Archives of Gerontology and Geriatrics 61 (2015) 33–38

Contents lists available at ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

Rectus femoris (RF) ultrasound for the assessment of muscle mass in older people

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ARTICLE INFO

Article history:
Received 5 September 2014
Received in revised form 21 March 2015
Accepted 25 March 2015
Available online 1 April 2015

Keywords:
Ultrasound
Rectus femoris
Sarcopenia

ABSTRACT

Background: Simple and reliable methods to evaluate muscle mass in older people are lacking.
Aim: To evaluate ultrasound as a measure of RF muscle mass and quality in healthy subjects of different ages and gender, assessing its concordance with dual energy X-ray densitometry (DEXA) and association with muscle strength and walking capacity.
Methods: We selected 54 adults of both genders, aged 20–55 years and 51 adults older than 60 years. Ultrasound images of the RF were obtained at the mid-thigh to measure its thickness and ultrasonographic density using a GE Logiq e equipment. Body composition was assessed by DEXA. Quadriceps isometric strength and 12 minutes' walk were also measured and gender specific *t* scores for older adults were calculated using the values obtained in adults.
Results: RF ultrasound measurements correlated significantly with lean body mass assessed by DEXA (Double energy X-ray absorptiometry). The concordance between both measures was also adequate. Older people had lower muscle mass and worse ultrasound parameters than adults. Older males with a *t* score for quadriceps strength of −2 or less, had a significantly higher RF grayscale density. Older males with a 12 minutes' walk *t* score of −2 or less and old males and females with a walking speed of 1 m/s or less had a lower RF thickness.
Discussion: There is a good concordance between RF ultrasound and DEXA.
Conclusions: Assessment of RF using ultrasound appears to be a reliable and accurate method to evaluate muscle mass in older people.

1. Introduction

Sarcopenia, or the inexorable loss of muscle mass and function which occurs with aging is an important cause of disability and a risk factor for falls among older people (Marschollek et al., 2009). The proposed working definition of this condition, includes measures of muscle mass, strength and function (Dam et al., 2014), stressing the fact that a low muscle mass is one of multiple factors that affect muscle functionality (Studenski et al., 2014). Muscle loss also affects acutely ill patients admitted to intensive care units and patients with cancer and is a dismal prognostic factor among ill people (Antoun, Borget, & Lanoy, 2013).

Older people experience a reduction in mass and quality of muscle. There is a progressive fatty infiltration of muscle, called myoesteatosis, which replaces muscle fibers, damages the remaining fibers, probably by oxidative damage, and also generates metabolic derangements such as insulin resistance (Song et al., 2004). We and others have shown previously that sarcopenia, expressed as a reduction in muscle mass measured by double energy X-ray absorptiometry (DEXA) or a functional derangement assessed as walking speed, is associated with an increased risk for mortality and disability (Bites et al., 2013; Bunout, De La Maza, Barrera, Leiva, & Hirsch, 2011). Simple and reliable measures of muscle mass and for timely detection of its changes associated with interventions, such as exercise training, are required. Anthropometry is very limited for these purposes, considering its inherent measurement errors (Bunout, Barrera, De la Maza, Leiva, & Hirsch, 2012). Functional parameters such as hand grip or quadriceps strength have a prognostic value and are commonly used to evaluate the functional capacity of older people (Al Snih, Markides, Ray, Ostir, & Goodwin, 2002; Sasaki, Kasagi, Yamada, & Fujita, 2007). However measurement of strength depends on the volitional response of participants and is affected

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http://dx.doi.org/10.1016/j.archger.2015.03.006
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by muscle or bone pain, limiting its usefulness, especially among people with osteoarthritis. There are also composite measures such as timed up and go, which are popular among geriatrists (Schoene et al., 2013).

Muscle mass can be assessed accurately using imaging methods. CT scans and magnetic resonance (MR) imaging are precise, however both methods are expensive and one implies exposure to ionizing radiation. Double energy X-ray examination (DEXA) accurately assesses lean mass and is used as a gold standard. It is inexpensive and has a low re-test error (Chen et al., 2007). Its drawbacks are that the large equipment required precludes its use in field studies and that in patients with fluid management problems, the measurements can altered (Georgiou et al., 1997).

