British Journal of Nutrition (2015), **114**, 1209–1217
doi:10.1017/S0007114515002585

Prediction and validation of total and regional skeletal muscle volume using B-mode ultrasonography in Japanese prepubertal children

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(Submitted 26 November 2014 – Final revision received 31 May 2015 – Accepted 15 June 2015 – First published online 4 September 2015)

Abstract

Very few effective field methods are available for accurate, non-invasive estimation of skeletal muscle volume (SMV) and mass in children. We aimed to develop regression-based prediction equations for SMV, using ultrasonography, in Japanese prepubertal children, and to assess the validity of these equations. In total, 145 healthy Japanese prepubertal children aged 6–12 years were randomly divided into two groups: the model development group (sixty boys, thirty-seven girls) and the validation group (twenty-nine boys, nineteen girls). Reference data in the form of contiguous MRI with 1-cm slice thickness were obtained from the first cervical vertebra to the ankle joints. The SMV was calculated by the summation of digitised cross-sectional areas. Muscle thickness was measured using B-mode ultrasonography at nine sites in different regions. In the model development group, strong, statistically significant correlations were observed between the site-matched SMV (total, arms, trunk, thigh and lower legs) measured by MRI and the muscle thickness × height measures obtained by ultrasonography, for both boys and girls. When these SMV prediction equations were applied to the validation groups, the measured total and regional SMV were also very similar to the values predicted for boys and girls, respectively. With the exception of the trunk region in girls, the Bland–Altman analysis for the validation group did not indicate any bias for either boys or girls. These results suggest that ultrasonography-derived prediction equations for boys and girls are useful for the estimation of total and regional SMV.

Key words: Skeletal muscle volume: MRI: Children: Ultrasonography: Prediction equations

Although body-composition studies have been developed and refined over more than 30 years, only a limited amount of information is available on total body skeletal muscle volume (SMV) and mass in children. Studies on body composition at the organ–tissue level in children have only indicated the proportional contributions of skeletal muscle (SM) mass to body weight[1], and the process of developing a prediction formula for SM mass is still on-going[2]. The development of SMV in children is greatly influenced by nutritional intake and the level of physical activities. Therefore, SM mass may be a very important index for the estimation of nutritional status and prediction of exercise performance during different growth stages, and is linked to the comprehensive estimation of lifestyle[3].

MRI is a precise, reliable and safe method for the measurement of total body SMV in children and adults[4,5]. However, the use of MRI for the estimation of SMV requires exclusive-use facilities and a great deal of time for image analysis. On the other hand, ultrasonography is a non-invasive and safe method for the measurement of the muscle thickness of the extremities and trunk in children[6]. Moreover, a compact-type ultrasonography machine is easily portable, which is important for use during field research and for the assessment of SMV in large groups of subjects. In addition, ultrasonography can be used for the determination of total and regional muscle thickness in various body types.

Our previous research enabled the development of ultrasonography-derived prediction equations for the estimation of total and regional (i.e. arm, trunk, thigh and lower leg) SM mass in adults, both men and women[7]. The SM prediction model for adults is only applicable in adolescents of approximately 14 years of age (over Tanner stage 2 and at peak height velocity) and is not valid in prepubertal children (Tanner stage 1 and not approaching peak height velocity)[5]. Based on these previous studies, the present study was performed to develop regression-based prediction equations for SMV using ultrasonography in Japanese prepubertal children and to investigate the validity of these equations.

Methods

Subjects

In total, 145 healthy Japanese prepubertal children, aged 6–12 years (determined according to the years completed since birth)

Abbreviations: SM, skeletal muscle; SMV, skeletal muscle volume.

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and of Tanner stage 1, were randomly divided into two groups according to their fat-free mass: the model development group (sixty boys, including eight overweight and three obese boys; thirty-seven girls, including eleven overweight girls)[8] and the validation group (twenty-nine boys, including seven overweight and two obese boys; nineteen girls, including six overweight girls)[8] (Table 1). All the subjects were recruited through reference by friends and acquaintances in Tokyo. At the time of enrolment, criteria (i.e. demographic and socio-economic status) for inclusion in this study were not defined. The maturational level of the subjects was assessed using the Tanner scale questionnaire[9]. All the subjects were physically active (i.e. they played outdoor games); however, the sample did not include any athletes. None of the subjects showed any known pathological condition and were not on any medication. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all the procedures involving human subjects/patients were approved by the Ethics Committee of Waseda University. Written informed consent was obtained from all the subjects and their guardians.

Body mass was measured using a digital balance to the nearest 0·1 kg, with the subjects wearing only minimal clothing, and height was measured using a stadiometer (AS ONE Co. Ltd) to the nearest 0·1 cm. BMI was calculated as body weight in kilograms per square of the height in metres (kg/m^2) (Table 1). Total fat mass was measured using dual-energy X-ray absorptiometry (DXA, Delphi A-QDR; Pediatric Whole Body version 12.4:3; Hologic Inc.) (Table 1).

