Prediction of Total Body Electrical Resistance based on Limb Muscle Thickness: comparison of Anthropometric and Ultrasound measurements

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Collaborations

This project and my graduate internship were possible because of several collaborations between the departments, organizations and people mentioned below.

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Last but not least, I want to thank my boyfriend, Bart, and family for supporting me.

Eline Boksem

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List of abbreviations

AER	Electrical Resistance of the Arm
AMA_{tsf}	Arm Muscle Area based on triceps skinfold
AMA_{us}	Arm Muscle Area based on ultrasound
BIA	Bioelectrical Impedance Analysis
BMI	Body Mass Index
CSA	Cross-Sectional Area
MFT	Mean Fat layer Thickness
LER	Electrical Resistance of the Leg
MMT_{arm}	Mean Muscle layer Thickness of the upper arm
$MMT_{arm+leg}$	Mean Muscle layer Thickness of the upper arm and leg
MMT_{leg}	Mean Muscle layer Thickness of the upper leg
SD	Standard deviation
SEE	Standard Estimated Error
TBER	Total Body Electrical Resistance
TBW	Total Body Water
US	Ultrasound

List of symbols

MBT	Mean Bone Thickness
MFT_{arm}	Mean Fat layer Thickness of the upper arm
FT_{biceps}	Fat layer Thickness of the biceps site of the upper arm
$FT_{triceps}$	Fat layer Thickness of the triceps site of the upper arm
H	Height
MAC	Mid-Arm Circumference
R	Resistance
r_{arm}	Radius mid-Arm
TSF	Triceps Skinfold
V	Volume
ρ	Specific resistance

Abstract

Introduction

In many different diseases the hydration state is disrupted. Recently, a new model has been proposed to assess hydration in man. In this model measured Total body electrical resistance (TBER) is compared to the individual's predicted normale TBER value (TBERnorm), which is inversely related to the arm muscle cross-sectional area (AMA) as assessed by conventional anthropometry. AMA is used to normalize the TBER for differences in muscle mass between subjects. However, its performance is limited in obese patients and it only corrects for the muscle mass in the arms.
Aim of the study: to investigate whether prediction of TBERnorm by AMA can be improved by ultrasound (US)-based parameters of muscularity.

Methods

Performance of the US and anthropometric methods was examined in 129 subjects (60 men and 69 women) ranging in age from 18 to 75 yrs and in BMI from 17.4 to 52.4 kg/m^2. Measurements were performed with a portable BIA device and US measurements were performed by B-mode ultrasonography. Muscularity of the arm and leg was calculated based on upper arm and leg circumferences and fat layer thicknesses of the limbs measured by ultrasound, to yield the mean muscle layer thicknesses of arm and leg ($MMT_{arm+leg}$). The linear correlation between TBER corrected for body height (TBER/L) and AMA was compared to the correlation between the resistance and the $MMT_{arm+leg}$.

Results

US-based $MMT_{arm+leg}$, correlated better with TBER/L for men (R^2=0.75, SEE= 20.2 Ω/m versus R^2=0.66, SEE=23.7 Ω/m) and women (R^2=0.78, SEE=23.1 Ω/m versus R^2=0.62, SEE=30.5 Ω/m) than with AMA. Multiple regression between TBER/L and $MMT_{arm+leg}$ combined with gender as input variables showed that gender adds no significant information(P=0.10). The correlation between $MMT_{arm+leg}$ and TBER/L improved even further for men and women together (R^2=0.85, SEE= 22.2 Ω/m).

Conclusion

In conclusion, US-based $MMT_{arm+leg}$ measurement shows potential to optimize the prediction of $TBER_{norm}$. It remains to be established whether the improved precision achieved by US measurements is clinically meaningful and outweighs the extra workload that is introduced.

Introduction

Hydration state imbalance

In many different diseases involving heart, kidney or hormonal processes, the hydration state is disrupted. Every year, 40.000 patients with heart failure and 2.000 patients with end-stage renal failure are diagnosed in the Netherlands.[1,2] Heart failure is characterized by a diminished blood flow because of a damaged pump function of the heart, leading to build-up of blood in the veins. Renal failure is defined as a decrease in capacity of the kidneys to excrete excessive fluid and waste products. In both cases, the fluid retention can lead to peripheral oedema, tiredness, dyspnoea caused by lung oedema, and may deteriorate ventricular function further. To reduce these symptoms these patients depend on medical treatment to stabilize their hydration state. Accuracy of the hydration state assessment is essential, since suboptimal treatment of overhydration will increase symptoms and weaken the heart, while dehydration can have serious consequences such as orthostasis and renal failure.[3]

Assessment of hydration status

The assessment of the hydration state in clinical practice is based on clinical characteristics found during anamneses and physical examination. This subjective method depends on the interpretations of nurse practitioners or specialists and has a limited accuracy.[4] A non-invasive objective method for the assessment of hydration status would be most welcome.

Bioelectrical impedance analyses

In the seventies, bioelectrical impedance analysis (BIA) was introduced as a promising non-invasive objective method for the assessment of total body water (TBW) and hydration status based on measurement of total body electrical resistance (TBER)[5,6]. It is based on the principle that TBER is inversely related to TBW if electrolyte concentrations are within the normal range. TBER can be measured fast, safe and non-invasively by applying a small alternating current to electrodes placed on the hand and foot. Additional electrodes on the shoulder and hip make it possible to measure the electrical resistance of the arm (AER) and leg (LER), separately. [7] Several studies in the nineties investigated the relation between impedance index (Height^2/Resistance) and total body water *in heathy subjects* measured by a stable isotope dilution method as reference and demonstrated a significant correlation ($R > 0.90$) with standard estimated error (SEE) of 2-4%[8,9].
Subsequently, many algorithms were developed to calculate the TBW based on TBER, using body height, weight and gender as additional input variables. However, imprecision remained to high with SEE ranging from (0.4-3.5 liters) which is much too high for clinical practice.[10] As a result the method was not introduced in clinical practice[11].

Total body electrical resistance

Recently, a modified approach has been developed to document hydration by Schotman et al. based on the concept that a normal TBER reflects normal hydration if electrolyte concentrations are within the normal range. To assess hydration in individual patients, the actually measured TBER is compared with the TBER normal value (TBER$_{\text{norm}}$) for this individual. A positive difference > 2 SD indicates dehydration, a negative difference < -2 SD indicates fluid excess. Such an approach requires that the TBER$_{\text{norm}}$ value can be reliably predicted on a personal basis. This can be achieved by standardizing TBER based on factors directly affecting TBER, i.e. the size of the conducting muscles and body height.[12] This was chosen to lower the inter-individual variation and make the method more precise and patient specific.

It is based on a cylinder model describing the relation between resistance and physical characteristics of the body composition.[7] The resistance is inversely related to the cross-sectional area (CSA) and proportional to the length (L) of the conductor, as shown in eq.1. This model was adjusted to the composition of the human body by incorporating volume as shown in eq. 2. The TBER is described by the specific resistance (ρ), the body height (H) and the conductive volume (V). [7]

$$R = \rho * \frac{H}{CSA} \ (eq. 1) \quad \rightarrow \quad TBER = \rho * \frac{H^2}{V} (eq. 2)$$

As bone and fat are poor conductors, and the resistance of the trunk is very low, 95% of TBER is determined by the size and configuration of the limb muscle compartment[13]. To determine the relative muscle content, an estimate of the arm muscle area (AMA$_{\text{tsf}}$) was calculated using the triceps skinfold and the circumference of the upper arm in gender specific equations developed by Heymsfield et al.[14] Schotman et al. showed a significant correlation between the AMA$_{\text{tsf}}$ and TBER, AER and LER in 100 healthy men (R^{2}=0.53, R^{2}=0.37 and R^{2}=0.11) and 113 healthy women(R^{2}=0.42, R^{2}=0.27 and R^{2}=0.23). With these correlations, a patient-specific normal value of TBER can be calculated, based on AMA$_{\text{tsf}}$. TBER$_{\text{norm}}$ can then be used as a person specific reference value to assess the degree of hydration, and as a target value for correction of hydration abnormalities in patients with diseases associated with either fluid deficit or excess. Although, the anthropometric approach showed potential for standardizing the TBER, there is room for improvement in the measurement of AMA$_{\text{tsf}}$. [12]

Arm Muscle Area

The main inaccuracy of the AMA$_{\text{tsf}}$ estimate is caused by the measurement of the triceps skinfold (TSF). The intra-observer coefficient of variation for AMA$_{\text{tsf}}$ ranges from 6.2% to 10.5%, while the inter-observer coefficient of variation ranges from 8.2% to 21%.[14–17] In addition, AMA$_{\text{tsf}}$ has proven to lose accuracy in obese subjects (BMI > 30kg/m^2), which means that the hydration state estimation based on AMA$_{\text{tsf}}$ is insufficient for at least 16.4% of the male and 20.4% of the female Dutch population over 50 years old[18,19].

Another issue is the poor correlation between AMA_{tsf} and Leg electrical resistance (LER).[12] This can be explained by the variation in body composition in the limbs: the arm muscle content may not be representative for leg muscle content. Instead of using the AMA_{tsf} to standardize LER, it might be better to use the muscle content of the leg. However, CT validated equations to calculate the muscle area of the leg, based on measurement of leg circumference and skinfold thicknesses are not available. The present study was designed to the develop a more accurate, non-invasive method to assess body muscularity and to obtain a more precise prediction of $TBER_{norm}$.

