# Novel Calibration of in vivo Body Composition Analysis by $^{1}$H MR-Relaxometry

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**Rationale -** Numerous methods such as chemical extraction or dual emission X-ray absorptiometry (DEXA) are commonly used for body composition analysis. Yet, absolute quantification of body composition using different modalities has produced rather diverging results in absolute terms[(1)]. Most recently, MR-relaxometry has been introduced for rapid and non-invasive body composition analysis in mice[(1,2)] and rats[(2)] in vivo. Here, we present two novel and independent calibration procedures in order to render body composition analysis by MR-relaxometry fully quantitative in absolute terms.

**Methods -** Proton $T_2$ MR-relaxometry at 4.7 T was performed in 400 rats (250-650 g) of different strains (Sprague-Dawley, Wistar, Zucker Fatty), ages (6-25 w) and dietary conditions as described previously[(2)]. Whole-body data acquisition was accomplished with a spectroscopic CPMG sequence with 256 echoes and TR/TE=10000/2.5 ms. Relaxograms obtained after inverse Laplace transformation provided the individual signal contributions of tissue water and fat. Analysis of fat composition was carried out in vivo using plain proton-decoupled $^{13}$C-MRS (at 50 MHz) as described previously[(3)]. Signal contributions from individual carbon moieties were determined by integration after automatic baseline correction. Statistical analyses were carried out with StatView (SAS Istitute Inc., Cary, USA) and results are given as mean ± standard error.

**Calibration by Cross-Sectional Analysis -** Total body weight must be explained by the sum of fat and lean mass (i.e. water plus all dry matter). Experimental data from carcass studies suggest that the mass of water is strongly correlated with lean mass. Hence, we tested the hypothesis that the lean mass-to-water ratio ($\beta$) is constant and body weight may be expressed in terms of fat and water contents or their respective MR-signal intensities $S_{fat}$ and $S_{water}$ determined by MR-relaxometry:

$$body\ weight\ =\ fat + lean\ mass\ =\ fat + \beta * water\ =\ \alpha_{inst} * {}^{1H}\rho_{fat} * S_{fat} + \beta * \alpha_{inst} * {}^{1H}\rho_{water} * S_{water}$$

with $\alpha_{inst}$ being the general sensitivity of the instrument and ${}^{1H}\rho_{water}$ and ${}^{1H}\rho_{fat}$ the proton densities of water and fat (Proton density is defined as molar mass divided by the number of protons). Multiple regression analysis on cross-sectional data of 400 rats (fig. 1) provided the parameters $\beta$ = 1.39±0.04 and ${}^{1H}\rho_{fat}$ = 8.9±0.2 g/mol (${}^{1H}\rho_{water}$ = 9.0 g/mol is readily known from the chemical properties of water and $\alpha_{inst}$ was determined in water reference samples). With these parameters we could demonstrate that body weight is indeed highly correlated (r>0.986) with the weighted sum of water and fat as postulated above.

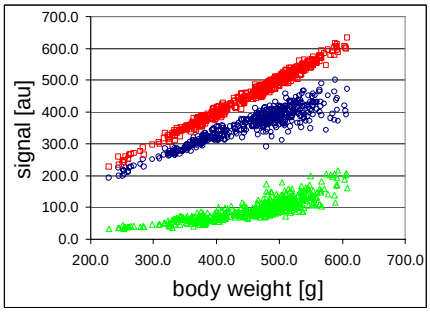


**Figure 1:** Body weight correlated with the signal intensities of fat (green), water (blue) and their weighted sum (red) determined in 400 rats using MR-relaxometry.

**Calibration by Fat Analysis -** Analytical knowledge of fat composition was used to corroborate the findings presented above. Whereas water is a pure chemical entity, tissue fat is a complex mixture of glycerides with acyl chains of different lengths and saturation. Hence, its proton density is a priory unknown but can be estimated from the weighted average of its pure constituents. Based on its capability to distinguish the different classes of triglycerides, $^{13}$C-MRS can report the chemical composition of fat in vivo[(3)] (fig. 2). From this analysis the proton density of fat in rats thus evaluates to ${}^{1H}\rho_{fat}$ = 8.8±0.2 g/mol. Moreover, $^{13}$C-MRS ascertained an average acyl chain length of 16.3±1.6 carbons and fractional contributions of saturated, mono- and doubly-unsaturated acyl chains of 27±3%, 22±2% and 51±3%, respectively.

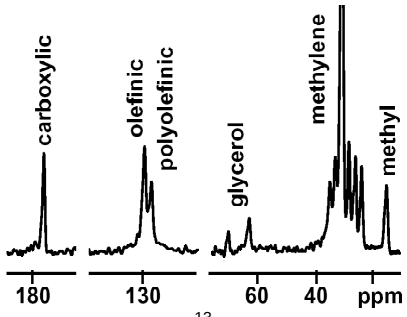


**Figure 2:** In vivo $^{13}$C-MR spectrum of fat in a rat.

**Conclusions -** With two independent calibration procedures we have firmly established the parameters ${}^{1H}\rho_{fat}$ (proton density of fat) and $\beta$ (lean mass-to-water ratio) required for absolute quantification of body composition by MR-relaxometry. Both procedures reported identical values. Moreover, ancillary information has been obtained as to the average chain length and chemical composition of fat. Backed by this calibration, MR-relaxometry now lends itself to accurate and rapid absolute body composition analysis in awake laboratory rodents.

## References

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