VARIATIONS IN BASAL LIVER AND MUSCLE GLYCOGEN IN TYPE II DIABETES DETERMINED USING ^{13}C-MRS

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Introduction

Measurement of glycogen concentration in skeletal muscles and liver using ^{13}C-MRS can provide an invaluable insight into energy storage especially in metabolic disorders such as Type II diabetes. It is therefore important to establish the reproducibility with which these measurements can be performed. We investigated the accuracy of measurement and variation with time of basal glycogen concentration in the calf muscles and liver using ^{13}C-MRS in both Type II diabetic and healthy subjects.

Method

The liver was studied in 10 Type II diabetic subjects (Age=62.3±9.4 years, Body mass index (BMI)=30.6±3.0 kg/m^2, Duration of diabetes=7.4±4.7 years, HbAlc=7.12±1.13%) and 10 healthy subjects (Age=54.5±9.3,BMI=27.8±3.6, HbAlc=5.4±0.2) and the calf was studied in 12 diabetic subjects (Age=63.8±6.3, BMI=30.7±3.0, Duration of diabetes=9.68±6.5, HbAlc=7.29±1.10) and 12 healthy subjects (Age=53.5±10.2, BMI=28.4±3.6, HbAlc=5.3±0.2). The subjects attended the Magnetic Resonance Centre on 3 separate occasions following an overnight fast; the second visit took place between 5 and 8 days after the first and the third visit took place between 21 and 28 days after the first. A 3T Philips Achieva system was used to acquire all MR data. On each visit an image was obtained from the torso using the body coil to allow identification of the region containing the liver. Spectra and images which were later used for quantification were then acquired from the liver and calf muscles using a surface probe containing a ^{13}C surface coil and quadrature ^{1}H coils.

The surface probe was placed over the region of interest and a T_1-weighted FFE image was obtained using the quadrature coils and a marker was placed at the centre of the probe enabled the distance between the coil and the region of interest to be found and used for quantification. ^{13}C spectra were acquired using a pulse-acquire sequence with CYCLOPS phase cycling, and decoupling was achieved using the WALTZ-16 sequence. The ^{13}C pulse was optimised to be 90° at a distance of 6cm from the coil for the liver and 3cm from the coil for the calf. Each spectrum resulted from 896 acquisitions with a repetition time of 1s, giving a temporal resolution of 15 minutes. All spectra were analysed with the MATLAB version of MRUI. Quantification was performed using a phantom with a known glycogen concentration which was positioned appropriately for liver and muscle.

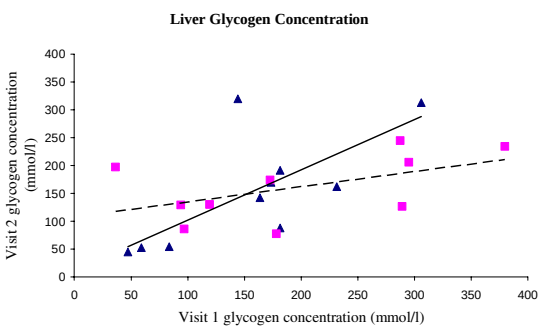
In 5 volunteers (2 healthy and 3 diabetic) multiple measurements were also taken on a single occasion to allow assessment of the variation in the measured glycogen concentration due to non-biological factors. 5 spectra were acquired in succession from the liver and calf muscles over a 1.5 hour period.

Results

Figures a. and b. show the correlation between glycogen concentration measurements taken on the first and second visits in the diabetic and healthy groups. Table 1 shows the correlation coefficients of glycogen concentrations measured on different visits in the liver and calf in both subjects groups. There was significant correlation between liver measurements taken on the first visit and second visit (a timescale of 5-8 days) in the healthy group, however in the diabetic subjects there was no significant correlation between measurements taken on these visits (fig a.). Between visits 2 and 3 (13-23 days) and 1 and 3 (21-28 days) there was no significant correlation in liver glycogen concentration in either the healthy group or the diabetic group.

In the healthy group, there was significant correlation between the calf glycogen concentrations measured over all three timescales. In the diabetic group, however, there was no significant correlation between the glycogen concentrations measured on any of the separate visits (fig b. shows comparison between visits 1 and 2).

a.



b.

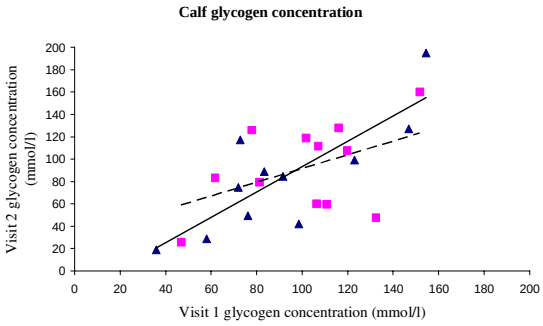


Fig 1: *Correlation between glycogen concentration measured on visits 1 and 2 in the liver (a) and calf (b) in healthy (▲) and type 2 diabetic (■) volunteers and the respective trendlines (—) and (---)*

For the repeat measurements, taken on a single day, the average coefficient of variation in glycogen concentration was determined to be 19% in the liver and 17% in the calf muscles. The average coefficient of variation between glycogen concentrations measured on different visits in the liver was 36% in the diabetic group and 33% in the healthy group; in the calf they were 26% in the diabetic group and 22% in the healthy group.

Discussion

The variation in glycogen concentration measured on the same day was 19% in the liver and 17% in the calf. Over this short time scale there should be little biological variation as subjects had fasted overnight and remained in the supine position. This variation can therefore by attributed to errors in the accuracy of the measurement. It was less than the variation seen between visits in both the calf muscles and liver and so suggests that a significant fraction of the latter variation seen between visits is of a biological nature.

In the healthy group, there was good correlation between the basal hepatic glycogen concentrations measured on visits 1 and 2 but not on visits 2 and 3 or 1 and 3. This suggests that fasting liver glycogen concentrations may vary significantly over a time period of 2 weeks. In the calf, there was correlation between the glycogen concentrations measured over all timescales investigated, with similar correlation coefficients for all, suggesting that basal glycogen concentrations in the calf muscles remain fairly constant over at least a 1 month period.

In the diabetic group, there was no correlation in the liver glycogen concentration between any of the visits, suggesting that liver glycogen can vary significantly over as little as a week in diabetics and that the variation may be much more than in non-diabetic subjects. In the calf muscles the glycogen concentrations also varied significantly between all visits, in stark contrast to the healthy group.

Liver glycogen concentration		
Duration between measurements (days)	**Correlation in healthy subjects (R)**	**Correlation in diabetic subjects (R)**
5-8	0.710 (p<0.021)	0.515 (p=0.182)
13-23	0.405 (p=0.246)	0.16 (p=0.645)
21-28	0.459 (p=0.182)	0.567 (p=0.087)
Calf glycogen concentration		
Duration between measurements (days)	**Correlation in healthy subjects (R)**	**Correlation in diabetic subjects (R)**
5-8	0.813 (p=0.002)	0.460 (p=0.132)
13-23	0.782 (p=0.004)	0.753 (p=0.083)
21-28	0.863 (p<0.001)	0.490 (p=0.106)

Table 1: *Pearson correlation coefficients of variation between glycogen concentrations measured on different visits.*

These results reveal the extent of the biological variation in basal liver and calf glycogen concentrations in Type II diabetics and age and BMI matched controls. It is particularly severe in the diabetic group and must be taken into account when drawing conclusions from measurement of basal glycogen.