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ORIGINAL ARTICLE

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Validity of ultrasonograph muscle thickness measurements for estimating muscle volume of knee extensors in humans

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Abstract This study aimed to investigate the suitability of using ultrasonograph muscle thickness (MT) measurements to estimate the muscle volume (MV) of the quadriceps femoris as an alternative approach to magnetic resonance imaging (MRI). The subjects were 46 men aged from 20 to 70 years who were randomly allocated to either a validation or a cross-validation group. In the validation group, multiple and simple regression equations, which used a set of MT values determined at mid-thigh and thigh length (l) and the product of π, $(MT/2)^2$, and l $[\pi\cdot(MT/2)^2\cdot l]$, respectively, as independent variables, were derived to estimate the MV measured by MRI. Because the two equations were cross-validated, the data from the two groups were pooled to generate the final prediction equations: MV $(cm^3) = (MT \times 311.732) + (l \times 53.346) - 2058.529$ as the multiple regression equation and MV $(cm^3) = [\pi\cdot(MT/2)^2\cdot l] \times 1.1176 + 663.040$ as the simple regression equation. In the multiple regression equation, MT explained 75% of the variation in the MV measured by MRI. The r^2 and the standard error of the estimate (SEE) of the equations were 0.824 and 175.6 cm^3 (10.6%), respectively, for the multiple regression equation and 0.829 and 173.7 cm^3 (10.5%), respectively, for the simple regression equation. Thus, the present results indicate that ultrasonograph MT measurements at mid-thigh are useful for estimating the MV of knee extensors. However, the observed SEE values suggest that the prediction equation obtained in this study may be limited to population studies rather than individual assessments in longitudinal studies.

Keywords Brightness mode ultrasound · Magnetic resonance imaging · Quadriceps femoris muscle · Field research

Introduction

There is a general consensus that aging has a greater effect on the musculature of the lower limbs than on that of the upper limbs (Bemben et al. 1991). For elderly people, muscle function of the lower limb is significantly correlated with performance estimates such as rising from a chair, stair climbing and walking (Bassey et al. 1992), and its reduction increases the risk of injury per fall (Whipple et al. 1987). The strength of the knee extensor muscle is a predictor of dependency and survival (Frontera et al. 2000), and so quantifying the amount of this muscle is important when investigating the effects of aging on muscle function and physical performance.

At present, computerized axial tomography (CT) and magnetic resonance imaging (MRI) of multiple sections of the body are widely used to measure skeletal muscle volume (MV). However, these methods are costly and time consuming, and are not applicable in field research on large numbers of subjects. We recently found that the MV of the upper arm, estimated from muscle thickness measurements using brightness mode (B-mode) ultrasonography, was highly correlated to the value measured by MRI (Miyatani et al. 2000). The technique of B-mode ultrasonography has the same advantages as a CT scan or MRI in making visible fat and muscle tissues without compression and radiation exposure, and is more suitable for field use and serial evaluation (Ishida et al. 1995). However, it is unknown whether the

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measurement of muscle thickness (MT) at a single site is useful for predicting the MV of a specific muscle group located in lower limbs.

In the present study, we tried to validate the use of ultrasonograph MT measurements for estimating the MV of knee extensors by comparing them with the data obtained from MRI multiple scans.

Methods

Subjects

A group of 46 healthy men aged 20 to 70 years were randomly allocated to either a validation ($n=23$) or a cross-validation ($n=23$) group. All were physically active to a moderate degree, but had not participated in any organized programmes of regular exercise for at least 1 year before the tests. Moreover, none was or had been an athlete, or was using a stick or other walking aid. The subjects were fully informed about the procedures to be used as well as the purpose of the study. Written informed consent was obtained from all the subjects.

