, $p < 0.05$). However, both genders had comparable gait speeds (1.2±0.2 m/sec vs 1.2±0.2 m/sec, $p=0.58$). Middle-aged male lost grip strength at -0.007 kg per year and the rate increased to -0.495 kg per year in the older male. The rate in middle-aged female was at -0.275 kg per year and -0.307 kg per year in older female. Middle-aged male lost gait speed at -0.003 m/sec per year and the rate increased to -0.007 m/sec per year in the older male, while middle-aged female lost at the rate of -0.002 m/sec per year and -0.013 m/sec per year in the older female (Table 1).

The prevalence of possible sarcopenia was studied in 1,081 eligible older adults. Anthropometric, mobility assessment, and the prevalence of possible sarcopenia in each age groups were reported (Table 2). The prevalence (95% CI) of possible sarcopenia in independent walking older adults was 19.4 (17.2-21.9)% in those with low grip strength or 43.0 (40.1-46.0)% in those with low gait speed. The prevalence increased with advancing age. By using low grip strength as a screening tool, the prevalence of the 60-69 years old adults was 11.5 (9.3-14.1)%, 26.4 (21.1-31.9)% of the 70-79 years, and 37.5 (29.8- 45.9)% of the 79 years and older (Table 2).

Table 1 Physical, Body Composition, and Mobility Changes in Independent Walking Adults

	Anthropometric Measurements 3,737 cases				Body Composition in Normal BMI 2,092 cases			Mobility Assessment in Normal BMI 1,139 cases		
	n	**Weight** (kg) Mean(±SD)	**Height** (cm) Mean(±SD)	**BMI** (kg/m^2) Mean(±SD)	n	**Muscle Mass** (kg) Mean(±SD)	**%Muscle** (%) Mean(±SD)	n	**Grip Strength** (kg) Mean(±SD)	**Gait Speed** (m/sec) Mean(±SD)
A. Peak Physical and Mobility function										
Young Adult (19–39-Year-Old)										
Male	426	69.9±12.9	171.9±6.4	23.6±3.8	242	50.5±5.1	77.8±4.3	49	42.5±7.0	1.2±0.2
Female	905	55.8± 12.1	159.3±5.7	21.9±4.4	578	35.6±3.4	67.0±4.5	125	26.5±5.2	1.2±0.2
	p	*< 0.05*	*< 0.05*	*<0.05*		*< 0.05*	*< 0.05*		*< 0.05*	0.58
B. Estimate Changes (Δ) Per Year										
	n	ΔWeight (kg/year)	ΔHeight (cm/year)	ΔBMI (kg/m^2/year)	n	ΔMuscle Mass (kg/year)	Δ%Muscle (% /year)	n	ΔGrip Strength (kg/year)	ΔGait Speed (m/sec/year)
Middle Age (40–59-Year-Old)										
Male	364	-0.139	-0.115	+0.022	180	-0.025	-0.020	106	-0.007	-0.003
Female	895	-0.085	-0.158	+0.034	482	-0.053	-0.087	266	-0.275	-0.002
	p	0.22	0.07	0.29		0.68	0.71		0.96	0.38
Older Age (60 Years and Older)										
Male	476	-0.389	-0.189	-0.019	262	-0.125	+0.030	249	-0.490	-0.007
Female	671	-0.328	-0.214	+0.018	348	-0.019	+0.076	344	-0.307	-0.013
	p	*<0.05*	*<0.05*	0.25		*<0.05*	*<0.05*		*<0.05*	*<0.05*

n = number of cases

Table 2 Physical and Mobility Changes in Independent Walking Older Adults

n	Weight (kg) Mean(±SD)	Height (cm) Mean(±SD)	BMI (kg/m²) Mean(±SD)	Grip Strength (kg) Mean(±SD)	Gait Speed (m/sec) Mean(±SD)	Low Grip Strength* # (%Case, Cases, SE, CI)	Low Grip Strength and Low Gait Speed*#$ (%Case, Cases, SE, CI)	Low Gait Speed* $ (%Case, Cases, SE, CI)
	Anthropometric Measurement			**Mobility Assessment**		**The Possible Sarcopenia**		
Overall, more than 60 years old: 1,081 cases						19.40%	12.70%	43.00%
						210/1,081	137/1081	465/1,081
						1.2	1.0	1.5
						(17.2-21.9)	(10.8-14.8)	(40.1-46.0)
A. 60-69 year : 669 cases								
Male	68.1±10.4	166.5±5.7	24.5±3.3	35.0 ± 6.6	1.1 ± 0.2	11.50%	7.20%	35.40%
276 cases								
Female	58.9±10.9	153.8±6.0	24.8±4.6	22.1 ± 4.4	1.0 ± 0.2	77/669	48/669	237/669
393 cases						1.2	1.0	1.8
p	*<0.05*	*<0.05*	0.36	*< 0.05*	*< 0.05*	(9.3-14.1)	(5.4-9.4)	(31.9-39.1)
B. 70-79 year: 276 cases								
Male	65.1±9.6	164.5±6.3	24.1±3.2	31.0 ± 6.9	1.0 ± 0.2	26.40%	17.70%	55.80%
111 cases								
Female	56.4±9.2	152.9±5.5	24.1±3.7	19.5 ± 4.3	0.9 ± 0.2	73/276	49/276	154/276
165 cases						2.6	2.3	3.0
p	*<0.05*	*<0.05*	0.92	*< 0.05*	*< 0.05*	(21.6-31.9)	(13.7-22.7)	(49.9-61.5)
C. More than 80 year: 136 cases								
Male	58.4±9.0	161.9±7.1	22.2±2.8	29.6 ± 7.2	1.0 ± 0.2	37.50%	29.40%	54.40%
53 cases								
Female	52.6±9.1	149.1±5.5	23.6±3.7	18.7 ± 4.9	0.9 ± 0.2	51/136	40/136	74/136
83 cases						4.2	3.9	4.3
p	*< 0.05*	*<0.05*	0.11	*< 0.05*	*< 0.05*	(29.8- 45.9)	(22.4-37.6)	(46.0- 62.5)