Possibilities of Ultrasound

Ultrasound (US) is a generally available and accepted technique to measure subcutaneous fat and muscle thickness. Wagner et al. investigated the inter-rater reliability for skinfold measurement by skinfold caliper and by US. The skinfold measured by caliper showed an intra-class correlation of 0.966 with a large 95% confidence interval of 0.328-0.991. The skinfold measured by US showed a comparable intra-class correlation of 0.987 with a much smaller 95% coefficient interval of 0.976 to 0.993, confirming the US technique to be more precise. [20]

The intra- and interrater reliability and validity of muscle thickness measurements by ultrasound was investigated by Thomaes et al. using CT-scans as a gold standard. The intra-rater reliability was found to excellent with an intra-class correlation of 0.97 with a 95% confidence interval between 0.92–0.99. Also, the validity against CT was found to be good with an intra-class correlation of 0.92 (95% confidence interval: 0.81–0.97). The average difference in muscle diameter compared to CT was 0.01 ± 0.12 cm.[21]

These data suggest that US could be a more precise and reliable technique to assess body muscularity, and could provide a better correlation between muscle area and resistance, and improve the standardization of $TBER_{norm}$, AER_{norm} and LER_{norm}.[12]

Aim of the study

The primary objective is to develop a more precise method to estimate the muscularity of the body using US in order to optimize the prediction of $TBER_{norm}$. Results of ultrasound measurements will be compared with the anthropometric assessment of AMA_{tsf}.

Methods

Healthy subjects

Performance of the US method was examined in 129 healthy adults (60 men and 69 women) with a BMI ranging from of 17.4 to 52.4 Kg/m^2. Exclusion criteria were 1) any condition or medication affecting hydration, 2) pregnancy, 3) prostheses, paraplegia or hemiplegia on measuring side.

Triceps skinfolds are often difficult to measure accurately in obese subjects because the thick layer of subcutaneous fat hampers proper folding of the skin. This is probably a major cause for measurement inaccuracies. To evaluate the added value of ultrasound measurements 46 obese subjects (of the 129 subjects) were included in the Dutch obesity clinic in Velp (Nederlandse Obesitas Kliniek). The group consisted of 21 men and 25 women who visited the outpatient clinic for screening for bariatric surgery, or for check-up three month after bariatric surgery.

BIA Measurements

Resistance measurements (TBER, AER, LER), were performed with a portable BIA device (BIA 101 Anniversary, Akern) with a 50 kHz 400 μA current. The BIA measurements were performed at the right side with the subjects in the supine position and limbs slightly abducted from the body after a 5-minute rest. The TBER, AER and LER were corrected for height.

Anthropometric Measurements – AMA_{tsf}

To determine the relative muscle content, an estimate of the arm muscle area (AMA_{tsf}) was calculated using triceps skinfold (TSF), and the mid-arm circumference (MAC) in gender specific equations developed by Heymsfield et al. (eq. 3 and eq. 4). [14]

$$Men:\ AMA_{tsf} = \frac{(MAC - \pi * TSF)^2}{4 * \pi} - 10.5 \quad (\text{eq.}\,3)$$

$$Women:\ AMA_{tsf} = \frac{(MAC - \pi * TSF)^2}{4 * \pi} - 6.5 \quad (\text{eq.}\,4)$$

The MAC was measured midway between the olecranon and the tip of the acromion by measuring tape. The triceps skinfold was measured at the same location, using a Harpenden skinfold caliper.

Ultrasound Measurements

The ultrasound measurements were performed using B-mode ultrasonography (Arietta Prologue; Hitachi Ltd., Tokyo, Japan), equipped with a 38 mm linear-array probe with a frequency range from 2-12 MHz. Two ultrasonic images were made of every measuring site (Arm: anterior and posterior site, Leg: anterior, posterior, lateral and medial sites).

The ultrasonic images of the upper arm were performed midway between the olecranon and the tip of the acromion, with the lower arm held in a 90-degree position. The anterior arm measurement was done with a 0 degrees shoulder rotation. The posterior sit of the arm was measured with a 90 degrees internal rotation of the shoulder, with the underarm resting against the thorax. The ultrasound images of the anterior, lateral and medial site of the upper leg were performed with the subjects in the supine position, legs relaxed, slightly bend at the knee, and midway between the patella and the iliac crest. The posterior site of the leg was measured at the same level with the knee bend in a 90-degree position.

The ultrasound images were stored in DICOM format. All the measurements and analyses were performed by the same investigator. Furthermore, age, gender and height were noted. Isolated muscle thicknesses were only measurable for a part of the population (38 males and females) due to a limited penetration depth of 10 cm. The protocol for all the measurements that were performed can be found in Appendix 1.

Dicom viewer software (MicroDicom viewer 3.0.1, Sofia, Bulgaria) was used to determine the thicknesses of the fat and muscle layers. Subcutaneous fat layer thickness was defined as the distance between the upper skin layer and the upper fascia of the most superficial muscles at the upper apex of the fat layer. Muscle thicknesses of the arm biceps and triceps and the anterior and lateral leg muscle thicknesses are defined as the distance between the upper fascia of the muscle and the bone cortex. Thickness of rectus femoris and rectus lateralis muscle was defined as the distance between the upper and the deep fascia of these muscles (figure 1).

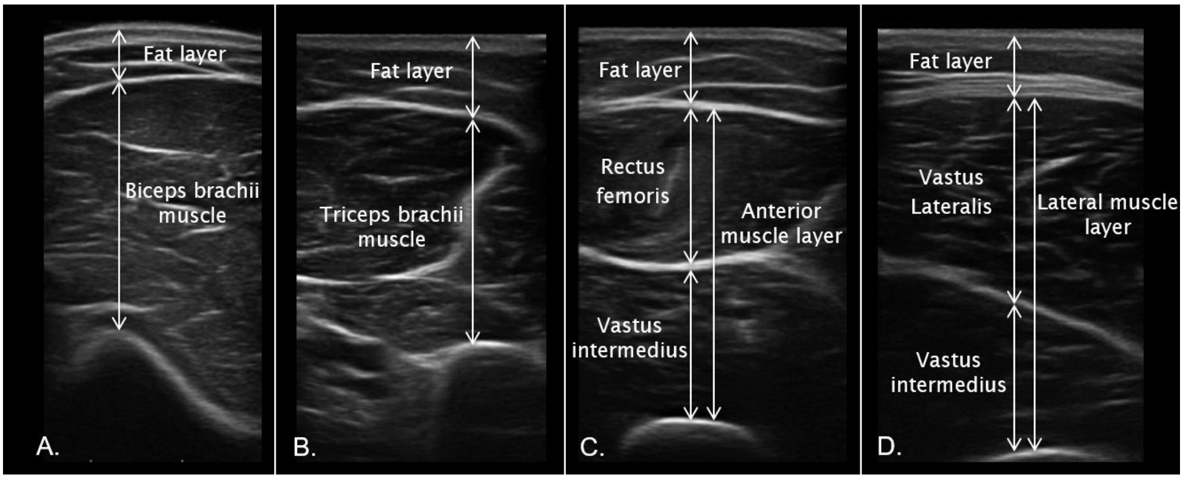


Figure 1. Ultrasound images of the mid-upper arm: A. the anterior side and B. posterior side.; Ultrasound images of the mid-upper leg: C. the anterior side and D. lateral side. For the arm we see the thicknesses of the fat layers, the biceps muscle layer and triceps muscle layer. For the leg we see the fat layers, the rectus femoris, vastus intermedius, vastus lateralis, anterior muscle layer and the lateral muscle layer.

Calculation of the mean muscle layer thickness

As shown in figure 2, the upper arm can be modelled as three cylinders, with the total arm muscle area as the conductive compartment. The upper arm radius (R) is the sum of three-layer thicknesses: mean fat layer thickness (MFT), the mean muscle layer thickness (MMT), and mean bone thickness (MBT).

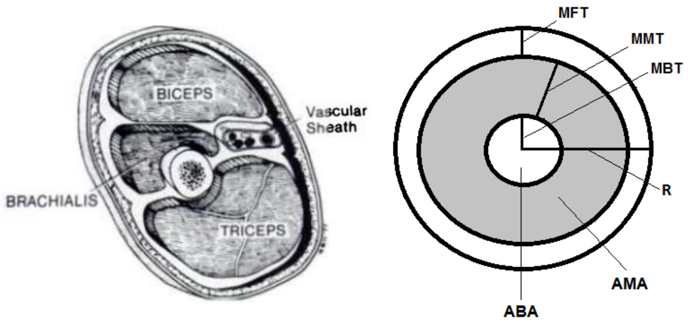


Figure 2. A schematic representation of the upper arm showing the fat layer around the various muscle groups of the humerus (left figure), and a symplified cylindrical model of the arm showing the mean fat layer thickness (MFT), the mean muscle layers thickness (MMT), the mean bone thickness (MBT), the total radius of the arm (R), the arm muscle area (AMA), the arm bone area (ABA) (right figure).

The collected measurements were used to determine the calculated mean muscle layer thickness of the upper arm (MMT_{arm}):

1. The radius(r_{arm}) is derived from the mid-upper arm circumference (MAC) (eq.5).

$$r_{arm} = \frac{MAC}{2*\pi} \qquad (eq. 5)$$

2. The mean fat layer thickness of the upper arm (MFT_{arm}) is calculated by averaging the biceps fat layer and the triceps fat layer, as measured by ultrasound (eq.6).

$$MFT_{arm} = \frac{FT_{biceps}+FT_{triceps}}{2} \qquad (eq. 6)$$

3. The MMT_{arm} can be calculated by subtracting the fat layer thickness (eq. 7).

$$MMT_{arm} = r_{arm} - MFT_{arm} \qquad (eq. 7)$$

The same method was used to calculate the mean leg muscle thickness. (MMT_{leg}): 1. the radius of the upper leg was obtained by measurement of the circumference of the mid-upper leg. 2. The mean fat layer thickness of the upper leg was calculated by averaging the anterior, posterior, lateral and medial fat layers at the mid-upper leg. 3. Lastly, the calculated mean muscle thickness of the mid-upper leg was determined by subtracting the mean fat layer thickness from the upper leg radius.

Finally, to obtain a muscle compartment value that might be more representable for whole body muscle, muscle thicknesses of the arm and leg were added up (eq. 8).

$$MMT_{arm+leg} = MMT_{arm} + MMT_{leg} \qquad (eq. 8)$$

Anthropometry compared to ultrasound

The accuracy of the AMA_{tsf} calculation based on TSF measurements is affected by BMI[15]. The reliability of skinfold measurement decreases linearly with skinfold thickness[15]. To investigate the impact of fat thickness on AMA_{tsf} accuracy, the AMA_{tsf} is compared to the AMA_{us}, which is calculated by using the US measured triceps subcutaneous fat layer thickness, times 2, in the equations developed by Heymfields et al.[14](eq. 2-3). The difference between AMA_{tsf} and AMA_{us} is then compared to the US triceps fat layer thickness.