MT measurements by ultrasonography

The thickness of the quadriceps femoris muscle at a single site on the right side was determined using a B-mode ultrasound apparatus (SSD-2000, Aloka, Japan) with a linear scanner (scanning frequency 7.5 MHz). The site was at the midpoint of the thigh, which was the distance from the greater trochanter of the femur to the articular cleft between the femur and tibiacondyles. The subjects stood with their arms and legs relaxed in an extended position during the measurement. A transducer with a 7.5 MHz scanning head was placed on the anterior thigh so as to be perpendicular to the tissue and underlying femur. The scanning head was coated with a water-soluble transmission gel, which provided acoustic contact without depressing the dermal surface. The subcutaneous adipose tissue-muscle and muscle-bone interfaces were identified from the ultrasound image, and the distance from the adipose tissue-muscle interface to the muscle-bone interface was defined as the MT. The muscles involved in MT measurement were the rectus femoris and vastus intermedius. The accuracy of MT measurements using this ultrasound technique had been established in an earlier study (Miyatani et al. 2000) by imaging a human cadaver. The repeatability for the MT measurement was assessed on 2 separate days in a pilot study on 10 adult men. The intraclass correlation coefficient for the test-retest of MT measurements was $r=0.972$. The coefficient of repeatability (Bland and Altman 1986) was 3.2 mm.

MV measurement by MRI

Cross-sectional images of the right thigh were obtained by MRI using a body coil (Signa 1.5T, GE Electronics, USA). Spin-echo, multi slice sequences with a slice thickness of 10 mm were used with a repetition of 900 ms and an echo time of 17 ms. Each subject lay supine in the body coil with his arms or legs extended and relaxed. Transverse scans were carried out with an interplaced gap of 0 mm, from the knee joint to the head of the femur. From each cross-sectional image of the thigh, an outline of the quadriceps femoris muscle was traced, and the trace was digitized using a personal computer (Power Machintosh 7500/100, Apple, Japan), and the anatomical cross-sectional area (CSA) was calculated. Subcutaneous adipose tissue and tendinous tissue, which were imaged in different tones from the muscle tissue, were excluded when digitizing. By summing the anatomical CSA of the muscle along its length and then multiplying the sum by the interval of 10 mm, the MV of the quadriceps femoris was determined as being that of the knee extensor muscle.

Data analyses

Two approaches were used to develop equations for predicting MV on the assumption that limb muscle has a uniform MT and CSA along the length of the limb. One was multiple regression analysis using MT and the thigh length (l, the distance from the greater trochanter of the femur to the articular cleft between the femur and tibiacondyles) as independent variables for estimating the measured MV. The other was a simple regression analysis for the relationship between the product of $\pi(=3.14159)$, $(\mathrm{MT}/2)^2$ and l[$(\pi\cdot(\mathrm{MT}/2)^2\cdot l$] and the measured MV. In the validation group, firstly, it was confirmed that the regression slope and intercept in the relationship between the estimated and measured MV values did not significantly differ from 1 and 0, respectively. Secondly, the predicted values of MV were calculated for individuals in the cross-validation group using the two equations derived for the validation group. When the equation was cross-validated, the data from the two groups were pooled to generate the final prediction equation. The standard error of the estimate (SEE) of the regression equation was calculated to evaluate the accuracy of the estimate by the equation obtained in each condition. The SEE was expressed as an absolute value and relative to the mean of the MV measured by MRI. In addition, the difference between the measured and estimated values (MRI minus ultrasonography) was also plotted against the mean MV of the two methods to examine for systematic differences, as described by Bland and Altman (1986).

Descriptive data have been presented as means and standard deviations (SD). The difference between mean values of a single estimate was tested for significance using a paired Student's t-test. Simple linear regression analysis was used to calculate the correlation coefficient (r). The probability level for statistical significance was set at $P<0.05$.

Results

Baseline characteristics

There were no significant differences between the validation and cross-validation groups in the age, anthropometric, MT and MRI measurements (Table 1).

