Absolute quantitation of glycogen by means of ^{13}C-MRS: A comparison of two different approaches

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Glycogen covers a substantial part of mammalian energy metabolism. Therefore, a determination of its concentration in absolute units is crucial. However, absolute quantitation of ^{13}C-MR spectra acquired by surface coils is difficult since X-nuclei generally lack a ubiquitous internal standard. This abstract expands two methods, which have been mainly used for the determination of relative concentrations so far, towards absolute quantitation of glycogen in human skeletal muscle: one that uses creatine as internal concentration standard, and one that is based on the superposition of a coil sensitivity map on anatomical images combined with a single reference measurement.

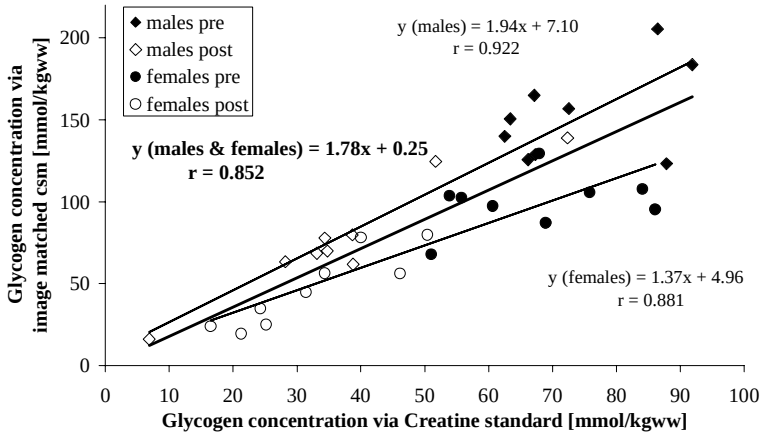
Introduction: Despite its large molecular weight, glycogen is 100% visible in ^{13}C MRS ([1] and Refs. therein) and the C1 resonance at 100.5ppm can be used for quantitation. Different approaches have been made to quantify glycogen, including calibration with anatomically shaped phantoms filled with a glycogen solution of known concentration ([1] and Refs. therein), the use of creatine as internal standard [2], and the use of sensitivity profiles of surface coils matched with MR images [3]. Since the latter two methods have mainly been used to determine signal ratios or institutional units, they are expanded and evaluated to allow for absolute quantitation.

Methods: In order to obtain a large range of glycogen concentrations, ^{13}C spectra were acquired from the thighs (*m.quadriceps femoris*) of 18 trained volunteers (9 m & 9 f) before and after a 3 h trial on a bicycle ergometer as described in detail in [4]. Measurements were performed on a 1.5 T whole body MR-scanner (GE SIGNA) with a double-tuned, flexible ^{13}C/^{1}H coil (Medical Advance, Milwaukee WI) using a pulse and acquire sequence (adiabatic excitation; TR=301ms; CW decoupling and NOE; 3x2000 acquisitions). An external acetone reference and two small, water filled tubes were fixed on the coil surface to determine coil loading and position of the ^{13}C coil. Signal intensities [S] of glycogen, creatine and acetone were determined by integration of peak areas and correcting for baseline contributions.

The *creatine standard method* uses total creatine (guanidino-carbon at 157ppm) as tissue specific concentration reference since it was reported that creatine shows only little inter-individual variation in muscle biopsies. Results in this abstract assumed a total creatine concentration in muscle tissue of 32 mmol/kgww. Metabolite specific T1 saturation and NOE were considered (approximate determination in separate experiments) according to the acquisition sequence applied in this study. In separate ^{1}H-MR spectra, it has been proven that no statistical differences between creatine concentrations have been found between males and females in this study [4].

The *image matched coil sensitivity map (csm)* is calculated in a Cartesian 3-dimensional matrix (n=100) using reciprocity principle and Biot-Savart's law with the following simplifications: idealized coil (wire geometry with rectangular shape 11x11 cm adapted to the ^{13}C-coil used yet without discrete components), ideal adiabatic pulse (90º, no relaxation), no interaction with tissue (no eddy currents or phase distortions). The csm is superimposed to MR images according to the coil position in the experiment with manually selected muscle tissue. A hypothetical signal intensity (α) can be defined as the sum of all volume elements from the selected region weighted with the csm at the respective position. α_{ex} of every individual in vivo experiment represents the influence of coil geometry and sensitivity on the measured signal and subsequently can be used to compare the signal with that of an arbitrarily shaped phantom filled with a glycogen solution of known concentration (α_{ph}). Glycogen concentrations are calculated by multiplication of the phantom concentration with the ratio $\alpha_{ex} \cdot S_{ex} / \alpha_{ph} \cdot S_{ph}$ of the weighted signals. Both signals are individually corrected for different coil loading using the acetone signal.

Results: The figure shows the correlation of both methods. Strong correlations were found for males (r=0.922), females (r=0.881), and all volunteers (r=0.852). Confidence intervals for all interceptions include 0, however, the slopes are significantly different from 1 in males, females, and all.



Discussion & Conclusion: Since a strong correlation was found between the two methods, it is concluded that both methods are suitable for quantitation in *institutional units* [i.u.], which are "absolute" as long as the identical method is applied. However, [i.u.] include a fixed yet unknown factor as compared to other units. Absolute concentrations in *standard units* [e.g. mmol/kgww] allow for a comparison between different methods, however, concentrations in standard units determined by the two methods differ significantly. The inclusion of pre- and post-exercise measurements guaranteed the ability to detect systematic effects over a wide range of concentrations. A separation of data from males and females helps to evaluate systematic influences of coil geometry under realistic and frequent conditions. Thigh muscles in males are typically placed closer to the coil than in female volunteers due gender-specific thickness of subcutaneous adipose tissue. This might explain the observed differences. The *creatine standard method* has the advantage that it uses an internal standard that is distributed almost identical to glycogen, thus avoiding coil sensitivity problems. However, this method strongly depends on accurate determination of T1 saturation factors and NOE for both creatine and glycogen. Reliable literature values can hardly be found and differ significantly. Additionally, literature values for muscular creatine content vary between 27.2 and 36.2 mmol/kgww as table 3 in [5] shows. The *image matched csm method* avoids these problems by measuring a reference solution. It seems that the simplification of the csm determination has little influence and adds only little scattering to the data. In addition, results from *the image matched csm method* are closer to generally accepted biopsy data than those from the *creatine standard method*.

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