Muscle ultrasound measurements have been proposed as an alternative to assess muscle mass. The method usually implies the measurement of muscle cross sectional thickness, especially of the RF (Sanada, Kearns, Midorikawa, & Abe, 2006). Using these methods, equations to predict total muscle mass using MR have been derived in Japanese participants (Miyatani, Kanehisa, Kuno, Nishijima, & Fukunaga, 2002). Muscle ultrasound density can be also measured (Fukumoto et al., 2012). Analogous to what occurs in the liver, this parameter should increase along with fat infiltration of the muscle and should provide an estimation of myoesteatosis (Cazzo, de Felice Gallo, Pareja, & Chaim, 2014). Ultrasound is of lower cost than DEXA and the size of the equipment allows its use in field studies or at bedside in hospitalized patients. RF was chosen to perform the measurements because it is commonly assessed in ultrasound studies (Tillquist et al., 2014) and changes in its cross sectional area after training are may be more prominent than in other muscles (Seynnes, de Boer, & Narici, 1985). Most studies have compared muscle volumes measured by different methods but have not included precise determinations of muscle mass. One study compared ultrasound with bioimpedance (Fukumoto et al., 2012), but this last method may be unreliable (Kim & Kim, 2013).

DEXA is the usual method to determine fat free mass in older people and is used to develop predictive equations to derive appendicular lean body mass (Baumgartner et al., 1998). Since there are few studies comparing DEXA with muscle ultrasound, we hypothesized that RF ultrasound would have a good concordance with total and appendicular lean body mass measured by DEXA. Therefore, the aim of this study was to assess if RF ultrasound examination has a good concordance with appendicular fat free mass measured by DEXA and is associated with muscle strength and performance.

2. Material and methods

Healthy individuals living in the community were invited to participate. Potential participants were enrolled in community centers and recreational facilities by direct contact. Exclusion criteria were being engaged in competitive sports, having chronic debilitating diseases such as heart or kidney failure, chronic infections or diabetes mellitus, neuromuscular diseases, ingestion of medications which could affect muscle function such as adrenal steroids or anabolic drugs, alcoholism and smoking more than five cigarettes per day. We selected participants of two age groups, namely adults aged between 20 and 55 years and older adults aged more than 60 years. For each age group an equal number of men and women were selected. All participants were fully informed about the experimental procedures, the purpose of the study and gave a written informed consent before participation. The ethics committee of the Institute of Nutrition and Food Technology from the University of Chile approved the study protocol.

The following assessments were performed on the same day to all participants:

1. Medical history taking to discard the presence of diseases or use of medications considered in the exclusion criteria.
2. Assessment of functional measures:
    (1) Quadriceps strength measured in a quadriceps table equipped with an isometric force transducer connected to a computer to register the maximal strength achieved in one repetition, using a previously published technique (Bunout et al., 2006). The subjects sat with their back and hips flexed at 85° and secured by straps and their knees flexed at 70°. The distance between the knee and the force transducer was kept constant for all measurements to avoid errors in torque calculation. Three consecutive measurements were done and the best value was registered. Strength was expressed in kg.
    (2) Twelve minutes' walk in which the subjects were encouraged to walk as fast as they could in a flat surface surrounding a basketball field during 12 minutes, in groups of at least three participants. The distance walked was recorded. A walking speed cutoff point of 1 m/s was used to define functional impairment.
    (3) Survey of physical activity using the international physical activity questionnaire (Grimm, Swartz, Hart, Miller, & Strath, 2012).
    (4) Measurement of body composition by double energy X-ray densitometry, using a Lunar General Electric iDEXA equipment. Total and segmental fat free, fat and bone masses were recorded.
    (5) RF ultrasound examination using a General Electric Logiq ultrasonographer. With the participant in the sitting position and the legs hanging relaxed, the probe was placed perpendicular to the skin without exerting compression, over the mid portion of the RF, calculated as half the distance between the anterior superior iliac spine and the lower edge of the patella. This landmark was chosen because it is easy to determine and reproducible between observers and is usually employed in other studies (Tillquist et al., 2014). Special care was taken to avoid any voluntary contraction of the RF. A GE 12L-RS 14.2 mm × 47 mm linear array ultrasound transducer probe was used, it was set to measure at a frequency of 8 MHz, with a gain of 58 dB and a depth of 5 cm. Left and right muscles were measured. The cross sectional thickness of the muscle was measured during the examination and the images were stored. All images were obtained by the same operator. Every measurement was made in triplicate.