Table 1 Baseline characteristics of validation and cross-validation groups [mean (SD)]

Variables	Validation group ($n=23$)	Cross-validation group ($n=23$)
Age (years)	37.2 (19.15)	36.0 (18.53)
Height (cm)	168.2 (7.59)	168.9 (8.14)
Body mass (kg)	67.3 (14.81)	68.8 (11.37)
Thigh length (cm)	38.8 (2.46)	38.8 (2.24)
Muscle thickness (cm)	5.3 (1.01)	5.3 (1.04)
Muscle volume measured by magnetic resonance imaging (cm^3)	1,679.0 (469.20)	1,637.5 (383.51)

Prediction equation derived from the validation group

Multiple regression analysis revealed that MT ($r^2 = 0.783$) and l ($r^2 = 0.393$) were significant ($P < 0.05$) contributors to the prediction of the MV measured by MRI. The multiple regression equation obtained in the validation group was the following:

$$MV\ (cm^3) = (MT \times 361.733) + (l \times 37.271) - 1687.216$$

where MT and l are in centimeters. In this equation, r^2 and SEE were 0.808 and 200.4 cm^3 (11.9%), respectively (Fig. 1 A). Simple regression analysis showed a correlation coefficient of 0.924 ($P < 0.05$) between $[\pi \cdot (MT/2)^2 \cdot l]$ and the measured MV and produced the equation: $MV\ (cm^3) = [\pi \cdot (MT/2)^2 \cdot l] \times 1.217 + 585.770$, with an r^2 and SEE of 0.854 and 175.3 cm^3 (10.4%), respectively (Fig. 1 B). In the two cases, analyses indicated that the slopes and intercepts in the regression equation between the measured and estimated MV values were not different significantly from 1 and 0, respectively.

Cross-validation of the prediction equation

The two equations derived from the validation group were used to estimate MV in the cross-validation group. In the cross-validation group, too, the slope and intercept of the regression equation between the measured and estimated MV values calculated from each of the multiple (Fig. 2 A) and simple (Fig. 2 B) regression equations were not significantly different from 1 and 0, respectively. Moreover, there were no significant differences between the slope and intercept values of regression lines for the validation and cross-validation groups in the relationship between the estimated and measured MV values for each equation. Hence, we pooled the data from the two groups to generate the final equations: $MV\ (cm^3) = (MT \times 311.732) + (l \times 53.346) - 2058.529$ as the multiple regression equation and $MV\ (cm^3) = [\pi \cdot (MT/2)^2 \cdot l] \times 1.176 + 663.040$ as the simple regression equation. In the final equation, too, analysis revealed that MT ($r^2 = 0.754$) and l ($r^2 = 0.356$) were significant ($P < 0.05$) contributors to the prediction of the MV measured by MRI. In addition, the correlation coefficient between $[\pi \cdot (MT/2)^2 \cdot l]$ and the measured MV was 0.910 ($P < 0.05$). The r^2 and SEE were 0.824 and 175.6 cm^3 (10.6%), respectively, for the multiple regression equation (Fig. 3 A), and 0.829 and 173.7 cm^3 (10.5%), respectively, for the simple regression equation (Fig. 3 B). In each condition, analysis indicated that the slope and intercept were not significantly different from 1 and 0, respectively.

The mean estimated MV derived from the multiple [1660.7 (386.2) cm^3] and simple regression [1658.5 (382.3) cm^3] equations did not significantly differ between the two equations, and from the measured MV [1658.3 (424.2) cm^3]. Moreover, the plots of Bland-Altman (1986)(Fig. 4 A, B) did not show a significant correlation between the mean of the estimated and measured MV values and the difference between the two variables.

Discussion

The main finding of this study was that MT explained 75% of the variation in the MV measured by MRI. No information on how the thickness of the quadriceps femoris muscle correlates with its volume is available

