Fat to Muscle Ratio Measurements with Dual Energy X Ray Absorbtiometry

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Abstract

Accurate measurement of the fat-to-muscle ratio in animal model is important for obesity research. An efficient way to measure the fat to muscle ratio in animal model using dual-energy absorptiometry is presented in this paper. A radioactive source exciting x-ray fluorescence from a target material is used to provide the two x-ray energies needed. The x-rays, after transmitting through the sample, are measured with an energy-sensitive Ge detector. Phantoms and specimens were measured. The results showed that the method was sensitive to the fat to muscle ratios with good linearity. A standard deviation of a few percent in the fat to muscle ratio could be observed with the x-ray dose of 0.001 mGy.

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22 **Introduction**

23 There is a need for accurate and cost effective measurement of fat to muscle ratio in the testing
24 of diabetic weight control drugs and obesity research. Currently micro-CT scans are used to measure the
25 fat to muscle ratios of the animal models, as weighing the animals is highly inaccurate due to outside
26 variables [1]. However, one problem with these micro-CT scans is the expenses and time necessary to
27 obtain results on a single mouse, about an hour itself, and a few more hours when taking into account
28 that the data obtained must be analyzed and processed. These animals need to be anesthetized, and it
29 would take a lot of time and effort to do this for each animal considering that animal experiments
30 typically involve a large number of them. Thus micro-CT scans are highly inefficient.

31 A more efficient way to measure the fat to muscle ratio is presented in this paper. X-ray
32 attenuation depends upon the x-ray energy, and this dependency differs between fat and muscle. Thus
33 by taking x-ray attenuation measurements of a specimen at two x-ray energies, the mass of each can be
34 solved. This method is called dual energy x-ray absorptiometry. Dual energy x-ray absorptiometry has
35 been widely used for differentiating between high-Z and low-Z materials, such as between bone and soft
36 tissue for bone densitometry [2], and recently Iodine and soft tissue for transvenous coronary
37 angiography [3-5]. Here, the use of dual energy x-ray absorptiometry is used to differentiate between
38 two soft tissue types, the fat and muscle, and to measure fat to muscle ratio.

39 Using a commercially available radioactive source that emits two x-ray energies, and an x-ray
40 detector that can discriminate between the energies, the dual energy x-ray absorptiometry can be easily
41 performed. An experimental apparatus was designed and constructed using a radioactive source made
42 by Amersham which provides the 22.105 and 59.5 keV x-rays and a Germanium detector made by
43 Canberra.

Phantoms of plexiglass, which is similar to fat in x-ray absorption, and water, which is similar to muscle, were designed and constructed. By varying the data acquisition time, measurements were made to determine the appropriate dosage to obtain accurate data. The amount of each component present was also changed to test the methods sensitivity and linearity.

Specimens of pork fat and muscle with different fat to muscle ratios were measured to validate this method's applicability for animal research as well. By layering the fat and muscle, a condition that is similar to the testing of live animals is created.

Theory

As x-rays travels through materials, they are attenuated by the material. X-ray attenuation is described by the Beer's law

$$I = I_0 \exp(-\mu T) \quad \text{Eq. 1}$$

where I_0 is the x-ray intensity before the material, I is the x-ray intensity after the material, μ is the linear attenuation coefficient of the material, and T is the thickness of the material.

Figure 1 shows x-rays transmitting a sample consisting of fat and muscle. The x-ray intensity after transmitting the material is

$$I = I_0 \exp(-\mu_f T_f) \exp(-\mu_m T_m) \quad \text{Eq. 2}$$

where μ_f and μ_m are the linear attenuation coefficients for fat and muscle, respectively, and T_f and T_m are the thicknesses of fat and muscle, respectively.

64

65 **Figure 1. This is the design used to measure the fat-to-muscle the ratio in the sample. The X-ray source**
 66 **is placed under the detector, with the specimen in between.**

67 In Eq. 1, the linear attenuation coefficient μ depends on the x-ray energy. Higher the x-ray
 68 energy corresponds to lower attenuation coefficient. Figure 2 shows the theoretial dependence of
 69 the linear attenuation coefficients of fat and muscle as function of x-ray energy.

Figure 2. X-ray linear attenuation coefficient for fat and muscle as function of x-ray energy.