The stored images were analyzed using Photoshop® software. Using the magnetic lasso, the muscle perimeter was delineated, not including the fascia. Using the measurements registry menu, the surface and the integrated density of the selected area were obtained and the grayscale density of the muscle was calculated as the ratio between the integrated density and the area (this division must be done since the integrated density is proportional to the area selected). Therefore the thickness and grayscale density of the right and left RF were obtained.

Intrarater error of ultrasound measures was assessed measuring RF by the same observer in 10 volunteers on 10 different days. Interrater error was assessed measuring RF in the same subject on the same day by 10 different observers, who were unaware of the measurements made by each other.

2.1. Data analysis

All statistical analyses were performed using Stata 12 for windows (Statacorp, College Station, TX 77845, USA). We evaluated the normality of variable distribution, using the Shapiro–Wilks test. As variables had a normal distribution, results are expressed as mean ± standard deviation. The significance of differences between two groups was calculated using Student's t-test. When more than two groups were compared, one way Anova with Bonferroni post hoc analysis, was used. Intra and inter observer variability of ultrasound measures were assessed measuring different individuals in 10 occasions by the same observer (intraobserver) and one individual in the same occasion by 10 different observers (interobserver). The results are expressed as % error (calculated as (standard deviation of observations/mean value of observations)*100) and intraclass correlation coefficients. Using means and standard deviations of twelve minutes' walk and quadriceps strength values of adults, gender specific t scores for these measures were calculated for older people. Comparisons were made between older participants with t scores of -2 or less with their counterparts with t scores over -2. The use of 2 standard deviations as cutoff point will indicate which individuals are allocated above or under 95% of the reference population and is extensively used, for example, to interpret bone densitometry. Correlations between parameters were calculated using Pearson's correlation coefficient. To determine concordance, body composition and ultrasound measures were normalized using two different methods (Organisation for economic co-operation and development, European Commission, 2008). Values were converted to ranks using the egen rank function of Stata 12 and were also converted to z scores, using the mean and standard deviation of the whole sample. The z score of RF density was multiplied by -1 to change its sign (and therefore have a positive correlation with appendicular fat free mass). Lin's concordance correlation coefficients were calculated using both normalization methods. The level of statistical significance was established at 0.05.

3. Results

We studied 54 adults (27 women) and 51 older adults (26 women). Demographic, anthropometric, body composition, ultrasound and functional data of participants are depicted in Table 1. There were significant differences between all groups in practically all parameters. Young men had the best measures, following in decreasing order by young women, old men and old women. The intra and interrater errors of ultrasound measurement of RF cross sectional thickness were 3.2 and 7.4%, respectively. The intraclass correlation coefficients for intra and interrater measurements were 0.81 and 0.67 respectively ($p < 0.01$). The intra and interrater errors for grayscale density were 14.3 and 14.5%, respectively. The figures for intraclass correlation coefficients were 0.64 and 0.5 respectively ($p < 0.01$).

The comparisons of ultrasonographic measurements in older people with t scores for functional parameters over or under -2, are depicted in Table 2. Among females, no differences in ultrasound or total or regional fat free mass were observed according to t scores for quadriceps strength and 12 minutes' walk. Among males, participants with a t score <-2 for quadriceps strength had a higher RF gray scale density and lower total and appendicular fat free mass. When comparing 12 minutes' walk t scores, the only difference observed was for RF thickness. Six older people (three women) had a walking speed of 1 m/s or less. Compared with their old counterparts, the former had a significantly lower RF cross sectional thickness (16.7 ± 1.6 and 19.6 ± 2.9 cm respectively, $p = 0.02$) but no significant differences in grayscale density (40.2 ± 7.8 and 37.7 ± 8.3 arbitrary units respectively, p = NS).

The correlation matrix between DEXA measures and RF ultrasound measures is depicted in Table 3. There were significant and high correlations between ultrasound and DEXA. However, the correlations of RF thickness with body composition were of greater magnitude than those of RF density. The results of the concordance analyses between z scores of average RF thickness and density and appendicular fat free mass are shown in Table 4 and Fig. 1(A and B). There was a significant concordance between DEXA and ultrasound measures. However the degree of concordance was higher for RF thickness.

4. Discussion

In this group of participants, we showed that RF cross sectional thickness had a low intra and interrater error, good correlation coefficients and concordance with total and appendicular fat

Table 1
Demographic, clinical and body composition features of studied participants (x ± standard deviation).