Statistical analysis

Results are shown as mean values ± standard deviation (SD). Differences between the male and female population was tested using a Mann-Whitney U Test, since the subject characteristics data generally showed a non-normal distribution. Overall a P-value < 0.05 was considered statistically significant. To correct for multiple comparisons, i.e. 22 subject characteristics, the Bonferroni correction was used, and this yielded a P-value cut-off of 0.002 to define statistical significance. The correlations between TBER/L, AER/L, LER/L and the AMA_{tsf}, the mean muscle thickness of different muscles of the upper arm and leg, and the calculated muscle thickness of the arm and leg were analyzed by linear regression. The squared correlation coefficient and the SEE for the relation between TBER/L and AMA_{tsf} were compared with the relation between TBER/L and the measured and calculated muscle thicknesses. Multiple regression test was performed to test if the measurement techniques of the AMA_{tsf} and MMT were affected by gender.

Results

Subjects characteristics

General characteristics of the 60 males and 69 females included in this study are shown in Table 1. Mean age, BMI, arm and leg circumference were similar for man and women. However, men were on average 0.12 ± 0.01 m taller than women (P<0.001), had thinner triceps SKF's (P<0.001) smaller fat layer thicknesses (P<0.001) and a higher AMA_{tsf} (P=0.001). Mean TBER/L was significantly lower in males (242 ± 40 Ω/m versus 315 ± 49 Ω/m for females, P<0.001).

Table 1. Subjects characteristics are expressed in a mean ± standard deviation (SD)). An independent t-test was used to compare means between male and female. Bonferroni corrected P-value < 0.002 is significant.

Characteristics	**Males (n=60)**	**Females (n=69)**	**P-value**
Age (yrs)	46.8 ± 15.7	41.3 ± 14.4	0.051
Weight (kg)	95.4 ± 26.2	84.2 ± 27.9	0.005
BMI (kg/m²)	29.2 ± 7.8	29.9 ± 10.1	0.650
Body Height (m)	1.80 ± 0.1	1.70 ± 0.1	<0.002
Arm circumference (cm)	34.7 ± 5.4	33.6 ± 7.5	0.080
Leg circumference (cm)	56.3 ± 6.4	57.6 ± 9.7	0.998
Triceps skinfold (mm)	16.1 ± 7.3	24.4 ± 10.3	<0.002
Arm Muscle Area(cm²)	59.5 ± 18.5	49.1 ± 22.1	<0.002
AER/L (Ω/m)	354 ± 64	487 ± 86	<0.002
LER/L (Ω/m)	231 ± 37	285 ± 42	<0.002
TBER/L (Ω/m)	242 ± 40	315 ± 49	<0.002
Fat layer thickness arm(mm):			
- *Biceps*	9.7 ± 6	15.0 ± 9.3	<0.002
- *Triceps*	10.8 ± 4.7	20.4 ± 9.3	<0.002
- MFT_{arm}	10.2 ± 5.1	17.7 ± 9.0	<0.002
Fat layer thickness leg(mm):			
- *Anterior*	11.1 ± 5.2	21.3 ± 10.3	<0.002
- *Lateral*	9.1 ± 7.0	21.5 ± 13.4	<0.002
- *Medial*	20.2 ± 9.3	30.9 ± 13.0	<0.002
- *Posterior*	9.5 ± 4.1	19.1 ± 7.3	<0.002
- MFT_{leg}	12.5 ± 5.9	24.2 ± 10.0	<0.002
MMT_{arm} *(mm)*	4.5 ± 0.5	3.0 ± 0.8	<0.002
MMT_{leg} *(mm)*	7.7 ± 0.7	6.8 ± 0.8	<0.002
$MMT_{arm+leg}$ *(mm)*	12.2 ± 1.1	10.3 ± 1.1	<0.002

Abbreviations: arm electrical resistance (AER), leg electrical resistance (LER), total body electrical resistance (TBER) and the mean muscle thickness (MMT).

Distribution

Age ranged from 18 to 75 yrs, BMI from 17.4 to 52.4 kg/m^2 and AMA_{tsf} from 16.8 to 113.7 cm^2. The obese subjects (BMI>30) were significantly older (48.5 ± 1.6 yrs) compared to the non-obese subjects (40.8 ± 1.9 yrs) with a P-value of 0.0043. The obese subjects also showed a significantly higher AMA_{tsf} (73.8 ± 2.3 cm^2) compared to the non-obese subjects (42.2 ± 1.5 cm^2) with a P-value < 0.001.

The mean fat layer thicknesses of arm and leg are shown in figure 3A-B, respectively. The mean fat layer thicknesses of the arm and leg were significantly higher in women than in man (P<0.001). Moreover, the range was much wider in women as compared to men.

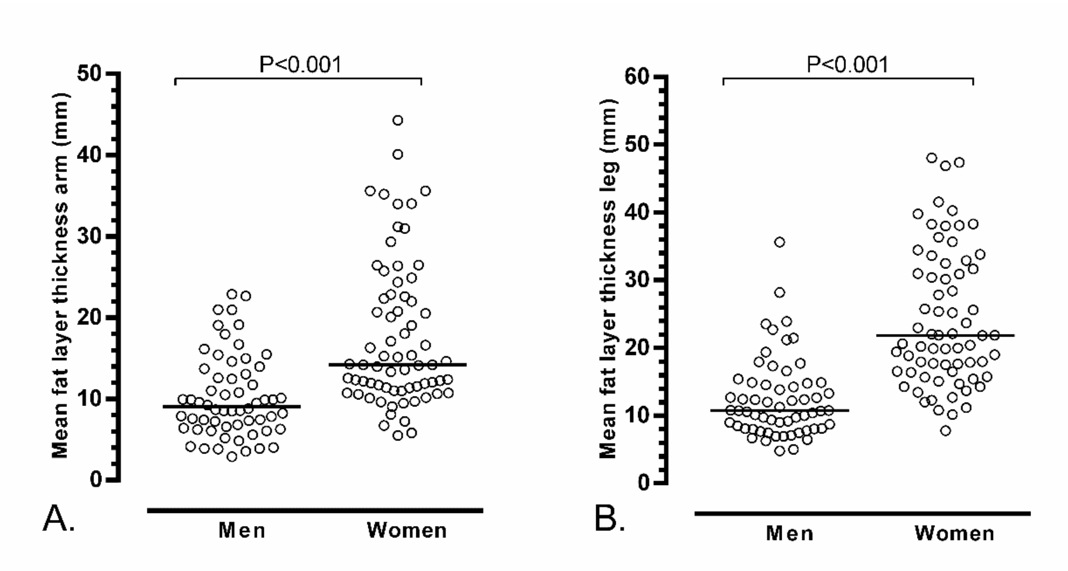


Figure 3A-B. Distribution of Mean arm fat layer thickness of A. the arm and B. the leg in 60 males and 69 females. The line represents the median value per group. The mean fat layer thickness of the arm and leg were significantly higher in women than in man (P<0.001).

TBER in male population

The results of the linear regression tests in the male population(n=60) between TBER/L, AER/L, LER/L versus AMA_{tsf} and calculated muscle layer thicknesses of arm and leg are presented in table 2. The isolated muscle thicknesses were only measurable for a part of the population (38 males) due to a limited penetration depth and are therefore not shown in table 2. The isolated muscle thicknesses all showed a poorer correlation with the TBER/L than AMA_{tsf}, see Appendix 2.

Table 2. Comparison of correlations (R^2) standard estimated error (SEE) for men (n=60) between the total body, arm and leg resistances (TBER, AER and LER, respectively) versus arm muscle area (AMA_{tsf}) based on skinfolds and calculated mean muscles thickness (MMT).

Men (n=60)	**TBER/L**		**AER/L**		**LER/L**	
	R^2	**SEE**	**R^2**	**SEE**	**R^2**	**SEE**
AMA based on skinfold	0.66	23.7 Ω/m	0.59	41.7 Ω/m	0.44	28.2 Ω/m
MMT_{arm}	0.69	22.5 Ω/m	0.65	38.3 Ω/m	0.47	27.5 Ω/m
MMT_{leg}	0.66	23.6 Ω/m	0.54	44.2 Ω/m	0.56	25.1 Ω/m
$MMT_{arm+leg}$	**0.75**	20.2 Ω/m	**0.65**	38.1 Ω/m	**0.58**	24.6 Ω/m

The AMA_{tsf} showed a correlation with TBER/L of R^2=0.66 (P<0.001), described by eq. 9. Correlations improved when the MMT was used. The highest correlation coefficient (R^2=0.75, P<0.001) was found between TBER/L and $MMT_{arm+leg}$ (eq. 10).

$$\mathrm{TBER/L} = -1.725 * \mathrm{AMA_{tsf}} + 346.8,\ \ \mathrm{R^2} = 0.66,\ \ \mathrm{SEE} = 23.7\ \Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\,9)$$

$$\mathrm{TBER/L} = -30.75 * \mathrm{MMT_{arm+leg}} + 616.8,\ \ \mathrm{R^2} = 0.75,\ \ \mathrm{SEE} = 20.2\ \Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\,10)$$

The distribution of the TBER/L, the correlation between TBER/L and AMA_{tsf} and the correlation between TBER/L and $MMT_{arm+leg}$ in males are shown in figure 4A-C, respectively. The precision of TBER/L prediction for individuals (SD=39.2 Ω/m) increased using the AMA_{tsf} (SEE=23.7 Ω/m) and further improved by using $MMT_{arm+leg}$ (SEE=20.2 Ω/m). The results were comparable for the segmental resistance, AER/L and LER/L, see Appendix 3.