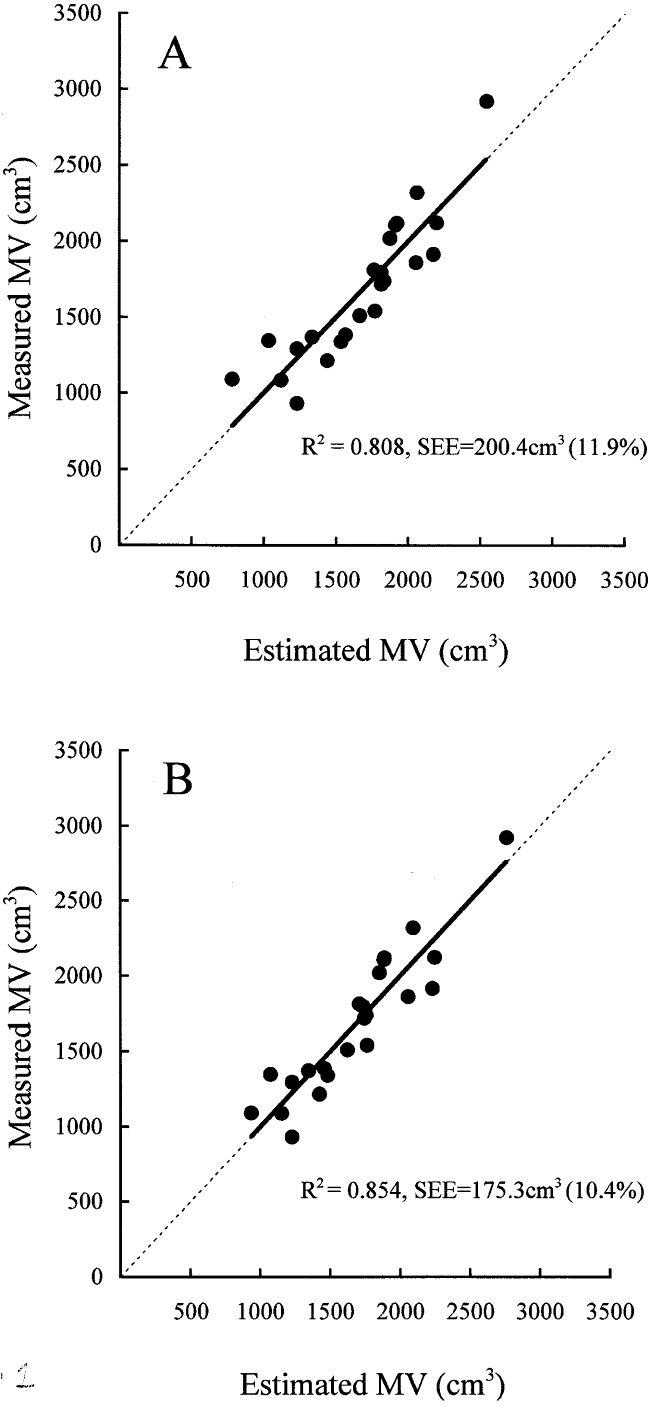

Fig. 1 Regressions between the muscle volume (*MV*) value estimated by either the multiple regression equation (**A**) or simple regression equation (**B**) and that measured by magnetic resonance imaging for the validation group. *Solid lines* regression lines, *dotted lines* lines of identity

from previous studies. The present result indicates that the MT determined at mid-thigh reflects the whole volume of the quadriceps femoris. In other words, this implies that ultrasonograph MT measurements at the mid-thigh are useful for estimating the total volume of the quadriceps femoris as an alternative approach to the use of MRI multiple scans.

The simple and multiple regression analyses resulted in equations with similar r^2 and SEE values for predicting MV, suggesting that the accuracy of the volume estimate is almost the same regardless of whether MT and CSA are taken as constant along the limb's length. From the findings of Abe et al. (1997), a high correlation ($r = 0.91$) exists between the muscle thickness and CSA of the quadriceps femoris, which were determined at the mid-thigh. If one examines the corresponding relationship in the present data, a significant correlation

