

High frequency energy absorption and the measurement of limb muscle

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High frequency energy absorption (HFEA) is being developed as a portable, inexpensive, non-invasive procedure for the measurement of muscle mass within cross-sections of limbs. The instrument consists of a flexible coil 2.5 cm wide of which the length can be adjusted over a 10 cm range. A series of coils of different lengths has been constructed that are jointly suitable for limbs with circumferences ranging from 20 to 75 cm. To measure HFEA, a coil of appropriate length is attached to a 9v battery that, through an oscillator, produces a frequency varying from 15 MHz (longest coil) to 40 MHz (shortest coil). Zero readings, with the coil set at the same circumference as the limb, are obtained before and after HFEA is measured and they are used to adjust the observed values. HFEA, in theory, is related to the number of electrolytes deep to the coil and almost all these electrolytes are in muscle. Good precision has been demonstrated and the instrument has been successfully validated against saline solutions. A previous model was validated against magnetic resonance images with good results (r^2 about 0.8). Further validation of the present model against magnetic resonance images is almost complete; these findings are presented.

Introduction

There is no doubt that total body muscle mass is important for physical performance and as a store of protein, which is one aspect of nutritional status; but the relationships of muscle mass to health and longevity are less certain. There is, however, suggestive evidence that low amounts of muscle mass are associated with increased mortality rates in the middle aged and elderly¹. This evidence is derived from large follow-up studies in which body mass index (BMI) (weight/stature²) or arm muscle area have been recorded at entry. The evidence for associations of muscle mass with morbidity and mortality rates is only suggestive due to the lack of studies of large samples over long periods in which accurate measures of muscle mass, or of variables that are closely related to muscle mass, were made at baseline.

A practical method for the accurate measurement of total body muscle in the living is not available at present. Calculations can be made from the excretion of creatinine in urine during 24-hour periods but the conversion factor is uncertain and probably varies with the amount of muscle that is present². Furthermore, this approach assumes constancy for the creatine content of muscle tissue and for the rate of conversion of creatine to creatinine. The day-to-day variation of about 6% in creatinine excretion, even when the dietary intake is fixed, casts doubt on the accuracy of these assumptions^{3,4}. Total body muscle mass can be calculated from the urinary excretion of 3-methyl histidine (3-MH) but much of the 3-MH in the body is not in muscle and there are large day-to-day variations in the amounts excreted^{5,6}. Nevertheless, Lukaski et al.⁷ reported that estimates of muscle mass from 3-MH are highly correlated with those from neutron activation.

Total body muscle mass can be measured by computed tomography (CT) and magnetic resonance imaging (MRI) but these procedures are expensive. Furthermore, many 'slices' are required leading to significant irradiation with CT. Most consider MRI preferable to CT because there is no irradiation and tissue differentiation is better⁸. Both these methods are important because they can provide accurate criterion or dependent values but, mainly because of cost, only small samples have been studied. Neutron activation can provide measures of total body nitrogen (TBN) and total body potassium (TBK) can be calculated from emitted gamma rays. Muscle mass can be calculated from TBN and TBK applying assumptions about the relative proportions of nitrogen and potassium in muscle and lean non-muscle tissue^{9,10}. Unfortunately, these relative proportions vary and the values obtained for muscle mass are systematically too low^{11,12}.

Difficulties in the measurement of total body muscle mass have led to the measurement of muscle volumes and areas within regions as implicit surrogates. Dual-energy X-ray absorptiometry (DEXA) can provide precise measures of the fat-free mass (FFM) of limbs excluding bone. This mass is almost entirely muscle. These measurements may, however, be inaccurate because they depend on a fixed relationship between skeletal mass and muscle mass in parts of limbs where three types of tissue (skeletal, muscle, adipose) are superimposed. Only with such an assumption can three tissues with different absorption characteristics be separated

using two energies. A further limitation is that DEXA equipment is expensive and it is not portable.