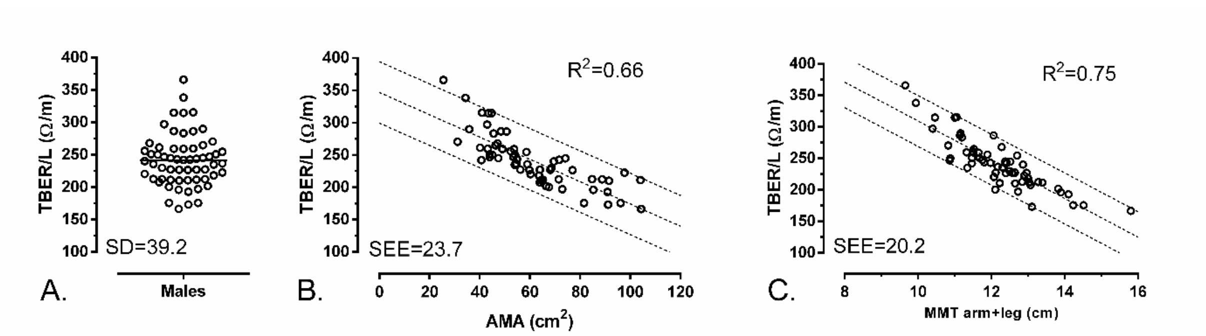


Figure 4. Graphs showing A. the distribution of the TBER/L, B. the correlation between TBER/L and AMA_{tsf} (R^2=0.66, SEE=23.7 Ω/m, P<0.001)and C. the correlation between TBER/L and $MMT_{arm+leg}$ (R^2=0.75, SEE=20.2 Ω/m, P<0.001) in 60 men.

TBER in female population

The results of the linear regression tests between TBER/L, AER/L, LER/L and anthropometric measurements in the total female population (n=69) are summarized in table 3. As in the male population, the isolated muscle thicknesses could only be measured in a part of the female population(n=41) because of a limited penetration depth of the scope and are therefore not shown in table 3. The isolated muscle thicknesses all showed a poor correlation with the TBER/L, see Appendix 2.

Table 3. Comparison of correlations (R^2) and standard estimated error (SEE) for women(n=69) between the total body, arm and leg resistances (TBER, AER and LER, respectively) versus arm muscle area (AMA_{tsf}) based on skinfolds and calculated mean muscles thickness (MMT).

Women (n=69)	**TBER/L**		**AER/L**		**LER/L**	
	R^2	**SEE**	**R^2**	**SEE**	**R^2**	**SEE**
AMA based on skinfold	0.62	30.5 Ω/m	0.53	59.0 Ω/m	0.39	33.2 Ω/m
MMT_{arm}	0.72	26.4 Ω/m	0.60	51.6 Ω/m	0.48	30.5 Ω/m
MMT_{leg}	0.67	28.6 Ω/m	0.52	59.6 Ω/m	0.48	30.6 Ω/m
$MMT_{arm+leg}$	**0.78**	23.1 Ω/m	**0.66**	50.3 Ω/m	**0.54**	28.7 Ω/m

The AMA_{tsf} showed a correlation with TBER/L of R^2=0.62 (SEE=30.5 Ω/m, P<0.001). Correlations improved when the mean of muscle thicknesses was used. The highest correlation coefficient was found between TBER/L and $MMT_{arm+leg}$ (R^2=0.78, SEE=23.1 Ω/m, P<0.001). The linear equations between TBER/L and AMA_{tsf} and TBER/L and $MMT_{arm+leg}$ are described in eq. 11 and eq. 12, respectively.

$$\mathrm{TBER/L} = -1.750 * \mathrm{AMA_{tsf}} + 400.6,\ \ \mathrm{R^2} = 0.62,\ \ \mathrm{SEE} = 30.5\,\Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\,11)$$

$$\mathrm{TBER/L} = -37.86 * \mathrm{MMT_{arm+leg}} + 705.9,\ \ \mathrm{R^2} = 0.78,\ \ \mathrm{SEE} = 23.1\,\Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\,12)$$

The distribution of TBER/L, the linear correlation between TBER/L and AMA_{tsf} and the linear correlation between TBER/L and $MMT_{arm+leg}$ for the female population is shown in figure 5 A-C, respectively. The results were comparable for the segmental resistance, AER/L and LER/L, see Appendix 3.

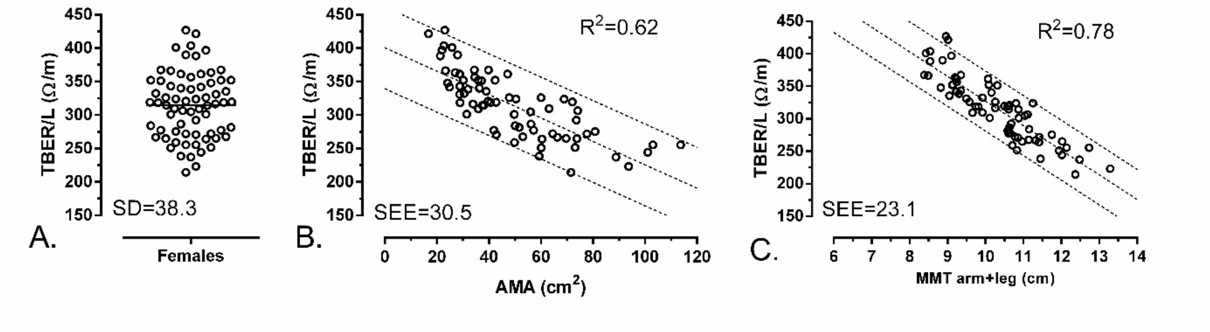


Figure 5. Graphs showing A. the distribution of the TBER/L, B. the correlation between TBER/L and AMA_{tsf} (R^2=0.62, SEE=30.5 Ω/m, P<0.001)and C. the correlation between TBER/L and $MMT_{arm+leg}$ (R^2=0.78, SEE=23.1Ω/m, P<0.001) in 69 women.

TBER in total population

Multiple regression with AMA_{tsf}, age and gender showed an age independency (P=0.404) and a gender dependency in the correlation with TBER/L (P<0.001). In contrast, multiple regression with $MMT_{arm+leg}$ and gender as input variables showed that gender had no significant impact on TBER/L (P=0.10), while age was significant(P=0.004). If the measurements in men and women are combined, the correlation between TBER/L and $MMT_{arm+leg}$ (R^2=0.75 for men and R^2=0.78 for women), increases to R^2=0.85 in the total population, with a SEE of 22.2 Ω/m (eq. 13).

$$\mathrm{TBER/L} = -36.44 * \mathrm{MMT}_{\mathrm{arm+leg}} + 688.8,\ \ \mathrm{R}^2 = 0.85,\ \ \mathrm{SEE} = 22.2\ \Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\ 13)$$

For the correlation between AER/L and MMT_{arm} and between LER/L and MMT_{leg} correlation coefficients and SEE's also improved (R^2=0.78, SEE=47.7.2 Ω/m and R^2=0.64, SEE=28.8 Ω/m, respectively). The linear relation between resistance (TBER/L, AER/L and LER/L) and MMT are described in eq.13-15 and shown in figure 6 A-C.

$$\mathrm{AER/L} = -89.03 * \mathrm{MMT}_{\mathrm{arm}} + 754.7,\ \ \mathrm{R}^2 = 0.78,\ \ \mathrm{SEE} = 47.7\ \Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\ 14)$$

$$\mathrm{LER/L} = -44.24 * \mathrm{MMT}_{\mathrm{leg}} + 578.2,\ \ \mathrm{R}^2 = 0.64,\ \ \mathrm{SEE} = 28.8\ \Omega/\mathrm{m},\ \ \mathrm{P} < 0.001 \quad (\mathrm{eq.}\ 15)$$

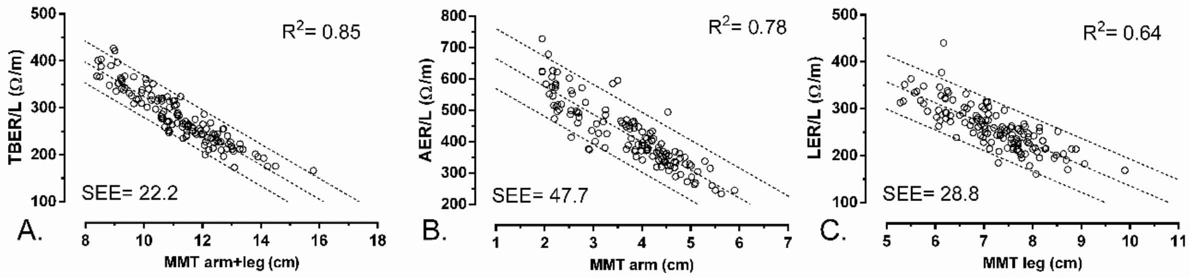


Figure 6. Graphs showing A. the linear correlation between TBER/L and $MMT_{arm+leg}$ (R^2=0.85, SEE=22.2 Ω/m, P<0.001), B. the linear correlation between AER/L and MMT_{arm} (R^2=0.78, SEE=47.7 Ω/m, P<0.001) and C. the linear correlation between LER/L and MMT_{leg} (R^2=0.64, SEE=28.8 Ω/m, P<0.001) in the total population(n=129).

Adding age to the equation leads to a very small improvement from R^2=0.85, SEE=22.2 Ω/m (P<0.001) to R^2=0.87, SEE=21.5 Ω/m (P<0.001).

Obesity related error

To explain the disappearance of gender dependency when AMA was replaced by MMT as the correcting variable, we examined the impact of triceps skin thickness on the calculation of AMA, by comparing the anthropometric AMA_{tsf} and the AMA calculated with ultrasound derived triceps skinfold measurements (AMA_{US}). A greater subcutaneous fat thickness was associated with a progressively increasing discrepancy between AMA_{tsf} and AMA_{US}, in both men and women (R^2=0.46 and R^2=0.78, respectively) (Figure 7).