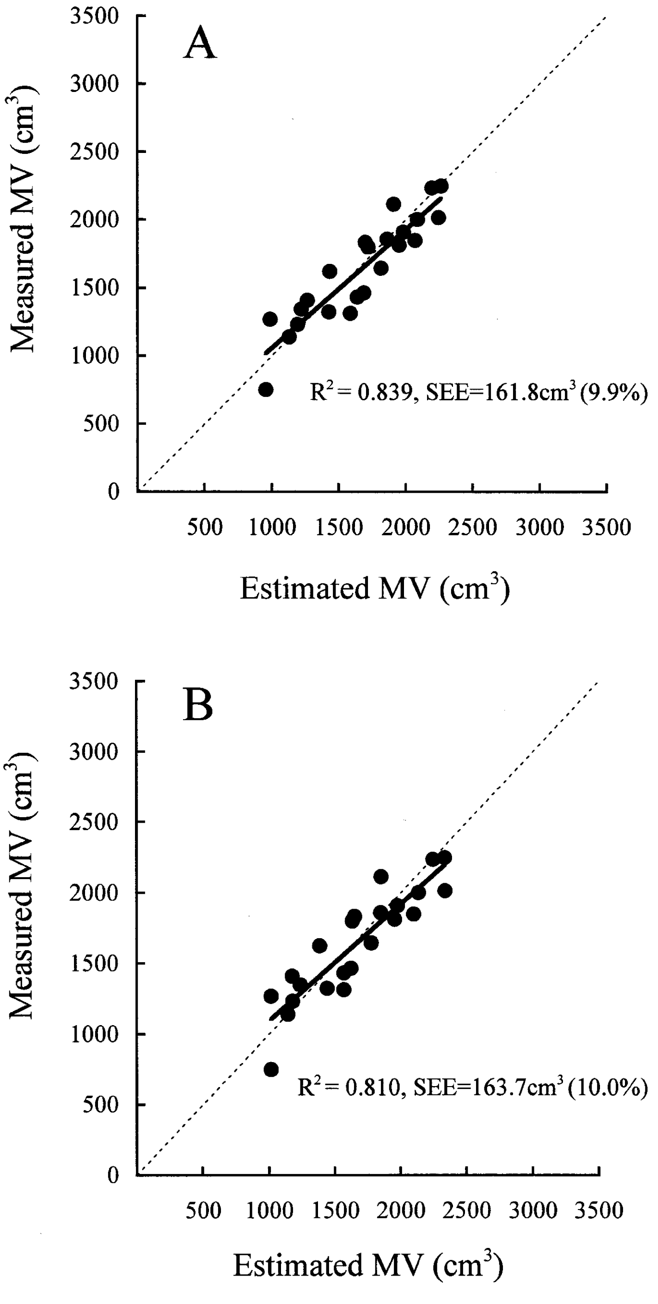

Fig. 2 Regressions between the muscle volume (*MV*) value estimated by either the multiple regression equation (**A**) or simple regression equation (**B**) and that measured by magnetic resonance imaging for the cross-validation group. *Solid lines* regression lines, *dotted lines* lines of identity