Skeletal muscle volume measured by MRI

The total body SMV was measured using a General Electric Signa EXCITE VI 1.5 T scanner (General Electric). A T1-weighted spin-echo, axial-plane sequence was performed with repetition time of 500 ms during breath-holding scans and normal-breathing scans and echo time of 13·1 ms. The subjects rested quietly in the magnet bore in the supine position, with their hands placed on their abdomen. For each subject, contiguous transverse images with slice thicknesses of 1·0 cm (interslice gap, 0 cm) were obtained from the first cervical vertebrae to the malleolus lateralis. Approximately five sets of acquisitions were obtained, extending from the first cervical vertebrae to the femoral head, while holding their breath (approximately 20 s/set). The other sets of acquisitions were obtained from the femoral head to the ankle joints during normal breathing[4]. All the images (approximately 100–150 slices/subject) were traced by a highly trained technician, from the SM segment, excluding the connective tissue, blood vessels, fat tissue and abdominal organs. MRI were analysed by ZedView software (LEXI Co. Ltd) for segmentation and calculation of cross-sectional tissue areas.

SMV was calculated by the sum of the cross-sectional area (cm^2), which was determined by tracing the images, and then multiplying the cross-sectional area with the slice thickness (cm). The estimated coefficient of validation (CV) for SMV measurements from a test–retest analysis was determined to be 2 %[4]. The SMV was also separated into discrete regions using anatomical landmarks that were visible in the scanned images: arm, from the axillary fossa to the styloid process of the radius; trunk, from the first cervical vertebra to the femoral neck; thigh, from the femoral neck to the articular surface of the medial condyle; and lower leg, from the articular surface of the medial condyle to the malleolus lateralis.

Predicted skeletal muscle volume by ultrasonography

Muscle thickness measured by B-mode ultrasonography was scanned using a real-time linear electronic scanner with a 5-MHz scanning head (SSD-1000; Aloka). The scanning head was covered with a water-soluble transmission gel that provided acoustic contact, without causing a depression on the

Table 1. Subject characteristics and muscle thickness measured by ultrasonography (Mean values and standard deviations)

	Development				Validation			
	Boys (*n* 60)		Girls (*n* 37)		Boys (*n* 29)		Girls (*n* 19)	
Prediction model	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	9	2	9	2	9	2	9	2
Standing height (m)	1·37	0·11	1·33	0·11	1·36	0·09	1·34	0·12
Body mass (kg)	33·3	9·3	31·1	8·8	33·9	10·0	31·7	9·3
BMI (kg/m^2)	17·4	2·8	17·1	2·7	18·0	3·5	17·4	3·1
Fat (%)	23·8	8·2	28·1	6·7	25·1	9·1	28·4	7·4
Muscle thickness (cm)								
Lateral forearm	1·5	0·3	1·3	0·2	1·5	0·2	1·3	0·2
Anterior upper arm	1·9	0·3	1·7	0·2	1·9	0·3	1·7	0·4
Posterior upper arm	1·9	0·5	1·8	0·4	2·0	0·5	1·9	0·5
Abdomen	0·8	0·2	0·8	0·2	0·8	0·1	0·8	0·2
Subscapular	1·6	0·4	1·5	0·5	1·5	0·4	1·3	0·4
Anterior thigh	3·7	0·5	3·7	0·6	3·8	0·6	3·6	0·6
Posterior thigh	4·4	0·6	4·2	0·5	4·4	0·6	4·1	0·7
Anterior lower leg	2·0	0·3	2·0	0·2	2·1	0·3	1·9	0·5
Posterior lower leg	5·0	0·5	4·7	0·6	5·0	0·6	4·8	0·6

skin surface. The scanner was placed perpendicular to the tissue interface at previously marked sites. Muscle thicknesses were obtained at nine sites from the anterior and posterior surfaces of the body, as previously described[6]. The sites included the lateral forearm, anterior and posterior upper arm, abdomen, subscapular, anterior and posterior thigh and anterior and posterior lower leg. The nine anatomical landmarks for the selected sites were defined as follows: 'lateral forearm' is located on the anterior surface, 30 % proximally between the styloid process of the wrist and the head of the radius, near the elbow; 'anterior upper arm' and 'posterior upper arm' are located on the anterior and posterior surfaces of the upper arm, 60 % distal between the lateral epicondyle of the humerus near the elbow and the acromial process of the scapula at the shoulder; 'abdomen' is located 2–3 cm lateral to the umbilicus on the right-hand side; 'subscapula' is at a distance of 5 cm directly below the inferior angle of the scapula; 'anterior thigh' and 'posterior thigh' are located on the anterior and posterior surfaces of the upper leg, midway between the lateral condyle of the femur near the knee and the greater trochanter at the hip; and 'anterior lower leg' and 'posterior lower leg' are located on the anterior and posterior surfaces of the lower leg, 30 % proximally between the lateral malleolus of the fibula near the ankle and the lateral condyle of the tibia near the knee. Muscle thickness, measured directly from the screen using callipers, was considered to be the distance from the adipose tissue–muscle interface to the muscle–bone interface. The reliability of image reconstruction and distance measurements was confirmed by comparing the ultrasonic and manual measurements of tissue thicknesses in human cadavers. For cadaveric studies, the CV from test–retest analyses was approximately 1 %[10].