n = number of cases

* = Estimated prevalence from the population of 35,400 elders, SE: standard error, CI: confidence interval

= M < 28 kg, F <18 kg

$ = < 1.0 m/sec

Discussion

Aging associates with many physical changes, including body composition, the percentage of muscle mass and adiposity[19,20]. Weight, height, and BMI decline with age; however, the decline rates vary among ethnicities[21]. The decrease in height of the older adults is a result of the vertebral body compression and loss of postural control muscle tone[22]. Our results showed that the weight and height of both genders declined during middle and older-age groups, however more prominent during older age. The older-age group lost at -0.4 kg for male (-0.3 kg for female) per year for weight, and -0.2 cm (both male and female) per year for height (Table 1). Because of a proportional loss of weight and height from the aging process, both gender BMI changes were subtle during older age (Table 1, 2).

Our body composition studies were conducted in adults with normal BMI who maintained walking ability and did not have chronic illness or over/undernutrition. A dual-frequency BIA is our preferred choice because of its accessibility and portability. The results showed certain features of body composition change due to the aging process. Male had significant changes in their body composition throughout their life. Young men had a higher skeletal muscle mass than young female (50.5+5.1 vs 35.6+3.4 kg, p <0.05). However, older men lost skeletal muscle mass at a faster rate than older female (-0.125 vs -0.019 kg/year, p <0.05), while changes in female were more subtle throughout life. There was evidence of increasing body adiposity, as reflected by decreasing in % muscle been observed during middle-age in both genders (-0.02% and -0.09% per year). Contrary to belief of gaining adiposity during older age, our studies observed evidence of losing adiposity during older age, as reflected by an increase in muscle percentage in both genders (+0.03% and +0.08% per year). This finding indicated a disproportionate loss of adiposity compared to muscle mass.

Aging also associates with the declination of muscle function and physical performance and even more prominent than physical and body composition declination. The declination in grip strength showed certain characteristics: female lost grip strength as of middle age (-0.275 kg/year). During older age, both genders lost their grip strength, and even faster rate than female (-0.490 vs -0.307 kg/year). (Table 1)

Locomotion or mobility is a key hallmark of healthy aging in older people[23,24]. Older adults who lose mobility will become dependent. Moreover, the loss of mobility is associated with increased risks of morbidity and mortality[25,26]. Mobility loss in older people can be associated with an impairment of multiple systems, including sensory perception, central and peripheral nervous system, and musculoskeletal system[27]. Mobility can be assessed by measuring muscle function and physical performance[28]. Muscle strength is the amount of force that the muscles produce in a single maximal effort. In the clinical setting, muscle strength is a measurement of choice for assessing muscle function. Grip strength is preferable and used as a surrogate for the overall muscle function. Overall, the grip strength is higher in male than female and peaks at the fourth decade. With advancing age, the grip strength declines with age and gender dependent rates[29]. Physical performance is the ability to carry out physical tasks, ranging from activities of daily living to more complex activities that require a combination of skills.[28] Physical performance tests are objective assessments of the whole-body function, which measure not only the musculoskeletal system but also the central and peripheral nervous system related to mobility. Impaired mobility performance, such as frailty, disability, or impaired activity of daily living (ADL) can be assessed by using objective physical performance tests. The tests can be as simple as a single objective measure, such as gait speed, or more complex measures, such as the chair stand test, short physical performance battery (SPPB), and timed up and go (TUG) test[28]. Gait speed is a quick, inexpensive, and reliable measure that can assess and monitor functional status as well as the overall health in a wide range of populations. As a result, gait speed is proposed as the sixth vital sign[30]. and as a biomarker for older adults[31]. Gait speed is one of the components of intrinsic capacity and should be monitored for life[32].