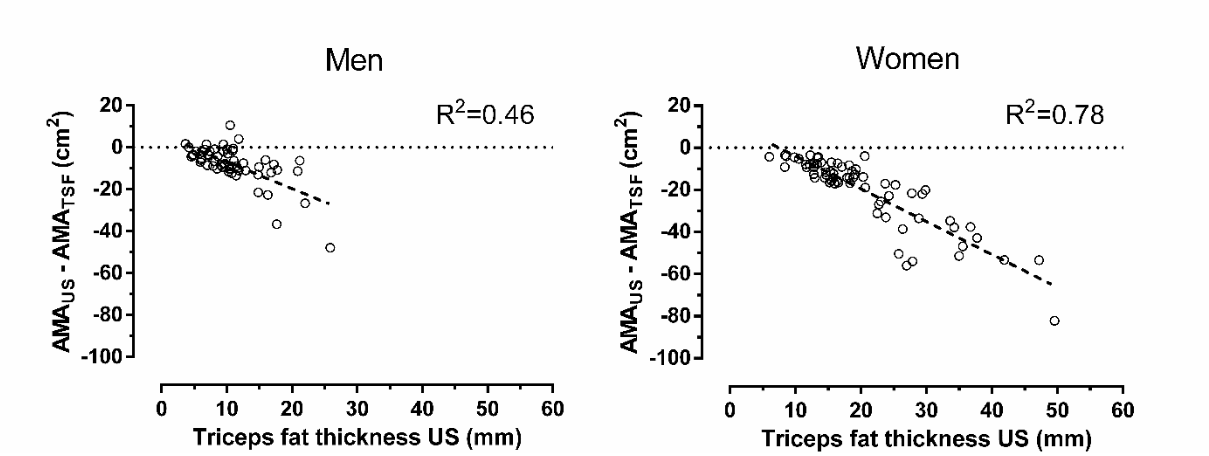


Figure 7. The graphs are showing the difference between the anthropometric and ultrasonic measurement of the arm muscle area (AMA_{tsf} and AMAus, respectively) against the triceps fat thickness measured with ultrasound for men(left) and women(right). A linear correlation was found for both men (R^2=0.46, P<0.001) and women (R^2=0.78, P<0.001).

The slopes of the lines were statistically similar in men and women (P=0.203). In subjects with a US measured skin thickness < 10 mm there was no significant difference between AMA_{tsf} and AMA_{US}. The correlation between the difference in AMA measures and the triceps subcutaneous fat layer thickness for the total population (n=129) a showed an R^2 of 0.76 (P<0.001).

Discussion

The primary objective of this study was to investigate whether muscularity assessed by US performed better than AMA assessed by anthropometry (AMA_{tsf}) in predicting an individual's TBER normal value. This is of major importance for the accuracy of hydration assessment using BIA. This study shows that the US-based $MMT_{arm+leg}$ measurements correlated significantly better with TBER/L (R^2=0.75, SEE= 20.2 Ω/m versus R^2=0.66, SEE=23.7 Ω/m for men, and R^2=0.78, SEE=23.1 Ω/m versus R^2=0.62, SEE=30.5 Ω/m for women) than AMA_{tsf}. Moreover, whereas muscularity assessed by AMA_{tsf} was found to be gender dependent, $MMT_{arm+leg}$ measurements proved to be independent of gender. This further improved and simplified the correlation between $MMT_{arm+leg}$ and TBER/L, described by a gender independent equation for men and women together with a R^2 of 0.85, and SEE of 22.2 Ω/m. Prediction of normal values for segmental resistances of the arm and leg was also improved by US-based measurements of muscularity. It remains to be established whether the improved precision achieved by US measurements is clinically meaningful and outweighs the extra workload that is introduced. AMA_{tsf} measurements take about 1 min, assessment of MMT arm and leg about 6 min.

Calculated muscle thickness

The improvement in the correlation between TBER and $MMT_{arm+leg}$ compared to AMA_{tsf} can be explained by several factors. First of all, the AMA_{tsf} only includes the muscularity of the arm, which would be no problem if there is little inter-individual difference in the proportion of muscularity between the arms and leg. However, a part of the population, sportsman, manual workers and patients with physical disabilities, can show a divergent proportion in the muscularity in arm and leg. To avoid this limitation, the muscularity of the leg should be also considered.

The second reason for the improvement in correlation is the fact that the skinfold measurement is less precise compared to the US fat thickness measurements. Wagner et al. showed that the skinfold measured with caliper showed a intraclass correlation (ICC) of 0.966 with a large 95% confidence interval (CI) of 0.328-0.991, whereas the skinfold measured by US showed a comparable ICC of 0.987 but with a much smaller 95% CI of 0.976 to 0.993.[20]

Furthermore, it is known that the AMA_{tsf} loses reliability in obese subjects (BMI>30 kg/m^2)[18]. This study showed that adding of a population with a thicker triceps fat layer, causes a significantly bigger difference between anthropometric and ultrasonic measurements of the AMA. These results suggest that people with a higher triceps fat layer thickness have a higher margin of error in the skinfold measurements, that results in an overestimated AMA_{tsf}. Using ultrasound instead of anthropometry increases the accuracy in patients with BMI>30 kg/m^2, which makes the BIA-approach also available for the obese population.

Gender (in)dependency

The correlation between AMA_{tsf} and TBER/L shows a gender dependency ($P<0.001$), which is mostly explained by the fact that the AMA_{tsf} loses validity in subjects with a higher fat layer thickness. The female population shows a significantly higher thickness of all the fat layers, including the triceps fat layer. The correlation ($R^2=0.76$) between the difference in AMA measures (anthropometry and ultrasound) and the fat layer thickness affirms the fact that the gender dependency in AMA_{tsf} is the result of measurements errors instead of biological gender-differences in the AMA_{tsf} and TBER/L correlation.

Limitations

The main limitation of the present study is that all measurements have been performed on a single occasion and by a single investigator. The intra- and inter-observer variation of the US measurements are currently not known. However, previous studies strongly suggest that reproducibility of skinfold thickness measurements by US are highly reproducible. Comparison of MRI and US measurements of subcutaneous adipose tissue yielded a strong correlation (R=0.79-0.95).[22] Moreover, intra- and inter-observer reliability of US fat layer thickness measurements was high, with an inter-class correlation of 0.99 and 0.989, respectively.[23] Observer variation in TBER measurements has been shown to be small with an intra- and inter-observer variation of 1.2% and 0.6%, respectively[15,24].

Recommendations

This study showed an improvement in the accuracy in the assessment of TBER, using MMT based on US, compared to AMA_{tsf}. However, there are still some unknowns to attend to before this method can be used in clinical practice. First of all, the validity needs to be tested, preferable against CT. It would also be interesting to see how much more there is to gain from using the muscle area determined by CT. Secondly, the population needs to be expanded including sportsmen and underweight adults, to enhance its applicability in de general population.

If this new BIA-approach proofs to be valid and reliable enough in a healthy population, it needs to be investigated if the method is also applicable for over/under hydrated patients. Over hydrated patients show edema, mostly in the feet and lower legs. However, excessively overhydrated patients can show edema in the upper legs, affecting the mid-upper leg circumference measurement. This can result in an overestimation of the MMT and therefore an under estimation of hydration state. A solution for this might be repeated circumference measurements after the edema has dissolved from the upper leg. Protocol for these scenarios need to be established before it can be used in clinical practice.

The feasibility of the new BIA-approach in terms of costs and logistics should be investigated before implementation. The costs are expected to be minimal since ultrasound machines are used in daily practice in almost every department, including the cardiology and internal medicine departments which admit the most patients suffering from hydration state imbalance. Challenging drawbacks to overcome are the logistics including finding and training medical personal to perform resistance and ultrasound measurements, analyze the data and consult the physician. Since some knowledge of ultrasound is necessary and the BIA-approach is applicable to more than one department, it is logical to include the radiology department in the implementation.

Conclusion

This study has shown that the limb muscularity based on US measurements is highly correlated with TBER in healthy, normally hydrated men and women, and in a gender independent manner. This correlation of the US-derived mean muscle thickness and the TBER in healthy subjects can be used to calculate the expected normal TBER in patients. The $TBER_{norm}$ can than then be used as a target for the treatments of patients with abnormal hydration. It remains to be established whether the improved precision achieved by US measurements is clinically meaningful and outweighs the extra workload that is introduced.