Based on previous research that developed regression-based prediction equations for the estimation of SM mass using ultrasonography in adults[7], the parameters of the ultrasonography-predicted equations for SMV in the present study were determined as muscle thickness in centimetres (cm) × standing height in metres(m). The following calculations were used: 'arm' = lateral forearm + anterior and posterior upper arm muscle thicknesses; 'trunk' = abdomen + subscapular muscle thicknesses; 'thigh' = anterior and posterior thigh muscle thickness; 'lower leg' = anterior and posterior lower leg muscle thickness; 'total' = 'arm' + 'trunk' + 'thigh' + 'lower leg'.

Statistics

All the results are presented as mean and standard deviations. For all the boys and girls, Lin's concordance correlation coefficient (CCC) between the SMV measured by MRI and predicted by ultrasonography in total and each region are calculated. The difference between the measured SMV and the predicted SMV was examined using paired *t* tests. The agreement between the measured and predicted values of SMV was further examined by plotting the differences in the predicted values against the means with the limits of agreement (mean difference ± 2 SD of the differences: 95 % limits of agreement, which gives an indication of the precision of the method), as suggested by Bland & Altman[11]. Statistical analyses were performed using SPSS for Windows (IBM SPSS version 22.0; SPSS Inc.) and MedCalc (version 15.4; MedCalc Software bvba). Differences were regarded as significant when the *P* value was <0·05.

Results

The physical characteristics and ultrasonography measurements of muscle thickness are summarised in Table 1. The mean height and weight values were comparable with the physical fitness standards of the Japanese people[12], indicating that the volume and distribution of SM in the subjects of the present study are representative of those in Japanese prepubertal children.

Strong significant correlations were observed between the site-matched SMV (total, arms, trunk, thigh and lower legs) measured by MRI and the muscle thickness × height measures obtained by ultrasonography in the model development group for both boys and girls (R^2_{adj} 0·57–0·93, P<0·01, standard error of the estimate (SEE) = 89–731 cm^3; Table 2; Fig. 1).

When these SMV prediction equations were applied to the validation groups, the measured total and regional SMV were very similar to the predicted values for both boys and girls (Table 3). The results of the Bland–Altman analysis for the

Table 2. Predictive equations for total body and regional skeletal muscle volume (SMV) measured by MRI from muscle thickness (MTH) using B-mode ultrasonography

SMV (cm^3)	Equation	R^2_{adj}	SEE
Boys (*n* 60)			
Total	$SMV_{MRI} = 384{\cdot}96 \times (MTH_{ultrasonography} \times Ht) - 3662{\cdot}10$	0·93	659
Arm	$SMV_{MRI} = 127{\cdot}09 \times (MTH_{ultrasonography} \times Ht) - 76{\cdot}44$	0·71	124
Trunk	$SMV_{MRI} = 992{\cdot}53 \times (MTH_{ultrasonography} \times Ht) + 363{\cdot}69$	0·65	565
Thigh	$SMV_{MRI} = 463{\cdot}47 \times (MTH_{ultrasonography} \times Ht) - 1624{\cdot}30$	0·84	419
Lower leg	$SMV_{MRI} = 176{\cdot}10 \times (MTH_{ultrasonography} \times Ht) - 539{\cdot}29$	0·92	91
Girls (*n* 37)			
Total	$SMV_{MRI} = 364{\cdot}87 \times (MTH_{ultrasonography} \times Ht) - 3523{\cdot}00$	0·89	731
Arm	$SMV_{MRI} = 132{\cdot}68 \times (MTH_{ultrasonography} \times Ht) - 139{\cdot}40$	0·80	89
Trunk	$SMV_{MRI} = 658{\cdot}79 \times (MTH_{ultrasonography} \times Ht) + 935{\cdot}72$	0·57	561
Thigh	$SMV_{MRI} = 425{\cdot}40 \times (MTH_{ultrasonography} \times Ht) - 1506{\cdot}70$	0·90	286
Lower leg	$SMV_{MRI} = 166{\cdot}19 \times (MTH_{ultrasonography} \times Ht) - 439{\cdot}17$	0·88	103

SEE, standard error of the estimate; total, arm MTH + trunk MTH + thigh MTH + lower leg MTH; SMV_{MRI}, predicted MRI SMV; $MTH_{ultrasonography}$, MHT measured by ultrasonography in centimetres (cm); Ht, height in metres (m); arm, lateral forearm MTH + anterior and posterior upper arm MTH; trunk, abdomen MTH + subscapular MTH; thigh, anterior and posterior thigh MTH; lower leg, anterior and posterior lower leg MTH.