Sarcopenia is a generalized, progressive age-related loss of skeletal muscle mass. The low amount of skeletal muscle mass in sarcopenia results in declined muscle function and/or impaired physical performance[10]. The diagnosis of sarcopenia in older adult requires the establishment of the low amount of skeletal muscle mass, especially in lower extremities in comparison to a healthy young to middle-aged adult, and low level of muscle function in an older adult[11,12]. Under current circumstances, the diagnosis of sarcopenia or severe sarcopenia will be conducted at acute to chronic care or at clinical research setting and requires the establishment of appendicular skeletal muscle mass or limb muscles by using either dual energy X-ray absorptiometry (DXA) or multifrequency bioelectrical impedance analysis (BIA). Older adult with either low amount of limb muscle mass, low grip strength or low physical performance will be diagnosed as sarcopenia. Severe sarcopenia will be diagnosed in older adult with low amount of limb muscle mass, low grip strength and low physical performance.

The concept of "possible sarcopenia" has been introduced for early identification of sarcopenia in older adults or those who are at risk of sarcopenia in the community. Early identification of possible sarcopenia can facilitate early intervention that can be beneficial to older adults. As aforementioned, the diagnosis of sarcopenia requires advanced diagnostic equipment for quantitative measurement of low extremity muscle mass which may not be widely available in the community or primary care setting. The diagnosis of possible sarcopenic older adult requires only either grip strength or physical performance. This is consistent with the WHO concept of healthy aging that shifts from disease-centered to functional-centered, by early detection and intervention to delay disability and dependency[8]. The deterioration of the mobility in older adults can be detected early at the community level by assessing the muscle function and physical performance. Possible sarcopenia was identified in the independent walking older adults (>60 years old) in our study, as either those who had low grip strength or who had low gait speed. Our studies showed that overall older adult from both genders, 19.4% had low grip strength and 43% had low gait speeds. The prevalence of possible sarcopenia by using grip strength in our study increased with age which ranged from 11.5% to 37.5% (Table 2).

Clinical implication of early identification of possible sarcopenia is early intervention. The aim is to minimize the deterioration of muscle mass and its function. Possible sarcopenic adult should be referred to acute to chronic care or clinical research setting where the quantitative appendicular skeletal muscle measurement available to confirm the diagnosis of sarcopenia. Possible sarcopenic adult should be benefited from lifestyle modification in diet and exercise. At least, older adult should adhere to WHO guideline of physical activity, should do at least 150–300 minutes of moderate-intensity aerobic physical activity; or at least 75–150 minutes of vigorous- intensity aerobic physical activity. Older adult should do muscle strengthening activities at moderate or greater intensity that involve all major muscle groups on 2 or more days a week[33]. Community base intervention of sarcopenic older adult at community level by using the combination of nutritional supplement and muscle strengthening exercise were confirmed by clinical trials [34,35].

This study had limitations due to many factors, such as the study design, tool, and external factors. This study was a cross sectional study. The cross-sectional study can provide snapshot information from multiple age ranges and variables under limited resources, budget and time. A long-term, observational study of individual's physical and mobility function changes throughout the course of life may provide a better individual trajectory of changes over a period of time. The reference technique for quantitative measurements of skeletal muscle is DEXA. An alternative is a multifrequency BIA. Both tools provide the measurement of the amount of skeletal muscle in the whole body or appendicular (limb) musculature. The low amount of appendicular skeletal muscle is associated with low motility performance in older adults and used as a diagnostic criterion of sarcopenia. Our study used a dual frequency BIA for quantitative measurement of skeletal muscle. This dual frequency BIA provided us the whole-body muscle information, instead of appendicular muscle mass only. However, the device was portable and more accessible which could be a trade-off. The Covid-19 pandemic was an external factor that limited our study's reliability and validity. Our study was conducted during the pandemic period and abruptly ended. The data analysis revealed a low number of cases in particular samples such as mobility assessment in normal BMI young adults, and in older age, more than 79-year-old.

Conclusion

In young adults, male had higher physical parameters (weight, height, BMI, skeletal muscle mass, percent of skeletal muscle mass) and mobility function (grip strength) than female. During middle age, body composition showed a slight increase in body adiposity. Other changes were subtle, and no differences shown between genders. The declination of hand grip strength is observed as of middle age. During older age, the physical changes were evident and even more prominent in mobility function: grip strength and gait speed. Deteriorations during older age were different between genders. Changes in BMI were subtle throughout the course of life. The prevalence of possible sarcopenia in older adults who independently walked in our community was high.

Ethical Approval Statement

The study was reviewed and approved by the Institution Ethics Committee (EC_KMITL_62_010) and was the part II from the study of age-related muscular mass changes in Ladkrabang District, Bangkok. Written informed consent was obtained from all subjects.

Author Contributions

NR and UJ designed the study with guidance from AS. NR and UJ carried out the data collection and data analysis. NR re-analyzed the data and wrote the manuscript. All authors read and approved the manuscript prior to submission for publication.

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Conflicts of Interest

All authors have no conflicts of interest to disclose.

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