References

1. Hartstichting. *Bekijk de Cijfers over Hart- En Vaatziekten*.; 2016.
2. Nierstichting. *Feiten En Cijfers over Behandeling Nierfalen*.; 2017.
3. Brater D. Diuretica therapy. *Best Pract Res Clin Rheumatol*. 1999;13(3):479-485.
4. Shepherd A. Measuring and managing fluid balance. *Nurs Times*. 2011;107(28):12-16.
5. Hoffer EC, Meador CK, Simpsons DC. Correlation of Whole Body Impedance with Total Body Water Volume. *J Appl Physiol*. 1969.
6. Thomasset A. Bio-electrical properties of tissue impedance measurements. *Lyon Med*. 1962;(207):107-118.
7. Kyle UG, Bosaeus I, De Lorenzo AD, et al. Bioelectrical impedance analysis - Part I: Review of principles and methods. *Clin Nutr*. 2004.
8. Kushner RF, Schoeller D a, Carla R Fjeld and LD. Is the impedance index (ht2 / R) predicting total body water ? *Am J Clin Nutr*. 1992;56(April):835-839.
9. Sergi G, Bussolotto M, Perini P, et al. Accuracy of Bioelectrical Impedance Analysis in Estimation of Extracellular Space in Healthy Subjects and in Fluid Retention States. *Ann Nutr Metab*. 1994;38(3):158-165. http://www.ncbi.nlm.nih.gov/pubmed/7979169. Accessed September 10, 2018.
10. Ellis KJ. Human Body Composition: In Vivo Methods. *Physiol Rev*. 2000;80(2):649-680.
11. Kyle UG, Bosaeus I, De Lorenzo AD, et al. Bioelectrical impedance analysis—part II: utilization in clinical practice. *Clin Nutr*. 2004;23(6):1430-1453.
12. Schotman JM, van Borren MMGJ, Kooistra M, Doorenbos CJ, de Boer H. Hydration Status Assessment in Patients on Hemodialysis, based on Bio-Electrical Impedance Analysis (BIA): Back to Basics (in preparation). 2016:8.
13. Faes T, Van der Meij H, de Munck J, Heethaar R. The electric resistivity of human tissues (100 Hz-10 MHz): a meta-analysis of review studies. *Physiol Meas*. 1999;1(20):R1-R10.
14. Heymsfield B. Anthropometric measurement of muscle mass: bone-free arm revised muscle equations for calculating area. *Am J Clin Nutr*. 1982;36(October):1-11.
15. Alex Roche BF, William Cameron Chumlea Ds, Guo S. Identification and Validation of New Anthropometric Techniques for Quantifying Body Composition. 1984;(July 1985).
16. Vicente-Rodríguez G, Rey-López JP, Mesana MI, et al. Reliability and intermethod agreement for body fat assessment among two field and two laboratory methods in adolescents. *Obesity*. 2012;20(1):221-228.
17. Klipstein-Grobusch K. Interviewer variability in anthropometric measurements and estimates of body composition. *Int J Epidemiol*. 1997;26(90001):174S - 180.
18. Forbes GB, Brown MR, Griffiths HJ. Arm muscle plus bone area: anthropometry and CAT scan compared. *Am J Clin Nutr*. 1988;47(6):929-931.
19. Volksgezondheidenzorg. Volwassen met overgewicht en obesitas. 2019.
20. Wagner DR, Cain DL, Clark NW. Validity and reliability of a-mode ultrasound for body composition assessment of NCAA division i athletes. *PLoS One*. 2016.
21. Thomaes T, Thomis M, Onkelinx S, Coudyzer W, Cornelissen V, Vanhees L. Reliability and validity of the ultrasound technique to measure the rectus femoris muscle diameter in older CAD-patients. *BMC Med Imaging*. 2012.

22. Abe T, Tanaka F, Kawakami Y, Yoshikawa K, Fugunaga T. Total and segmental subcutaneous adipose tissue volume measured by ultrasound. *Med &amp Sci Sport &amp Exerc*. 1996;28(7):908-912.

23. Schlecht I, Wiggermann P, Behrens G, et al. Reproducibility and validity of ultrasound for the measurement of visceral and subcutaneous adipose tissues. *Metabolism*. 2014;63(12):1512-1519.

24. Vettorazzi, Carolina; Smits, Els; Solomons NW. The interobserver reproducibility of bioelectrical impedance analysis measurements in infants and toddlers. *J Pediatr Gastroenterol Nutr*. 1994;19(3):277-282.

Appendix 1. Meetprotocol AMA, spierecho's en weerstandmetingen

1. Voorbereiding echo

Benodigdheden voor de ultrasound metingen:

- Ultrasound machine met B-mode
- Lineare transducer met frequentie tussen 7,5-12 Mhs
- Gel
- Doekjes om gel te verwijderen
- Ontsmettingsmiddel voor de transducer
- Pen om meetlocatie aan te geven

Werkwijze

I. De echomachine moet worden aangezet door de stekker in het stopcontact te doen en op de aan knop te drukken. Het kan even duren voordat de machine het doet.
II. Maak een nieuwe map voor de patiënt aan met studienummer.
III. De ultrasound machine moet ingesteld staan **op "musculoskeletal meausrements+ far view",** de diepte begint met 6cm en de gain begint op 45% maar beide kunnen aangepast worden op de patiënt.
IV. Leg de scoop, gel en de tissues klaar.

2. Voorbereiding proefpersoon

I. Leg uit waarom dit onderzoek gedaan wordt en wat het zal inhouden.
II. Vraag naar protheses, nier-of hartdoeningen en zwangerschap. Als er geen exclusiecriteria bij zaten wordt de meetzijde bepaald.
III. Laat de patiënt zijn trui uit doen, sieraden afdoen en op de onderzoeksbank plaats nemen.

3. AMA bepalen

Benodigdheden AMA-meting:

- Harpenders caliper
- Meetlint
- (Markeer)stift

Werkwijze

I. De lengte van de bovenarm (acromion-olecranon) wordt opgemeten met een meetlint en in het midden (de midline) wordt een markeringsstreepje gezet met de stift. Hiervoor ligt de arm te rusten op het been in een hoek van 90 graden.
II. Vervolgens wordt met een meetlint de omtrek van de bovenarm gemeten tot op de millimeter. Hierbij dient het meetlint tegen de onderzijde van de markering gelegd te worden. *Zet aan de onderzijde van het meetlint aan de voor en achterzijde van de arm streepjes, zodat je precies weet waar er geëchood moet worden.*
III. Met behulp van een Harpenders caliper wordt de huidplooidiktes van de bovenarm gemeten ter hoogte van de markering. Hierbij dient de arm ontspannen te zijn om gemakkelijker onderscheid te kunnen maken tussen de spier- en vetlaag. De posities van de huidplooidikte is de triceps (TSF): posterieure zijde van de arm.
IV. Houdt de huidplooi goed vast tussen duim en wijsvinger, schud de plooi wat los van de onderliggende spierlaag. Positioneer de Harpenders caliper loodrecht op de huidplooi en laat vervolgens de kracht van de caliper los op de huidplooi. De caliper dient niet te dicht op het lichaam te worden gepositioneerd en tevens niet te dicht bij de punt van de huidplooi.
V. De huidplooidikte dient afgelezen te worden op 1 mm, 1 à 2 seconden nadat de volledige kracht aan de huidplooi is gegeven.

VI. De meting wordt per positie 2 maal uitgevoerd, waaruit de gemiddelde huidplooidikte wordt bepaald.

4. Echo arm

Werkwijze:

I. Breng voldoen gel aan op de transducer en plaats de transducer transversaal op hoogte van het aangegeven meetpunt op de bicepszijde, in het midden van de voorzijde van de bovenarm.

II. Probeer een minimale druk uit te oefenen met de transducer zodat de dikte van de spier en vetlagen niet beïnvloed wordt. Haal de scope langzaam van de arm af en maak een afbeelding op het moment dat er aan de zijkanten van de afbeelding zwart in beeld komt maar de lagen nog steeds te onderscheiden zijn. Dit betekent dat er net geen contact meer is met de huid. Een afbeelding maak je door op freeze te drukken en dan op store.

III. Maak op deze manier 2 afbeeldingen.

IV. Laat nu de patiënt de arm in een 90 hoek op de buik leggen.

V. Plaats de transducer transversaal op hoogte van het meetpunt op de tricepszijde, in het midden van de voorzijde van de bovenarm. Probeer een minimale druk uit te oefenen met de transducer zodat de dikte van de spier in beïnvloed wordt. Zorg er weer voor dat de spier en vetlagen goed te onderscheiden zijn van de rest van de afbeelding.

VI. Maak 2 goede afbeeldingen door op freeze te drukken en dan op store.

VII. Maak nu de arm schoon.

5. BIA-metingen

Meetmethode bio-impedantie meting

De benodigdheden voor de bio-impedantie meting zijn:

- Bio impedantie analysator, gecertificeerd en draagbaar, de BIA 101 (Quantum IV, RJL systems, Clinton Township, MI, USA)
- 8 bia-elektrodes (biatrodes)
- Alcohol (Desinfectans) en gaasjes voor de reiniging
- Meetlint
- (Markeer) stift

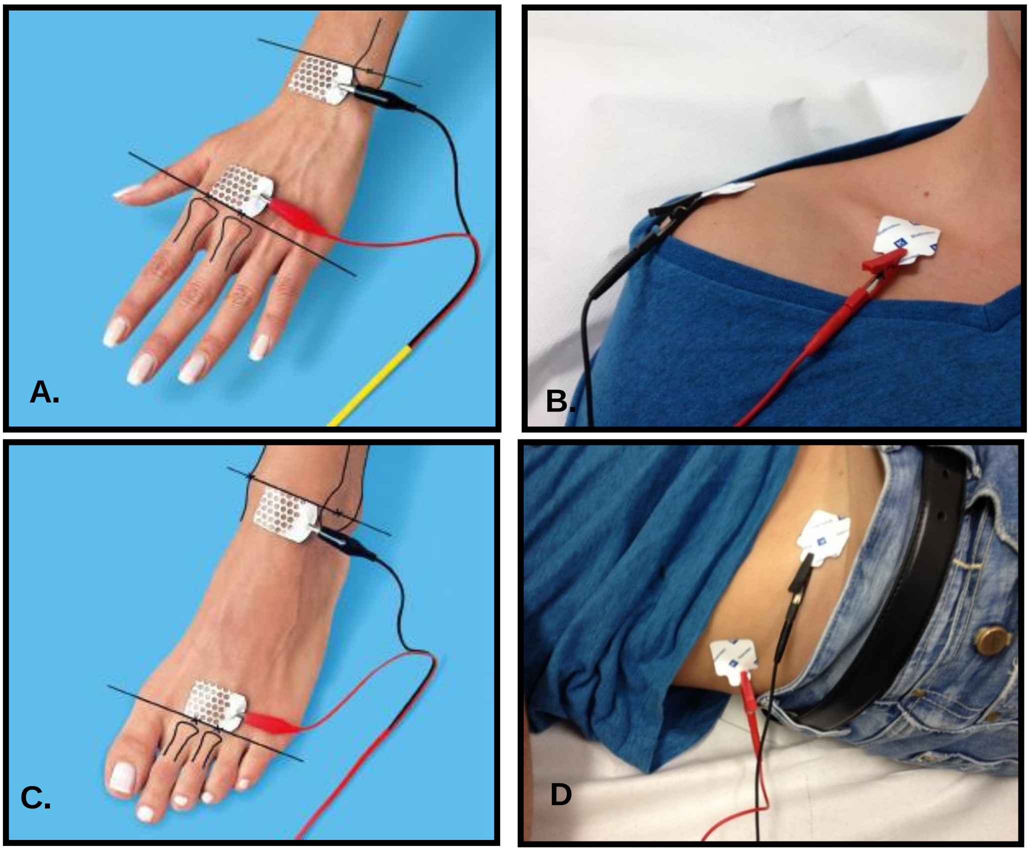
Werkwijze:

I. Laat de patiënt nu zijn broek uit doen en gaan liggen op de onderzoeksbank in een hoek van 30-45 graden.

II. Huid schoonmaken indien nodig.

III. Plaats de bia-elektroden op de juiste plaatsen.

   a. Voor de hand: 1 net onder het metacarpofalangeale gewricht en 1 in het midden van de posterieure zijde van de pols. (Figuur A1-1A)
   b. Voor de schouder: 1 op de distale punt van het acromion en 1 5 cm vanaf het acromion op het acromioclavicualair gewricht. (Figuur A1-1B)
   c. Voor de voet: 1 op het metatarsofalageale gewricht in het midden van de voet en 1 op de ventrale zijde van het enkelgewricht. (Figuur A1-1C)
   d. Voor de heup: 1 op de spina iliaca anterior superior en 1 op de bovenrand van de trochanter major van het femur. (Figuur A1-1D)



Figuur A1-1 Juiste plaatsen voor de BIA- elektrodes.