	(A) Young women ($n = 27$)	(B) Young men ($n = 27$)	(C) Older women ($n = 26$)	(D) Older men ($n = 25$)	Significance of differences between groups[a]
Demographic and functional measures					
Age (years)	32.4 ± 7.1	34.6 ± 6.7	72.5 ± 5.8	74.5 ± 6.5	
Weight (kg)	60.5 ± 6.5	78.9 ± 11.0	70.6 ± 11.1	75.5 ± 10.3	Only C and D not different
Height (cm)	159.2 ± 6.6	171.2 ± 5.9	152.5 ± 6	165.6 ± 7	All groups different
Body mass index (kg/m^2)	24 ± 3	26.9 ± 3.7	30.4 ± 4.3	27.6 ± 3.4	Only A and D not different
Physical activity (MET-minutes/week)	1119.8 ± 848.5	1871.7 ± 1490.4	729.4 ± 413.8	1225.3 ± 1243.8	A vs B B vs D
Twelve minutes' walk (m)	1061.3 ± 116.4	1145.3 ± 155.9	860.6 ± 106.8	959.8 ± 188.4	A vs C B vs C C vs D
Right quadriceps strength (kg)	37.4 ± 6.6	48.7 ± 11.9	24.9 ± 6.4	35.8 ± 7.6	Only A and D not different
Left quadriceps strength (kg)	35.4 ± 5.1	51.7 ± 11.4	27.2 ± 9	42.1 ± 12.5	All groups different
Quadriceps ultrasound measurements					
Right RF thickness (mm)	21 ± 2.2	26.9 ± 3.5	18.2 ± 2.3	21.6 ± 3.1	Only A and D not different
Left RF thickness (mm)	19.7 ± 1.7	25.5 ± 3.7	16.6 ± 1.7	20.6 ± 2.8	Only A and D not different
Average RF thickness (mm)	20.3 ± 1.8	26.2 ± 3.4	17.4 ± 1.8	21.1 ± 2.7	Only A and D not different
Right RF density (arbitrary units)	26.6 ± 8	21.8 ± 4.7	39.8 ± 8.5	34.4 ± 10.1	Only C and D not different
Left RF density (arbitrary units)	30 ± 6.3	23.7 ± 6	42.4 ± 8.6	35.1 ± 6.1	All groups different
Average RF density (arbitrary units)	28.3 ± 6.7	22.7 ± 4.9	41.1 ± 8	34.8 ± 7.2	All groups different
Duble energy X-ray densitometry (DEXA)					
Total fat free mass (kg)	35.7 ± 3.2	53.3 ± 5.9	36.3 ± 4.7	47.9 ± 5.5	Only A and C not different
Total fat mass (kg)	23 ± 4.8	23.5 ± 5.8	32.7 ± 6.9	25.4 ± 6	Only A and B not different
Lower limb fat free mass (kg)	11.9 ± 1.3	18.5 ± 2.7	11.7 ± 1.9	15.5 ± 2.3	Only A and C not different
Lower limb fat mass (kg)	8.2 ± 1.7	6.5 ± 1.8	9.7 ± 2	6.2 ± 1.9	Only B and D not different
Appendicular fat free mass (kg)	15.3 ± 1.6	24.9 ± 3.5	15.5 ± 2.4	20.9 ± 3.1	Only A and C not different
Appendicular fat mass (kg)	10.5 ± 2.0	8.6 ± 2.3	13.1 ± 2.6	8.3 ± 2.4	Only B and D not different

[a] $p < 0.05$ or less on one way ANOVA and Bonferroni.

Table 2
Ultrasound and body composition parameters among older people with t scores for functional measures over or below two standard deviations of values in young people.

	Quadriceps strength[a]			Twelve minutes' walk		
	Females					
	t score <-2 ($n=15$)	t score ≥-2 ($n=11$)	p[b]	t score <-2 ($n=9$)	t score ≥-2 ($n=17$)	p[b]
RF[a]						
Cross sectional thickness (cm)	17.4 ± 1.9	17.4 ± 1.7	NS	16.8 ± 1.7	17.7 ± 1.8	NS
Gray scale density (AU)	41.4 ± 8.0	40.7 ± 8.3	NS	42.4 ± 7.0	40.4 ± 8.6	NS
DEXA						
Total fat free mass (kg)	35.2 ± 5.1	37.9 ± 3.7	NS	35.3 ± 5.6	36.9 ± 4.2	NS
Appendicular fat free mass (kg)	14.8 ± 2.5	16.4 ± 1.9	NS	14.8 ± 3.1	15.8 ± 1.9	NS
	Quadriceps strength[a]			Twelve minutes' walk		
	Males					
	t score <-2 ($n=4$)	t score ≥-2 ($n=21$)	p[b]	t score <-2 ($n=6$)	t score ≥-2 ($n=19$)	p[b]
RF[a]						
Cross sectional thickness (cm)	19.5 ± 2.3	21.4 ± 2.7	NS	18.4 ± 2.6	21.8 ± 2.4	0.02
Gray scale density (AU)	41.1 ± 5.0	33.6 ± 7.0	0.05	37.7 ± 8.2	34.3 ± 7.1	NS
DEXA						
Total fat free mass (kg)	42.0 ± 3.7	48.9 ± 5.1	0.02	46.1 ± 6.8	48.2 ± 5.4	NS
Appendicular fat free mass (kg)	17.9 ± 2.7	21.5 ± 2.8	0.02	19.6 ± 3.9	21.2 ± 2.9	NS