The chemical composition of fat and muscle assumed for simulating Figure 2 is listed in table 1 [6].

Composition	Fat (mass percentage)	Muscle (mass percentage)
Hydrogen	11.2%	10.2%
Carbon	57.3%	12.3%
Nitrogen	1.1%	3.5%
Oxygen	30.3%	72.9%
Sulfur	.06%	.5%
Sodium		.08%
Magnesium		.02%
Phosphour		.2%
Calcium		.007%
Potassium		.008%

Table 1. Chemical composition of fat and muscle used for theoretical calculation of x-ray linear attenuation coefficients

Figure 2 shows that the dependence of fat and muscle on the x-ray energy is non-linear. Thus, by measuring the x-ray transmission (I_l and I_h) at two different x-ray energies (E_l and E_h), one can solve the thickness of the fat and muscle.

$$I_l = I_{0l} \exp(-\mu_f T_f) \exp(-\mu_m T_m) \quad \text{Eq. 3}$$

$$I_h = I_{0h} \exp(-\mu_{hf} T_f) \exp(-\mu_{hm} T_m) \quad \text{Eq. 4}$$

where μ_{lf} and μ_{lh} are the linear attenuation coefficients of fat at E_l and E_h , respectively, and μ_{lm} and μ_{hm} are the linear attenuation coefficients of muscle at E_l and E_h , respectively.

Taking the natural logarithm of Eqs. 3 and 4 results in two linear equations

$$\begin{aligned} \ln(I_l / I_{0l}) &= -\mu_{lf} T_f - \mu_{lm} T_m \\ \ln(I_h / I_{0h}) &= -\mu_{lh} T_f - \mu_{hm} T_m \end{aligned} \quad \text{Eq. 5, Eq. 6}$$

$\ln(I_l / I_{0l})$, defined as a , can be experimentally measured by performing x-ray attenuation measurement at x-ray energy E_l . Similarly, $\ln(I_h / I_{0h})$, defined as b , can be measured at x-ray energy E_h . With these two measurements and knowing the theoretical linear attenuation coefficients of fat and muscle at these two energies, Eq. 5 and 6 can be solved to obtain the thickness of fat and muscle in the sample:

$$T_f = \frac{-a\mu_{hm} + b\mu_{lm}}{\mu_{lf}\mu_{hm} - \mu_{lh}\mu_{lm}} \quad \text{Eq. 7}$$

$$T_m = \frac{a\mu_{hf} - b\mu_{lf}}{\mu_{lf}\mu_{hm} - \mu_{lh}\mu_{lm}} \quad \text{Eq. 8}$$

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96 **Experimental Setup and Data Acquisition**

97 An experimental apparatus was designed and constructed to measure x-ray attenuation of
98 samples at two x-ray energies simultaneously. Fig. 1 shows the design of the apparatus consisting of
99 radioactive source providing x-rays at 22.105 and 59.5 keV, an energy-sensitive x-ray detector, a copper

100 collimator that also serves as a sample support, and a detector mount that allows adjustment of the
101 distance between the source and detector. The collimator is 3-mm thick oxygen-free copper with a
102 circular hole of 10 mm drilled in the center. The detector has circular 5 mm thick Germanium sensor
103 with diameter of 20 mm that is cooled by liquid nitrogen in the Dewar above. The Dewar is filled twice
104 daily to maintain cryogenic temperature at the sensor through the duration of the experiment.

105 The x-ray source is a sealed Americium 241 manufactured by Amersham. The Americium 241
106 emits 59.5 keV x-rays. A Silver target is used for the source to provide 22.163 keV K α 1 and 21.99
107 keV K α 2 characteristic x-rays, with the flux-weighted energy being 22.105 keV. Thus with the silver
108 target, x-rays of 2 energies, 22.105 and 59.5 keV are emitted. The measured flux of the x-rays are 680
109 ph/s and 500 ph/s at 22.105 and 59.5 keV, respectively, at the detector position.

110 The x-ray detector is 2008S by Canberra. The distance adjustment between the source and
111 detector is achieved by X95 (Newport) slide. In the final configuration used to acquire the data
112 presented in this paper, the distance is 75 mm. The collimator-to-source distance is 35 mm. Fig. 3 shows
113 a picture of the experimental setup.