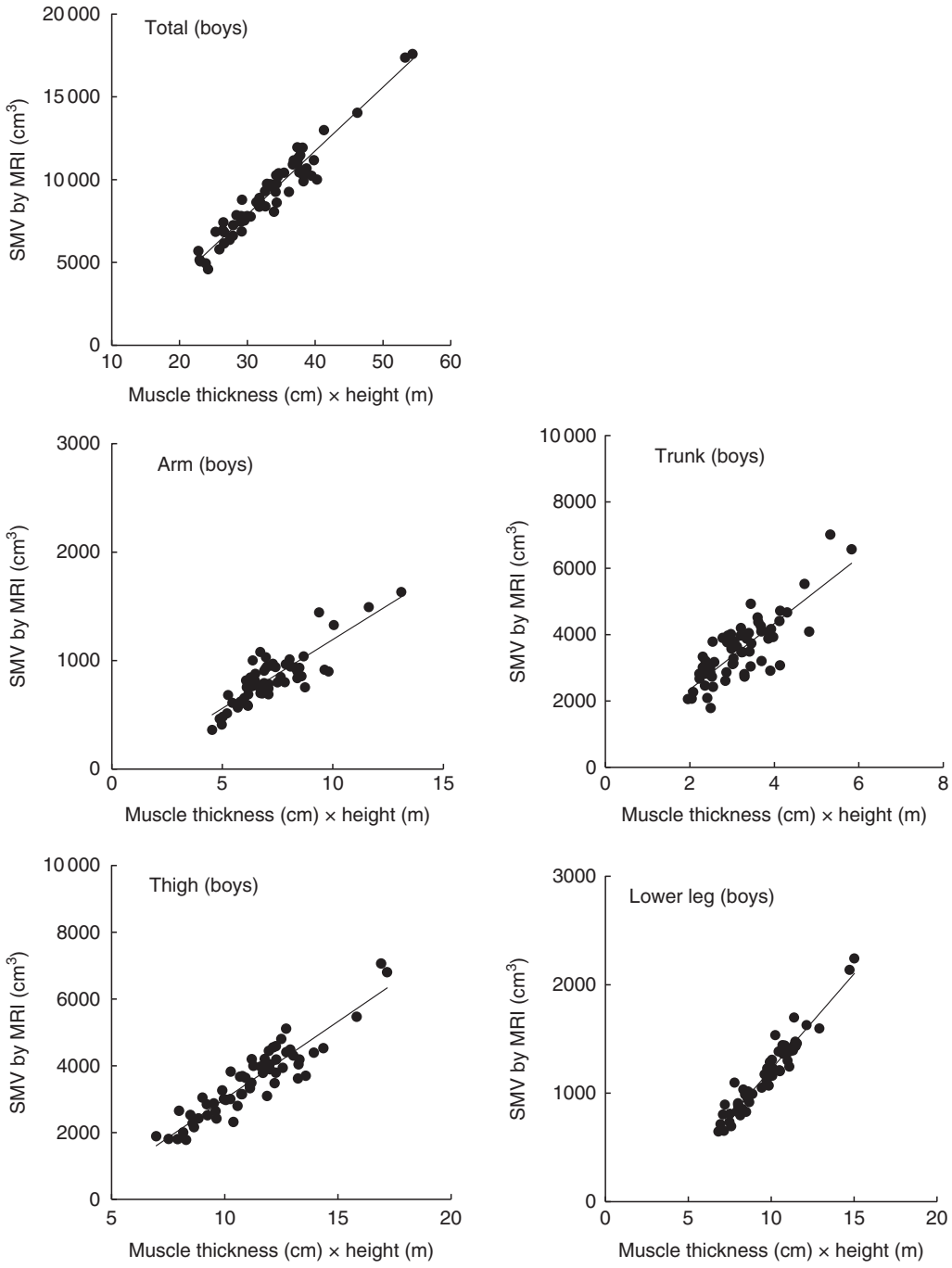

Fig. 1. *(Continued on following page)*

validation development group did not indicate any bias for either boys or girls, with the exception of the trunk region in girls (r –0·98, P<0·01; Fig. 2).

Discussion

In the past 15 years, several attempts have been made to develop prediction equations for the estimation of SM mass in children. In 2005, Poortmans *et al.*[13] reported that the determination of total body SM mass in children and adolescents can be validated with satisfactory confidence by simple anthropometric measurements or assessment of 24-h urine creatinine excretion. Although this was the first study designed to establish formulae for total body SM mass in children and adolescents on the basis of two measurements, it had the following limitations: the small sample size (n 39; aged 7–16 years), lack of a validation study and the use of adult DXA