[a] Average of both lower limbs.
[b] Probability for differences between groups.

Table 3
Correlation matrix between ultrasound and muscle composition parameters.

	RF thickness			RF density			Fat free mass	
	Right	Left	Average	Left	Right	Average	Total	Lower limb
Left RF thickness	0.873							
	<0.01							
Average RF thickness	0.9687	0.9667						
	<0.01	<0.01						
Left quadriceps density	−0.6001	−0.5954	−0.6178					
	<0.01	<0.01	<0.01					
Right quadriceps density	−0.5879	−0.5123	−0.569	0.8078				
	<0.01	<0.01	<0.01	<0.01				
Average quadriceps density	−0.6244	−0.5805	−0.6229	0.946	0.9552			
	<0.01	<0.01	<0.01	<0.01	<0.01			
Total fat free mass	0.6766	0.6911	0.7066	−0.4713	−0.3579	−0.4333		
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		
Lower limb fat free mass	0.71	0.7318	0.7448	−0.5114	−0.4014	−0.4774	0.9755	
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	
Appendicular fat free mass	0.7067	0.728	0.7411	−0.4921	−0.3843	−0.4582	0.9869	0.9948
	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 4
Lin's concordance correlation analysis between z scores for fat free mass measured by DEXA and z scores for RF ultrasound parameters.

	Concordance normalizing data as ranks		Concordance normalizing data as z scores	
	Between RF thickness and appendicular fat free mass	Between RF ultrasound density and appendicular fat free mass	Between RF thickness and appendicular fat free mass	Between RF ultrasound density (*−1) and appendicular fat free mass
Lin concordance correlation coefficient (rho)	0.681	−0.453	0.741	0.458
95% confidence intervals	0.578–0.785	0.606–0.299	0.654–0.828	0.306–0.611
p	<0.01	<0.01	<0.01	<0.01
Average and standard deviation of difference	0 ± 24.3	0 ± 51.9	0 ± 0.72	0 ± 1.041
Correlation between difference and mean[a]	0	0	0	0
Bradley-Blackwood F[b]	0	0	0	0
p	1	1	1	1

[a] A value near 0 implies concordance.
[b] Non significance implies concordance.

free mass measured by DEXA. The measurement error of grayscale density was higher and its concordance with DEXA measures was weaker.

In the present study we considered that older people with muscle strength and walking capacity below two standard deviations of values obtained in young people of the same gender (t scores), had sarcopenia from a functional point of view. This definition is analogous to that used to define osteoporosis using bone density data obtained by DEXA (Janssen, Heymsfield, & Ross, 2002). Using this division, comparisons within older

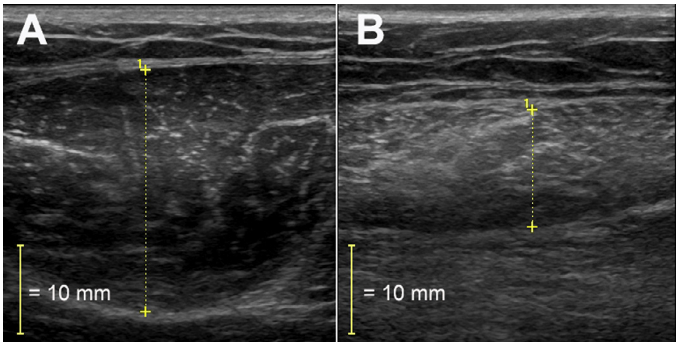

Fig. 1. Examples of ultrasound images of RF of (A) Young participant. (B) Old participant. The yellow dotted line represents the caliper that measures the thickness in the ultrasound equipment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