Fig.3. Experimental setup.

Background spectrum was acquired without the sample for 3600 s (T_0) to assure a good quality background. Then the sample was added and a spectrum was acquired for time T which can be different from T_0 . The background spectrum does not change with time, thus only one background is needed for all the measurements. Figure 4 shows a typical spectrum of the background in solid line, and spectrum with sample using the same acquisition time of 3600 s in dashed line. It can be seen that the lower-energy x-rays are attenuated more than the high energy x-rays.

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126 **Figure 4. Typical raw data spectrum with (dashed line) and without (solid line) the sample showing the detector**
127 **counts vs. channel number. The channel number is proportional to the x-ray energy.**

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129 The I_0 and I at both energies in Eqs 5 and 6 are derived from experimental data by summarizing
130 the counts under the Silver K_{α} peak for low energy x-rays, and the Am241 peak for high energy x-rays.
131 Then Eqs. 7 and 8 are used to calculate the thickness of fat and muscle in the sample. An IDL program
132 was developed to perform the data analysis automatically. The program requires user to simply enter
133 the file names for the background and sample spectra, and the acquisition time used for each spectrum.

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Phantom Studies

Fat and muscle was first simulated using a phantom constructed with water and plexiglass. The theoretical x-ray attenuation coefficients for water, fat, muscle and plexiglass are shown in table 2 for 22.105 and 59.5 keV x-rays. The table shows that the x-ray attenuation of water is similar to that of muscle at both energies, and that the plexiglass is similar to fat. The advantage of using phantom is that we can continuously and accurately vary the thickness of the compositions to study the signal-to-noise ratio of the measurement, and to find out the optimum measurement time. Phantom also offer the ability to conduct long, detailed experiments since they do not decay over time.

Element	μ at 22.105 keV (cm^{-1})	μ at 59.5 keV (cm^{-1})
Fat	0.4579	0.1976
Muscle	0.6408	0.2058
Pexiglas	0.5456	0.2293
Water	0.6292	0.2068

Table 2. Linear attenuation coefficients of fat, muscle, Plexiglas and water shows the similarity between the linear attenuation coefficients of fat and Plexiglas, and between that of muscle and water.

Figure 5. Design of the phantom used for the study that allows varying plexiglass thickness via the plexiglass sheet and water thicknesses.

Fig. 5 shows the design of the phantom, along with a representative picture of the phantom in one configuration. Adding or removing plexiglass sheets on the top changes the thickness of the plexiglass, adjusting the water level changes the thickness of the water.

Measurements were made with 3 phantoms consisting of 3 mm plexiglass + 9 mm water, 11 mm plexiglass + 9 mm water, and 11 mm plexiglass + 19 mm water. For each phantom, data was taken with acquisition times of 120, 225, 300, 450, 900, 1800 and 3600 s. For each acquisition time, 10-100 independent acquisitions were made and the data was analyzed to yield the water and plexiglass thickness and the water-to-plexiglass ratio. Since the multiple acquisitions were made with each acquisition time, the standard deviation of the thicknesses and ratio were also calculated. The data quality improves with increasing acquisition time. This is evident in the plot of the standard deviation of

plexiglass-to-water ratio divided by the ratio, versus acquisition time, shown in Fig. 6. Fig. 6 shows that at below 400 s acquisition time, the data is noisy due to Poisson statistics. With 900 s acquisition time, plexiglass to water ratio can be determined with an accuracy of about 5%, which is adequate for specimen measurement. Thus we choose 900 s as the standard acquisition time for subsequent measurements with fat and muscle specimen. The high-quality data of the phantom studies with long acquisition time also shows that the method differentiates the 3 phantoms with good accuracy and linearity.

Figure 6. Accuracy of the plexiglass-to-water ratio versus acquisition time. The accuracy is represented by the standard-deviation of the derived ratio divided by the ratio.

174 Specimen Studies

175 Specimen of pork with different fat-to-muscle ratios were prepared by cutting fillets of 5 mm
176 thick by 40 mm square of pure fat and pure muscle. Six fillets are then combined to form specimen of
177 30 mm total thickness, with 0 fat + 30 mm muscle, 5 mm fat + 25 mm muscle, 10 mm fat + 20 mm
178 muscle and so on. Each 30 mm thick sample was measured with an exposure time of 900s and the
179 thicknesses of fat and muscle were calculated.