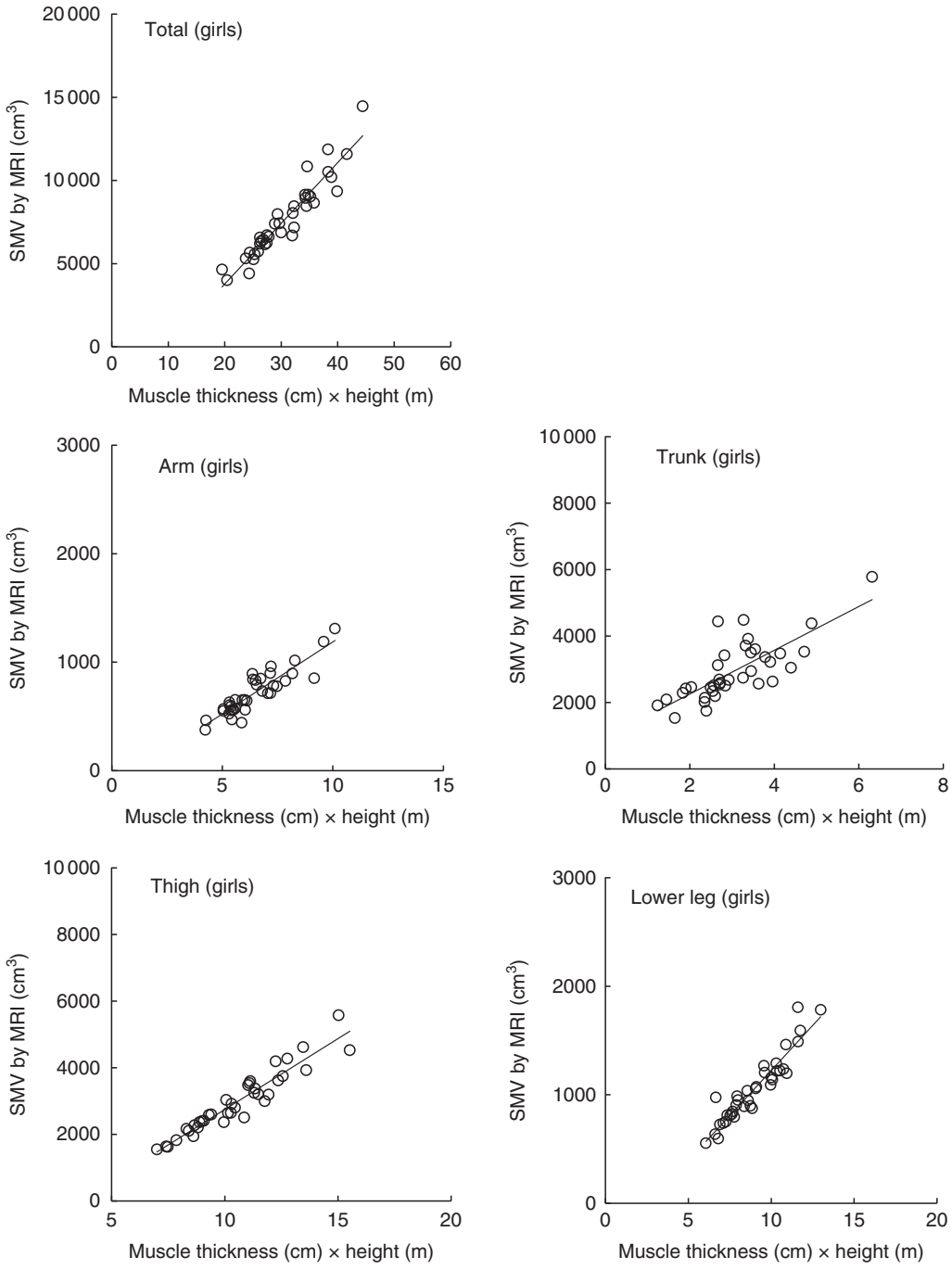

Fig. 1. *(Continued from previous page)* Relationship between the skeletal muscle volume (SMV) measured by MRI and the muscle thickness × height measured by ultrasonography: ●, boys (*n* 60); ○, girls (*n* 37).

equations for SM mass as reference data. Prediction equations for total SM mass specific to children, using MRI measurements as the reference data, have been previously reported by Kim *et al.*[2]. However, these equations were also developed using a small sample size (*n* 65; thirty-six boys and twenty-nine girls, aged 5–14 years) and validated in only eighteen subjects (ten boys and eight girls). In the present study, we developed, for the first time, to our knowledge, ultrasonography-derived prediction equations for boys and girls by using a larger sample and validation group (model development group, sixty boys and thirty-seven girls; validation group, twenty-nine boys and nineteen girls; aged 6–12 years) and MRI data as reference. Thus, we avoided some of the limitations of previous studies that attempted to estimate total body SMV in prepubertal children. Furthermore, the development of ultrasonography-derived prediction equations for the estimation of regional (arm, trunk, thigh and lower leg) SMV is important for future development and expansion of maturation research.