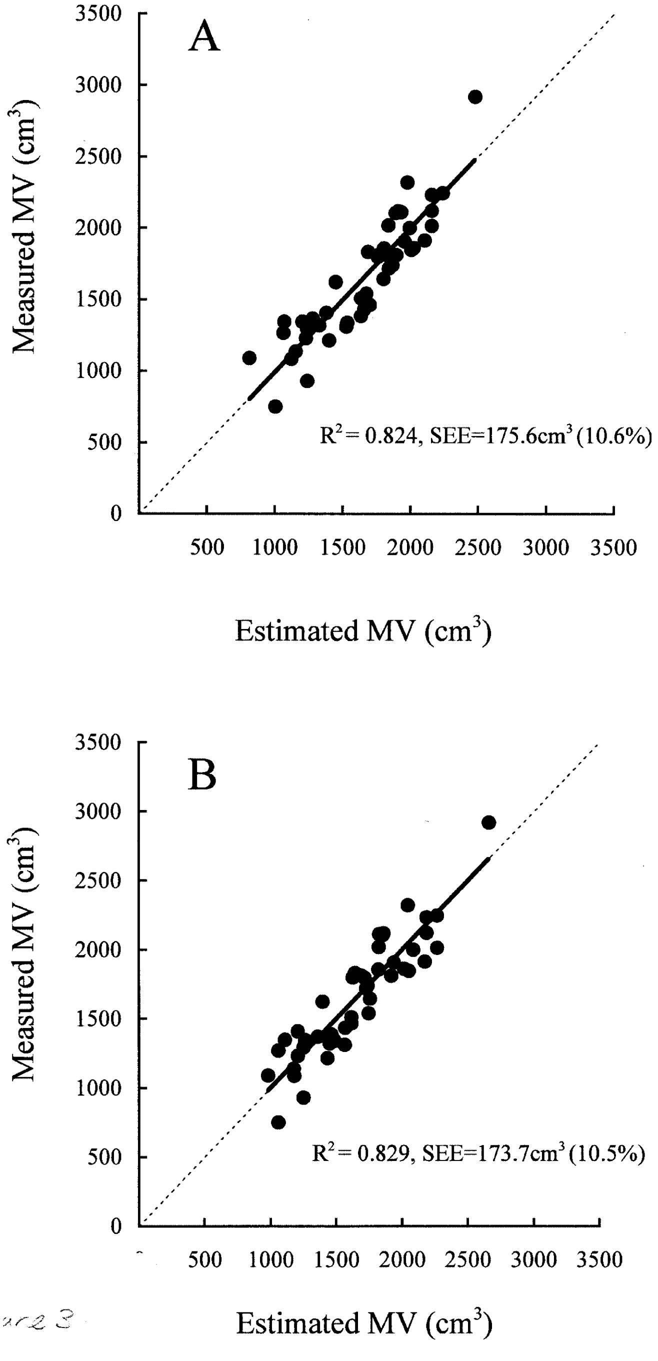

Fig. 3 Regressions between the muscle volume (*MV*) value estimated by either the multiple regression equation (**A**) or simple regression equation (**B**) and that measured by magnetic resonance imaging for the pooled data of validation and cross-validation groups. *Solid lines* regression lines, *dotted lines* lines of identity

($r = 0.881$, $P < 0.05$) is also observed. Hence, the similarities in the r^2 and SEE values between the two equations can be attributed to the linearity in the relationship between MT and CSA at the same level on the thigh.

Bland-Altman plots (Bland and Altman (1986) showed no significant systematic difference in the MV estimates obtained with the final equations, indicating that the estimates are independent of muscle size. However, we must draw attention to the observed SEE which corresponded to about 11% of the mean value of MV measured using MRI. According to the report of Akima et al. (2000), the change in the volume of knee extensors after 20 days of bed rest was 8%. On the other hand, hypertrophic changes in the quadriceps CSA, which have been observed in previous studies using short-term (8—10 weeks) resistance training, are less than 9% (Higibie et al. 1996; Hisaeda et al. 1996; Jones and Rutheford 1987; Narici et al. 1989). Judging from the magnitude of SEE, therefore, it seems that the equation derived in this study for predicting MV cannot account for hypertrophic and/or atrophic changes in the relatively short term.