Cross-sectional areas of FFM in the limbs can be obtained by CT or MRI. If the thickness of the 'slice' is known, these areas can be converted to volumes but this does not increase the amount of information provided. After bone is excluded from the cross-sectional images, almost all the FFM is muscle. Intramuscular adipose tissue may accumulate in the elderly. These intramuscular accumulations can be estimated by algorithms that separate pixels depending on their density, but, since this intramuscular adipose tissue is dispersed in small local amounts, these measurements may be inaccurate. The values obtained from CT may lose accuracy due to beam hardening and to shadows caused by bone while MRI data are influenced by the settings chosen. Nevertheless, precision is high for MRI and CT and there is close correspondence between areas measured with electronic pencils and those obtained from computer programs that recognize pixel densities. Both CT and MRI are important to determine the accuracy of other procedures that aim to measure cross-sections but it is unlikely they will be applied in surveys or clinical settings. Consequently, an alternative procedure is needed.

The cross-sectional area of FFM at a particular level in a limb can be calculated from the limb circumference and a skinfold thickness at the same level. This approach is useful despite the fact that it is based on invalid assumptions that cross-sections of limbs are circular and that an annulus of adipose tissue of uniform thickness surrounds the FFM which is centrally placed within a cross-section of a limb. Additionally, the usual equation for these calculations estimates the cross-sectional area of 'muscle plus bone,' although this is typically called 'muscle area.' Simplistic adjustments that subtract a sex-specific constant can be made that approximately remove the contribution of bone¹³. After these adjustments, the anthropometric method still overestimates the muscle area by about 7%^{14,15}. Despite these limitations, anthropometric arm muscle areas are significantly correlated with creatinine excretion and limb muscle mass^{2,16,17}. The anthropometric approach might be improved by substituting ultrasonic measures of subcutaneous adipose tissue for skinfold thicknesses. The ultrasonic values are at least as precise as skinfold thicknesses and they are not influenced by variations in compressibility^{18,19}.

Since anthropometry is the only established procedure for the regional measurement of muscle mass that is based on the use of portable inexpensive equipment, and since this procedure is based on incorrect assumptions and is inaccurate, an attempt was made to develop and validate the use of high frequency energy absorption (HFEA) for this purpose.

Theory and instrumentation

The HFEA approach assumes that the absorption of high frequency electromagnetic energy from a coil placed around a limb depends on the number of electrolytes in the tissues and fluids deep to the coil. There are few electrolytes in bone and adipose tissue. It is assumed that the concentrations of electrolytes in extra-cellular fluid are near constant and that the ratio intracellular fluid/extracellular fluid is fixed in the limb musculature. If these assumptions were correct, HFEA could provide accurate measures of limb muscle mass deep to a coil. These assumptions are similar to those for total body electrical conductivity (TOBEC) which is used to measure total FFM²⁰ and which, like HFEA, is believed to be insensitive to bone and adipose tissue²¹.

For the measurement of HFEA, flexible silver-coated coils are employed that are 2.5 cm wide and adjustable in length. Consequently, they can match limb circumferences over a 10 cm range for each coil. Collectively, these coils can be applied to limbs with circumferences from 20 to 75 cm. To measure HFEA, a coil of appropriate length is attached to an oscillator powered by a 9v battery that produces a frequency varying from 15 MHz (longest coil) to 40 MHz (shortest coil). The coil is placed around the limb and tightened as if the limb circumference were being measured; and the circumference is recorded. The coil is removed, set at the same circumference, and placed on a non-conductive flat surface to calibrate the reading to zero. The coil is then placed around the limb at the same location as previously and HFEA is measured after which the coil is again placed on the non-conducting surface to check if the instrument is still calibrated to zero. Any difference from zero is recorded and the reading is adjusted.

Laboratory data were obtained by placing a coil around one of 12 cylindrical beakers of different sizes that held demineralized water mixed with varying proportions of normal saline (0%, 25%, 50%, 75% or 100%). The HFEA readings (mv) were near zero for demineralized water. At each concentration of saline, the HFEA readings increased monotonically with beaker circumference with slight deceleration. The HFEA values from different coils but of similar lengths, eg 20–30 cm long, 30–40 cm long, for a particular beaker and solution were almost identical.