Our prediction equations for total body SMV had a high R^2_{adj} value (boys, 0·93; girls, 0·89) and a moderate SEE for both boys

Table 3. The measured and predicted skeletal muscle volume (SMV) in total body and regional segments for validation in boys and girls (Mean values and standard deviations)

	Boys (*n* 29)									Girls (*n* 19)								
	Measured		Predicted		Mean difference*					Measured		Predicted		Mean difference*				
SMV (cm^3)	Mean	SD	Mean	SD	Mean	SD	*P*†	*d*	CCC	Mean	SD	Mean	SD	Mean	SD	*P*†	*d*	CCC
Total	8942	2841	9113	2241	171	764	0·24	0·07	0·94	7804	2461	7688	2339	−117	607	0·41	0·05	0·97
Arm	825	194	851	198	27	100	0·16	0·14	0·86	719	232	743	208	25	85	0·22	0·11	0·92
Trunk	3453	780	3495	795	42	398	0·58	0·05	0·87	2982	929	2798	519	−183	529	0·15	0·24	0·73
Thigh	3484	986	3579	1026	94	457	0·28	0·09	0·89	3030	1015	2905	905	−125	289	0·08	0·13	0·95
Lower leg	1180	323	1164	295	−16	97	0·37	0·05	0·95	1074	346	1084	344	9	149	0·79	0·03	0·91

d, Cohen's *d*; CCC, Lin's concordance correlation coefficient between measured and predicted SMV.
* Mean difference: calculated as (predicted – measured SMV).
† *P* value for paired *t* tests: measured *v.* predicted SMV.

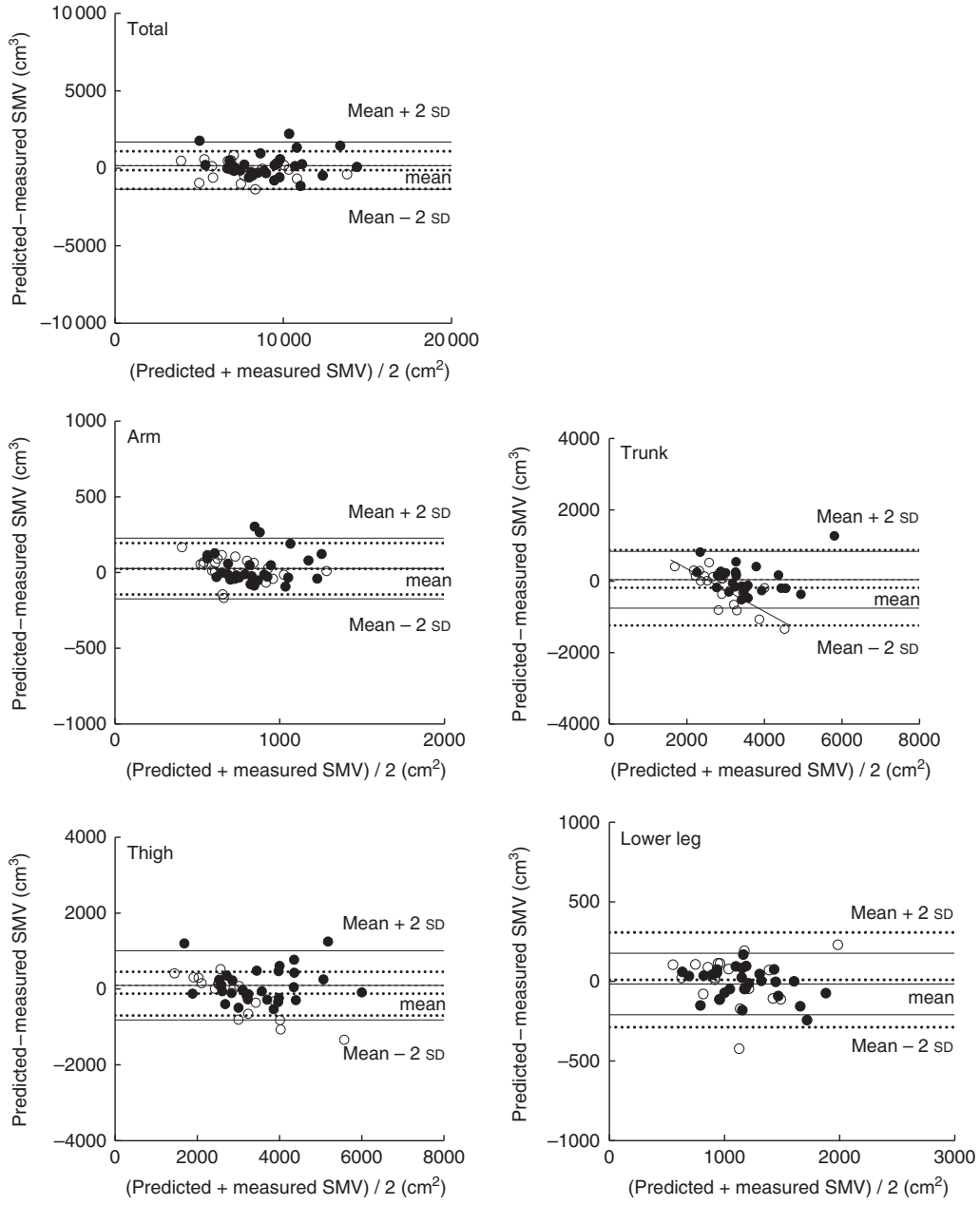

Fig. 2. Bland–Altman analysis for the validation group. ●, boys (*n* 29); ○, girls (*n* 19). Mean difference ± 2 SD: solid line, boys; dotted line, girls.