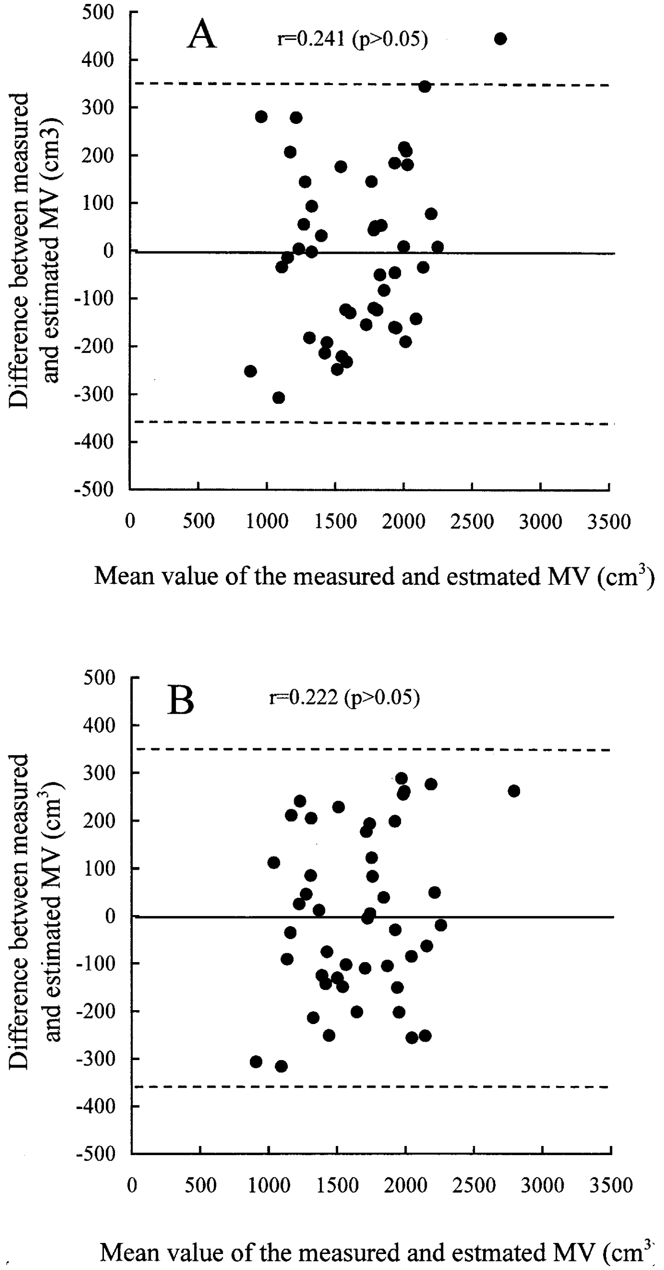

Fig. 4 Differences between the muscle volume (*MV*) measured by magnetic resonance imaging and that estimated by either the multiple regression equation (**A**) or simple regression equation (**B**) compared to the mean MV determined by the two methods. *Dashed lines* indicate the levels of mean ±2SD

Furthermore, the SEE in each equation was high compared to that reported previously for the total volume estimate of the upper arm muscle, 7% (Miyatani et al. 2000), which was derived from a simple regression analysis as used in this study. The reason for the greater SEE may be the individual variations in the shape of the quadriceps femoris and/or the size of each of the four constituents. The estimation of MV by ultrasonography was based on the assumption that limb muscle has a uniform MT or CSA along the length of the limb, but this is not true. Moreover, the changes induced by mass reduction (Katch and Hortobagyi 1990) or resistance training (Narici et al. 1989, 1996) in the CSA of a muscle vary from level to level on the limb. For example, Narici et al. (1996) reported that the gain in muscle CSA of the quadriceps femoris, produced by resistance training with knee extension exercise, is significantly different between and within the four constituents. Hence, it is reasonable to assume that the shape of the whole quadriceps femoris and/or size of the four constituents vary among individuals with the extent to which the quadriceps femoris is exercised during daily life. This implies a difficulty in predicting MV accurately from the MT determined at a single level on the limb. In addition, the muscles used for the thickness measurement were limited to the rectus femoris and vastus medialis. Akima et al. (2000), however, reported that the vastus lateralis had the largest volume among the four constituents of the quadriceps femoris. Therefore, the muscle groups used to measure MT will also influence the accuracy of the volume estimate even if the measurement level on the limb is the same.

Jones and Pearson (1969) have developed an anthropometric method for predicting the lean tissue (muscle-plus-bone) volume of the lower limb, in which the circumference and skinfold values at multiple levels were used to attenuate the influence of limb shape on the volume estimate. Overand et al. (1993) reported that the strongest predictive variable for the CT-measured quadriceps volume was muscle-plus-bone volume calculated from the circumference and skinfold values at five levels on the thigh, and its use resulted in prediction equations with r^2 and SEE values of 0.96% and 3%, respectively, for young men and 0.80% and 7%, respectively, for elderly men. Although their results have not been cross-validated, the observed SEE values are lower than those obtained in this study, suggesting that increasing the number of levels for MT measurements will improve the accuracy of the volume estimate. Further investigations of this point are needed.

In summary, the present results indicated that, as an alternative approach to using MRI multiple scans, ultrasonograph MT measurements are useful in estimating the volume of knee extensor muscles. However, the observed SEE values indicate that the prediction equations obtained in this study may be limited to population studies rather than individual assessments in longitudinal studies.

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