IV. Meet de lengte van het armsegment, van binnenste tot binnenste elektrode. Meet de lengte van het beensegment, van binnenste tot binnenste elektrode. Noteer de gemeten waarden. Meet tot op de halve centimeter nauwkeurig.

V. Sluit nu de impedantie meter aan. De rode kabel moet aan de distale(buitenste) zijde, de zwarte aan de proximale(binnenste) zijde.

VI. Zet de impedantie meter aan op de standaardinstellingen. Wacht totdat de waarde op het scherm gestabiliseerd is. Noteer voor elk van de gemeten segmenten de weerstand en de reactantie in Ohm.

VII. Belangrijk: zorg dat de patiënt zo stil mogelijk ligt met de armen langs, maar niet tegen het lichaam. En de benen iets gespreid.

VIII. Als alle benodigde BIA-waarden zijn verkregen kunnen de elektroden verwijderd worden.

6. **Echo bovenbeen**

<u>Werkwijze</u>

I. Meet de lengte tussen bovenrand van de trochanter major van het femur en bovenrand van de patella. Geef de meetlocatie aan op de helft van deze lijn met een stip. Meet de omtrek van het bovenbeen door het meetlint tegen de onderzijde van de stip aan te leggen. Gebruik het meetlint om op de dezelfde hoogte de meetpunten aan te geven op de laterale, mediale en de posterior zijde.
II. Probeer een minimale druk uit te oefenen met de transducer zodat de dikte van de spier en vetlagen niet beïnvloed wordt. Haal de scope langzaam van de voorzijde van het been af en maak een afbeelding op het moment dat er aan de zijkanten van de afbeelding zwart in beeld komt maar de lagen nog steeds te onderscheiden zijn. Dit betekent dat er een minimale druk op de huid wordt uitgeoefend. Een afbeelding maak je door op freeze te drukken en dan op store.
III. Maak op deze manier 2 afbeeldingen. Alle lagen (rectus femoris, vastus intermedius, subcutane vetlaag, and perimuscular fascia) moeten goed in beeld zijn.
IV. Doe hetzelfde voor de laterale en mediale zijde.
V. Voor de posterior zijde moet de patiënt zijn been optillen en gebogen neezetten.
VI. Maak 2 goede afbeeldingen door op freeze te drukken en dan op store.
VII. Zorg dat de patiënt zijn been kan schoon maken met tissues, waarna hij/zij zich weer kan gaan aankleden.

7. **Afsluiten echo**

I. Zet de patiëntenmap over op de USB.
II. Sluit het programma af door op end te drukken en dan op de uitknop.
III. Maak de transducer schoon met het ontsmettingsmiddel.

8. **Analyse beelden**

I. Sluit de USB aan op de computer en open de verzamelmap DICOMDIR met het programma MicroDicom viewer.
II. Meet met de measure Distance tool, de afstand tussen de verschillende lagen. In figuur A1-2 staan de vet en spierlagen afgebeeld.
III. Vul de waarden in het excel sheet in.

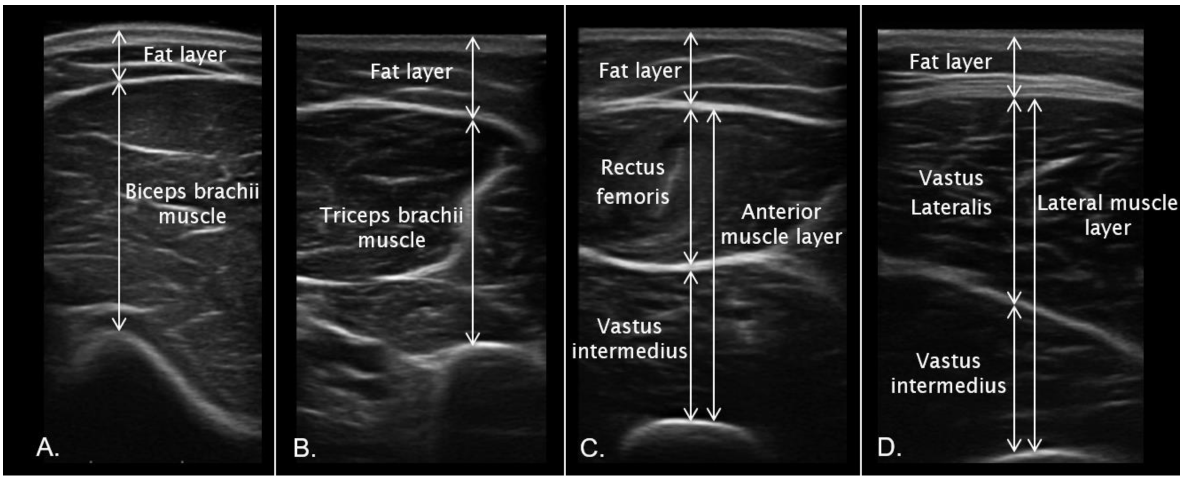


Figure A1- 2 Ultrasound images of the mid-upper arm: A. the anterior side and B. posterior side. Ultrasound images of the mid-upper leg: C. the anterior side and D. lateral side. The distances between the white arrows show for the arm the thicknesses of the fat layers, the biceps muscle layer and biceps muscle layer. For the leg we see the rectus femoris, vastus intermedius, vastus lateralis, anterior muscle layer and the lateral muscle layer.

Appendix 2. Correlation between TBER and Muscle thickness

Direct US measurements of separate muscle layers thicknesses was only possible in a part of the population, because of a limited penetration depth of the scope. This paragraph shows the results of 38 men and 41 women, for which imaging of the biceps, triceps, femoral and lateral muscle layer was possible.

Muscle thickness measurements in men

The results of the linear regression tests in males between TBER/L versus AMA_{tsf}, US measurements of muscle layer thicknesses of arm and leg and calculated mean muscle thickness are presented in table A2-1. Correlations with isolated muscle thicknesses were poor. Correlations improved when the mean of muscle thicknesses of arm was used(R^2=0.45). However, the AMA_{tsf} and the $MMT_{arm+leg}$ showed to be superior (R^2=0.66 and R^2=0.72, respectively).

Table A2-1. Comparison of correlation(R^2) for males(n=38) between the TBER and the AMA_{tsf}, US measurements of muscle thickness of different muscles and calculated muscle thicknesses are express by a correlation coefficient. Only statistically significant correlations (P<0.05) are shown.

Correlation (R^2)	**TBER/L**
AMA_{tsf}	0.66
Muscle thickness of:	
- *Biceps*	0.40
- *Triceps*	0.32
- *Rectus femoris*	0.24
- *Anterior quadriceps*	0.23
- *Rectus lateralis*	-
- *Lateral quadriceps*	0.22
Mean of 2 arm muscle thicknesses	0.45
Mean of 2 leg muscle thicknesses	0.30
$MMT_{arm+leg}$	0.72

Muscle thickness measurements in women

The results of the linear regression tests in females between TBER/L, versus AMA_{tsf}, US measurements of muscle layer thicknesses of arm and leg and calculated mean muscle thickness are presented in table A2-2. Correlations with isolated muscle thicknesses were also poor in women. The mean thickness of the arm muscles showed a better correlation (R^2=0.52), compared to the AMA_{tsf} (R^2=0.41). The $MMT_{arm+leg}$ however, showed the highest correlation(R^2=0.63).

Table A2-2. The correlation for females(n=41) between the TBER and the AMA_{tsf}, US measurements of muscle thickness of different muscles and calculated muscle thicknesses are express by a correlation coefficient. Only statistically significant correlations ($P<0.05$) are presented.

Correlation (R^2)	**TBER/L**
AMA_{tsf}	0.41
Mean muscle thickness of:	
- *Biceps*	0.22
- *Triceps*	0.39
- *Rectus femoris*	0.20
- *Anterior quadriceps*	0.25
- *Rectus lateralis*	-
- *Lateral quadriceps*	-
Mean of 2 arm muscle layer thicknesses	0.52
Mean of 2 leg muscle layer thicknesses	0.20
$MMT_{arm+leg}$	0.63

Appendix 3. Results - Segmental resistance

Male population

The correlation of AER/L and AMA_{tsf} increased from R^2=0.59 (SEE=41.7 Ω/m, P<0.001) to R^2=0.65 (SEE=38.3 Ω/m, P<0.001) when $MMT_{arm+leg}$ was used as measure of muscularity, and remains R^2=0.65 (SEE=38.1 Ω/m, P<0.001), when MMT_{arm} was used. Graphs showing the distribution of AER/L, the correlation between AER/L and the AMA_{tsf} and the correlation between AER/L and MMT_{arm} can be seen in figure A3-1 A-C, respectively.