(659 cm^3, 7·2 % of the mean measured SMV for the model development group) and girls (731 cm^3, 9·5 % of the mean measured SMV for the model development group). The R^2 was low and SEE value was high in the present study compared with the respective values obtained in a previous study, which used DXA to predict SM mass in children (R^2 value, 0·98; SEE, 0·565 kg, approximately 5 % of the mean measured SM mass)[2]. However, the prediction model in the present study yielded a similar R^2 value and a low SEE, compared with the corresponding values yielded by the ultrasonography-derived prediction equations for the estimation of total and regional SM mass and volume in adults[7]. Based on the estimation accuracy and the ease of obtaining measurements, ultrasonography-derived prediction in prepubertal children has a great potential as a technique for the assessment of total and regional SMV, especially in field settings.

In the research setting and clinical settings, ultrasonography-derived equations may be necessary for both prepubertal children and adults. According to the previous study, the increase in the SMV and mass is a key factor in deciding whether child or adult SM volume and mass equations are applicable[5]. In a previous study that estimated total SM mass using MRI, the SM mass:standing height ratio for prepubertal children (index of SM maturation; boys, 7·0 kg/m; girls, 7·7 kg/m) differed from that of adolescents (boys, 12·1 kg/m; girls, 9·4 kg/m)[5] and adults (men, 13·0 kg/m; women, 8·4 kg/m)[4]. Moreover, the SM prediction model for adults might only be applicable in adolescents aged approximately 14 years, over Tanner stage 2, and at peak height velocity[5]. In the present study, the ratio for prepubertal children aged 6–12 years (boys, 6·9 kg/m; girls, 6·1 kg/m, calculated from the assumed density of 1·041 g/cm^3 for SM in the validation group[14]) did not approach the previously reported value for adolescents and adults. The present results and previous findings indicate that the use of ultrasonography-derived equations in prepubertal children and adults depends on age and Tanner stage.

A number of limitations of the present study need to be addressed. First, these equations were developed for Japanese children and may not apply to children from other countries; a similar limitation related to race was also acknowledged by Kim *et al.*[2]. Second, the results of the present study indicate that the ultrasonography-derived prediction equation for girls for the trunk region resulted in a rather high Cohen's *d*, low CCC and an underestimation in children with a larger SMV; therefore, this bias needs to be considered during application of these equations. Third, ultrasonography-derived prediction equations were suitable for total SMV at the individual level, but the rather high degree of variability for regional SMV suggested limited applicability at the individual level. Further work is needed to improve the accuracy of the prediction equations.

The results of this study indicate that ultrasonography-derived prediction equations are useful for the estimation of total and regional SMV in prepubertal boys and girls. Our previously developed prediction equations for total and regional fat mass in children using B-mode ultrasonography[15] have enabled concurrent estimation of total and regional SMV and fat mass in a single assessment.

Acknowledgements

The authors sincerely thank the subjects who participated in this study and their guardians.

This study was supported in part by The Ministry of Education, Science, Sports and Culture of Japan (grant no. 18800054, 22700623 and 24680069).

The contribution of each author to the manuscript was as follows: T. M. designed and conducted the research, analysed the data and wrote the paper; M. O., Y. H., S. T. and S. S. conducted the research.

None of the authors has any conflicts of interest.