subjects can be made. We preferred to compare older adults with their own young counterparts rather than using international definitions of sarcopenia, because our own data and reports from Mexico indicate that the cutoff points using in Europe and North America, may not be applicable to our population (Velázquez Alva Mdel, Irigoyen Camacho, Delgadillo Velázquez, & Lazarevich, 2013). The lack of differences in ultrasound parameters among females and the scarce differences among males with and without functional impairment is not surprising. We (Bunout et al., 2004) and others (Newman et al., 2003) have shown that the association between functional and morphological parameters of sarcopenia, although significant, is weak. In our previous report, we also observed that this association was stronger among males than females. In the report by Newman et al. (2003), the R^2 of the regression models for muscle strength and lean body mass did not surpass values of 0.3 and the figures were also higher in men. Therefore, in the future, the prognostic value of ultrasound measurements, in terms of disability and mortality should be tested, rather than their cross sectional association with functional measures. We already know that walking capacity has the capacity to discriminate people with disability (Dangour et al., 2011). It remains to be seen if ultrasound measurements have a better predictive value.

Previous reports have shown that there is a good inter-rater agreement when muscle cross sectional thickness is measured by ultrasound, even by minimally trained or untrained professionals (Thomaes et al., 2012; Zaidman, Wu, Wilder, Darras, & Rutkove, 2014). Therefore it can become a measure that does not require experienced raters and can be obtained at the bedside. Moreover, the Lin concordance analysis shows a good concordance between DEXA and ultrasound cross sectional thickness, further validating the technique.

DEXA does not measure the quality of muscle. Therefore the significant although weak concordance between appendicular fat free mass measured by DEXA and ultrasound grayscale density may reflect the fact that there is a progressive loss of muscle mass and higher muscle fat infiltration with aging (Miljkovic et al., 2013). Thus, fat intramyocellular fat accumulation should be inversely proportional to muscle mass. The higher intra and interrater error of grayscale density measures is worrisome. It is possible that refining its measurement, placing the transducer in a fixed position and establishing criteria to define the area of interest where this parameter is calculated, may reduce this error. This measurement imprecision is analogous to what happens when fatty infiltration of the liver is assessed by ultrasound. The method, although widely used, has a sensitivity and specificity to detect fatty liver ranging from 60 to 90% and a low reproducibility (Schwenzer et al., 2009). In the future, the concordance of muscle echogenicity with a good measure of intramyocellular fat such as MR imaging (Perseghin, Scifo, De Cobelli, Pagliato, & Battezzati, 1999) or computed tomography (Larson-Meyer et al., 2006), should be tested. This is imperative since muscle fat accumulation is associated with insulin resistance (Corcoran, Lamon-Fava, & Fielding, 2007), a common problem in the elderly. If ultrasound proves accurate enough to measure muscle fat, it could be used to assess the impact of myosteatosis on metabolic derangements associated with older age.

Recently, muscle ultrasound has been used to assess loss of muscle mass among critical care patients. The loss of RF cross sectional area predicted multiorgan failure and was associated with a reduction in protein fractional synthesis rate (Puthucheary et al., 2013). Furthermore, if RF thickness changes as early as eight weeks after training, as shown by Housh, Housh, Johnson, and Chu (1992), it could become an early and sensitive measure to determine the success of training programs in older people. However, the inhomogeneous hypertrophy that occurs in thigh muscles after training should prompt the search for other muscles such as the vastus lateralis as indicators of a successful training (Ema, Wakahara, Kanehisa, & Kawakami, 2014). We have observed, that although training improves muscle strength, no changes are observed in fat free mass measured by DEXA (Bunout et al., 2005).

In summary RF ultrasound examination has a good concordance with DEXA measurement of appendicular fat free mass and could be a useful measure to estimate lower limb muscle mass.

Financial support

FONDECYT grant # 1130284.

Authors' contribution

Juan Berger, Daniel Bunout, Gladys Barrera, María Pía de la Maza and Sandra Hirsch involved in conception and design of the study; generation, collection, assembly, analysis and/or interpretation of data; drafting or revision of the manuscript and approval of the final version of the manuscript.

Sandra Henriquez involved in conception and design of the study, drafting or revision of the manuscript and approval of the final version of the manuscript.

Laura Leiva involved in generation, collection, assembly, analysis and/or interpretation of data and approval of the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflict of interests.

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