Precision

Five observers made duplicate HFEA measurements of the arm, calf and thigh of 9–10 subjects. Although the measurements were made under carefully controlled conditions with great attention to detail, there were some large intra-observer and inter-observer differences in the HFEA readings expressed as percentages of normal saline mixed with deionized water. The coefficient of variation (CV) values for the inter-observer differences were 12.1% for the arm, 4.1% for the calf and 4.0% for the thigh (Table 1). These data indicate that the coil values are unstable due either to inappropriate design of the coils or interference from other sources of electromagnetic energy. The large differences may have been related to the extent to which the coils had been used; the values from some coils became unstable after about 300 measurements. Due to these results, the immediate plans for future work relate to improving the design of the coils.

Table 1. Intra- and inter-observer differences (%) in measures of HFEA expressed as concentrations of normal saline in deionized water. (n=9 to 10).

Observer	Arm		Calf		Thigh	
	mean	sd	mean	sd	mean	sd
Intra-observer						
Observer A	4.32	5.54	1.24	1.72	1.62	2.16
Observer B	4.21	3.91	2.09	1.25	2.22	1.47
Observer C	7.65	10.50	1.21	0.54	2.12	1.62
Observer D	7.98	11.35	1.72	1.32	2.30	2.36
Observer E	5.09	6.58	2.20	2.92	1.79	3.28
Inter-observer	6.23	7.28	1.92	1.61	2.09	1.81
CV for interobserver	12.1%		4.1%		4.9%	

Accuracy

In vivo accuracy data for the evaluation of accuracy were obtained by applying MRI to cross-sections of the mid-arm, mid-calf, and of the thigh at the junction of its middle and

distal thirds. These images were made of 15 males and 15 females aged 11–50 years who were considered healthy. The data were obtained from inversion-recovery water-suppressed images using the following protocol: excitation and signal reception using a body coil, an imaging matrix of 205 x 256, TR = 1000 ms, TE = 26.5 ms, T1 = 250 ms, FOV = 240 mm, slice thickness = 2.5 cm, 1 slice, NA = 2, and foldover suppression on at 1.5 Tesla (Philips Gyroscan 515/hp). The areas of muscle on the images were converted to volumes using the known thickness of the tissues from which the MRI signals were obtained (2.5 cm).

These MRI volumes were used in a regression equation of the form:

$$\text{(MRI muscle volume = a + b HFEA + c circumference (cm) + c age (years) + sex (males = 1; females = 2),}$$

where the HFEA value is the proportion of normal saline in deionized water. These conversions were obtained from the laboratory data that were recorded using beakers. Since some of the independent variables were not significant (circumference not significant for arm, only the HFEA value significant for the thigh). Consequently, the regression equations will be altered in further analyses. The R^2 values were high being 0.87; 0.90 and 0.86 for the arm, calf and thigh, respectively. The root mean square errors were relatively large for the thigh and the CV varied from 18.4% for the arm, to 9.8% for the calf and 11.3% for the thigh (Table 2). Since age and sex were significant in the regression equations for the arm and calf, it is postulated that intramuscular adipose tissue, which could vary with age and sex, may have affected the HFEA data but not the MRI data.

Table 2. Comparisons between measures of muscle volume from magnetic resonance images of cross-sections of limbs and estimates from HFEA.

Limb	R^2	RMSE (mm ²)	CV (%)
Arm	0.87	23.1	18.4
Calf	0.90	21.9	9.8
Thigh	0.86	46.2	11.3

Conclusion

As a result of these exploratory studies, it is considered that the basic HFEA approach is sound but there is some concern about the results relating to precision and accuracy. Since these differences between readings are probably due to inadequacies in the coils, the work planned for the immediate future will focus on redesign of the coils.

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