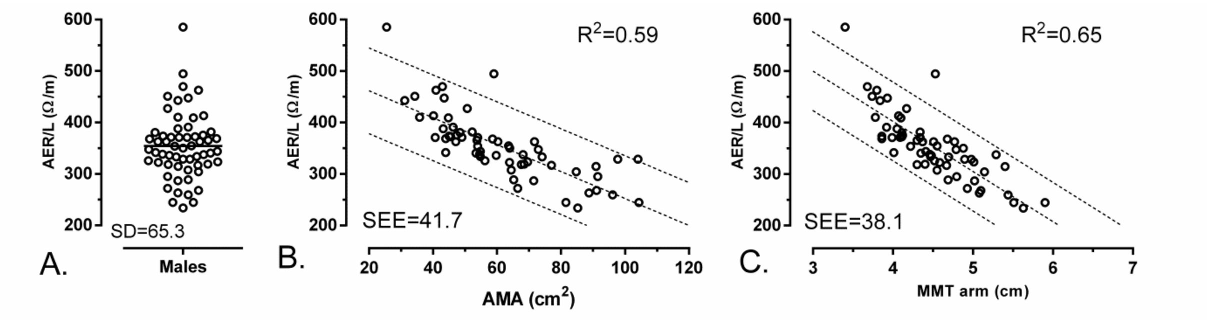


Figure A3-1. Graphs showing A. the distribution of AER/L, B. the linear correlation between AER/L and AMA_{tsf} (R^2=0.59, SEE=41.7 Ω/m, P<0.001) and C. the linear correlation between AER/L and MMT arm (R^2=0.65, SEE=38.1 Ω/m, P<0.001).

The LER/L correlates good with MMT_{ler} (R^2=0.56, SEE=25.1 Ω/m, P<0.001) and with $MMT_{arm+leg}$ (R^2=0.58, SEE=24.6 Ω/m, P<0.001), which was a considerable improvement compared to the AMA_{tsf} derived correlation (R^2=0.44, SEE=28.2 Ω/m, P<0.001). graph showing the distribution of LER/L, the correlation between LER/L and the AMA_{tsf} and the correlation between LER/L and MMT_{leg} can be found in figure A3 - 2A-C, respectively. Table A3-1 summarizes the R^2, SEE, the linear equation and the P-value for males(n=60) between the AER and LER versus AMA_{tsf} and MMT.

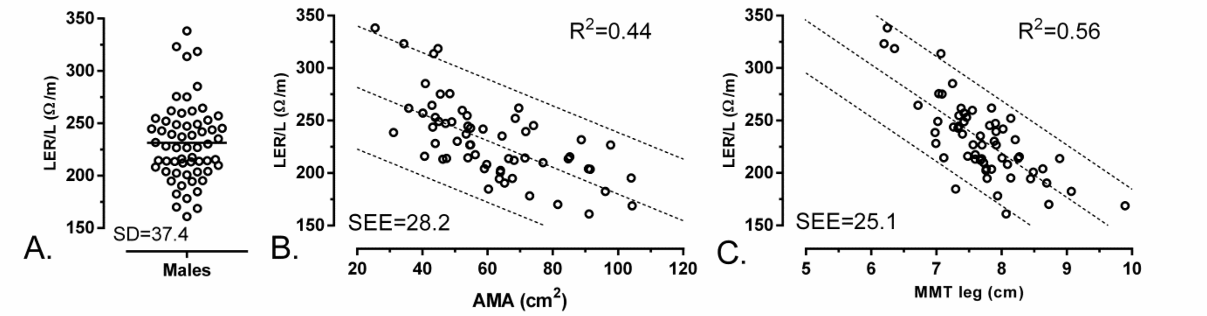


Figure A3-2. Graphs showing A. the distribution of LER/L, B. the linear correlation between LER/L and AMA_{tsf} (R2=0.44, SEE=28.2 Ω/m, P<0.001) and C. the linear correlation between LER/L and MMT leg (R^2=0.56, SEE=25.1 Ω/m, P<0.001).

Table A3-1. The linear equation, the correlation coefficient (R2), the standard estimated error (SEE) and the P-value in men(n=60) for the correlation between the arm and leg resistances (AER and LER, respectively) versus arm muscle area (AMA_{tsf}*) based on skinfolds and calculated mean muscles thickness (MMT).*

Men (n=60)	R^2	**SEE**	**P-value**
AER = 514-2.62× AMA_{tsf}	0.59	41.7 Ω/m	<0.001
AER = 530-45.5×MMT_{arm}	0.65	38.1 Ω/m	<0.001
LER = 312-1.32× AMA_{tsf}	0.44	28.2 Ω/m	<0.001
LER = 368-19.5×MMT_{leg}	0.58	24.6 Ω/m	<0.001

Female population

The correlation with AER/L improved from AMA_{tsf} (R^2=0.53, SEE=59.0 Ω/m, P<0.001) to MMT_{arm} (R^2=0.64, SEE=51.6 Ω/m, P<0.001). Graph showing the distribution of AER/L, the correlation between AER/L and AMA_{tsf} and the correlation between AER/L and MMT_{arm} can be found in figure A3 - 3A-C, respectively.

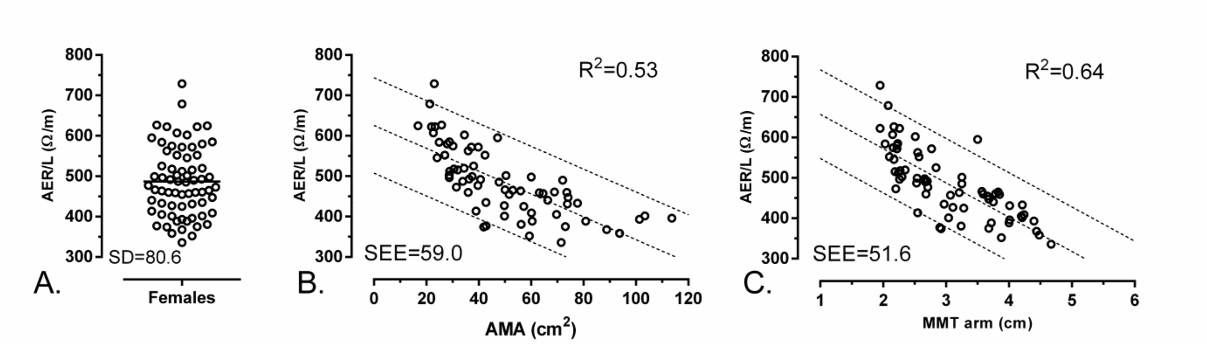


Figure A3 - 3. Graphs showing A. the distribution of AER/L, B. the linear correlation between AER/L and AMA_{tsf} *(R^2=0.53, SEE=59.0 Ω/m, P<0.001) and C. the linear correlation between AER/L and MMT arm (R^2=0.64, SEE=51.6 Ω/m, P<0.001).*

The LER/L correlation improved slightly from AMA_{tsf} (R^2=0.39, SEE=33.2 Ω/m, P<0.001) to MMT_{leg} (R^2=0.48, SEE=30.6 Ω/m, P<0.001). A graph showing the distribution of LER/L, the correlation between LER/L and the AMA and the correlation between LER/L and MMT_{leg} can be found in figure A3- 4 A-C. For the outlier in figure A3- 4C. is no explanation except for the fact that this was the youngest subject, turned 18 yrs old two months before the measurements. After removal of this outlier, the linear correlation between LER/L and the MMT_{leg} improved(R^2=0.54, SEE=25.9 Ω/m, P<0.001) while the linear correlation between LER/L and the AMA_{tsf} decreases slightly (R^2=0.39, SEE=30.0 Ω/m, P<0.001). Table A3-2 summarizes the R^2, SEE, the linear equation and the P-value between the AER and LER versus AMA_{tsf} and MMT.

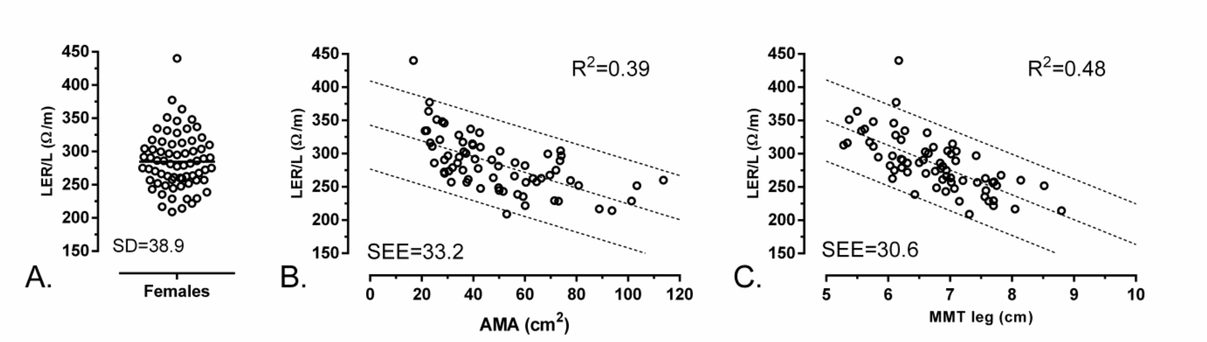


Figure A3 - 4. Graphs showing A. the distribution of LER/L, B. the linear correlation between LER/L and AMA_{tsf} (R^2=0.39, SEE=33.2 Ω/m, P<0.001) and C. the linear correlation between LER/L and MMT leg (R^2=0.48, SEE=30.6 Ω/m, P<0.001).

Table A3-2. The linear equation, the correlation coefficient (R2), the standard estimated error (SEE) and the P-value in women(n=69) for the correlation between the arm and leg resistances (AER and LER, respectively) versus arm muscle area (AMA_{tsf}) based on skinfolds and calculated mean muscles thickness (MMT).

Women (n=69)	**R^2**	**SEE**	**P-value**
AER = 626-2.83× AMA_{tsf}	0.53	59.0 Ω/m	<0.001
AER = 742-84.8×MMT_{arm}	0.66	50.3 Ω/m	<0.001
LER = 343-1.186× AMA_{tsf}	0.39	33.2 Ω/m	<0.001
LER = 537-37.31×MMT_{leg}	0.54	28.7 Ω/m	<0.001