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Appendix

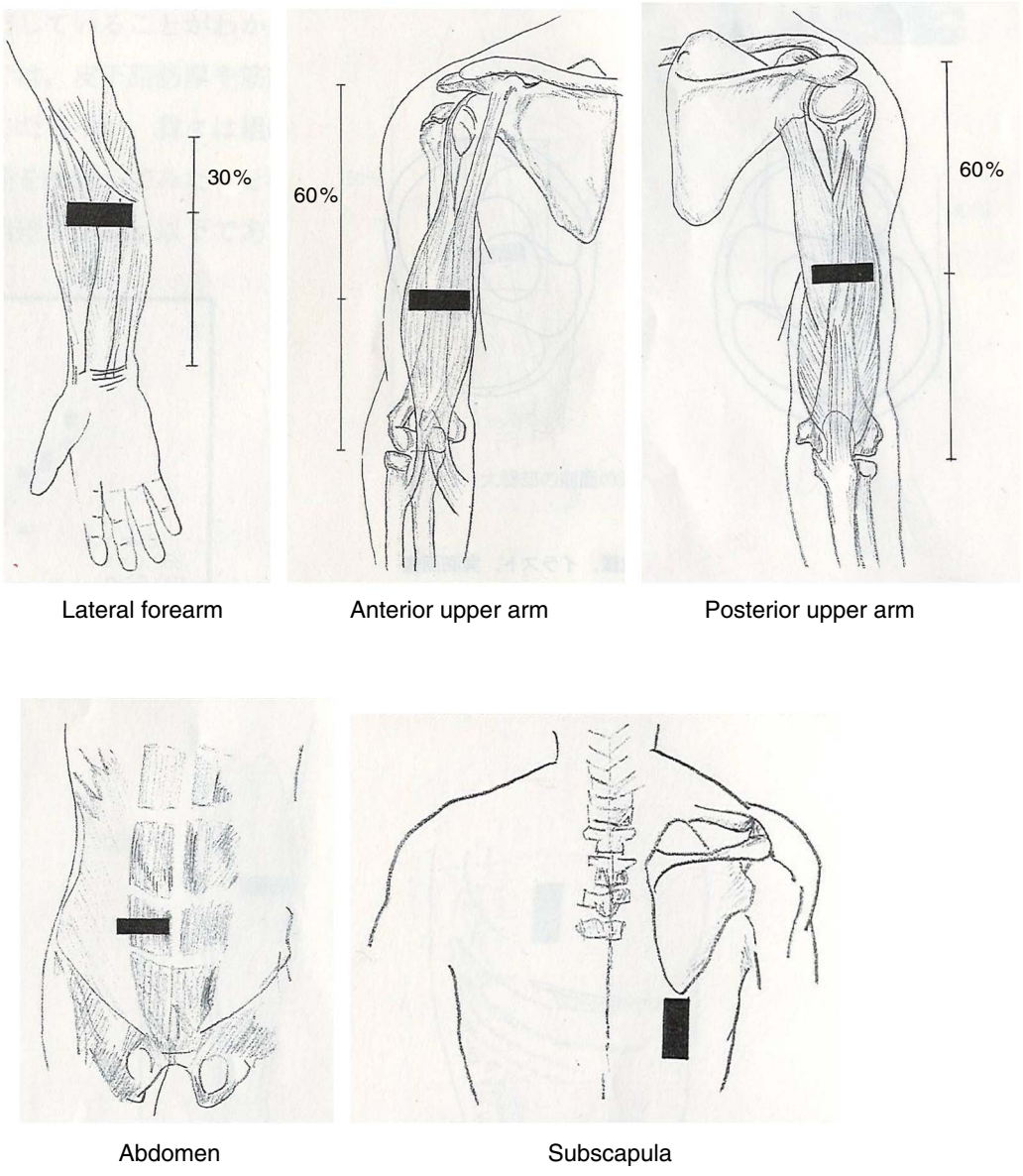
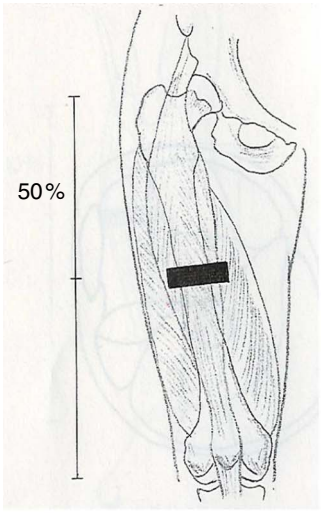
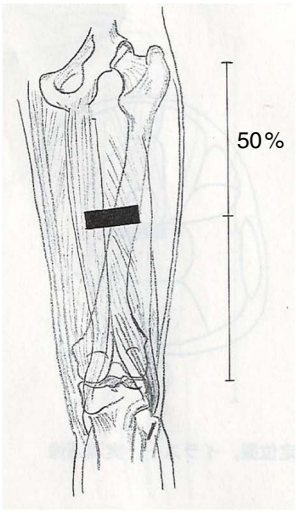


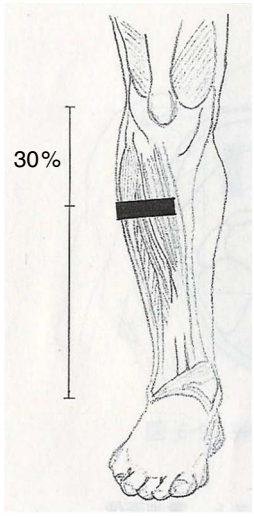
Fig. A1. The nine anatomical landmarks measured by B-mode ultrasonography (reprint permitted by Kyorin-syoin).



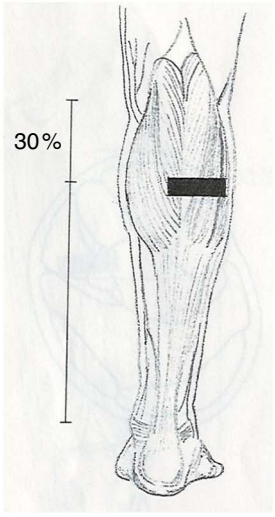
Anterior thigh



Posterior thigh



Anterior lower leg



Posterior lower leg

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