180 Figure 7 shows the resulting measured cross section (density times measured thickness) for fat
181 (+) and muscle (*) versus theoretical cross section calculated using the known thickness. The dashed
182 line is a guide to the eye for the case when measurement agrees perfectly with theory. The error bars
183 represent standard deviation of measured cross section, 0.2 g/cm^2 , for a 30 mm specimen measured for
184 10 times using the same experimental procedures.

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Figure 7. Measured fat (+) and muscle (*) weight versus true weight of the specimen with 900 s acquisition time. The solid line and dotted line represent the result of linear fitting to the fat and muscle data, respectively.

The random deviation of the experimental results from theory is attributed to inconsistency and inherent inhomogeneity of the specimen slabs. The use of theoretical linear attenuation coefficient contributes to the systematically higher measured fat cross sections, by approximately 0.1 g/cm^2 . If experimental attenuation coefficients were used, the systematic error would be reduced to zero. Nevertheless, Fig. 7 shows that with 900 s measurement time, the method is able to detect fat and muscle with acceptable accuracy and linearity. Note that the 30 mm specimen thickness is similar to the thickness of lab rats and mice.

With an exposure time of 900s, the incident x-rays flux on the sample are 5×10^5 ph/s for both x-ray energies. This results in x-ray dose of 0.00084 and 0.00015 mGy for 22.105 and 59.5 keV x-rays, respectively. The total x-ray dose is 0.00099 mGy.

Conclusions and Discussions

The results of phantoms of plexiglass and water with total thickness of 12 to 40 mm and a variety of Plexiglas-to-water ratio validates the method's utility for measurement in a two-component system with excellent linearity and good sensitivity that improves with longer acquisition time. The study allowed determination of optimum acquisition time to minimize dosage while obtaining accurate data. Specimens of pork fat and muscle with different fat to muscle ratios were measured to validate this method's applicability for animal research as well. By using and layering fat and muscle, a condition that is similar to the testing of live animals is created.

The results showed that the method was sensitive to the fat to muscle ratios with good linearity. A standard deviation of a few percent in the fat to muscle ratio could be observed with the x-ray dose of 0.001 mGy. This is compared with the standard micro-CT dose of about 1 mGy. The x-ray source that was used for calibration was fairly weak, and a source that is a hundred times stronger is commercially available. The current experiment uses a measurement time of 900 seconds, however, with a stronger source, the time could be less than 10 seconds. and the cost would be low enough that it will most likely become main-stream in most laboratories. Also, the animals would be allowed to freely move under the x-ray emitter, so anesthetization would be unnecessary.

Further studies with rat specimen and eventually live animals are needed to fully validate the method's utility for obesity research using small animals. Since animal consists of bone in addition to fat

219 and muscle, the method may need to be extended to include 3 energies in order to solve 3 unknown
220 quantities.

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229 **References**

- 230 [1] S. Judex, Y.K. Luu, E. Ozcivici, B. Adler, S. Lublinsky, and C. Rubin, *Methods*, 50 (2010) 14-19.
- 231 [2] S. Cummings, D. Bates, D. Black, *J. Am. Med. Assoc.* **288** (2002) 1889-1897.
- 232 [3] E. Rubenstein, J.C. Giacomini, H. J. Gordon, A.C. Thompson, G. Brown, R. Hofstadter, W. Thomlinson,
233 and H.D. Zeman, *Nucl. Instrum. Meth. in Phys. Res. A* **291** (1990) 80-85.
- 234 [4] H. Elleaume, S. Fiedler, F. Esteve, et. al., *Phys. Med. Biol.* **45** (2000) L49-L43.
- 235 S. Ohtsuka, Y. Sugishita, T. Takeda, Y. Itai, J. Tada and K. Hyodo, *Br. J. Radiol.* **72** (1999) 24–8.
- 236 [5] Z. Zhong, D. Chapman, W. Thomlinson, F. Arfelli, R. Menk, *Nucl. Instrum. Meth. in Phys. Res. A* **399**
237 (1997) 480-498.
- 238 [6] B. Hasegawa, *Physics of Medical X-ray Imaging* (1987), chapter 4, Medical Physics Pub. Corp.