VARIATIONS IN BASAL LIVER AND MUSCLE LIPID LEVELS IN TYPE II DIABETES DETERMINED USING ^{1}H MRS

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Introduction: Recent evidence suggests that insulin resistance in type II diabetes associated with excessive triglyceride levels in tissues such as the liver and skeletal muscle[1]. Although studies have previously been carried out to establish reproducibility in IMCL in lean and overweight subjects and over shorter time spans[2], there are few previous studies observing reproducibility of lipid measurements in either liver or calf, and especially in diabetic subjects where results might be expected to be more variable due to metabolic abnormalities. This study was undertaken in order to establish the reproducibility of liver and calf lipid measurements in both healthy and type II diabetic subjects over periods of 1 month.

Method: For both liver and calf lipid, three measurements were taken over a 28 day period, on days 1, 5-8 and 21-28. All subjects were requested to record their dietary intake for 3 days prior to each scan and to abstain from alcohol and exercise. The evening before each scan, subjects were requested to eat the same meal, followed by a 12h overnight fast. On each visit, measurements of blood glucose, plasma insulin and HbA1C were also taken. All ^{1}H MRS measurements were acquired on a Philips Achieva 3T system using a transmit/receive body coil.

Results are presented as lipid/water ratios as water levels are assumed to be constant in hepatic and skeletal muscle tissue under baseline conditions.

Liver lipid reproducibility: Ten healthy subjects (mean ± SD: age = 55±10 years, BMI = 27.8±3.5 kg/m^2, HbAlc=5.3±0.2%) and ten type II diabetic subjects (age = 62±10 years, BMI = 30.6±3.0 kg/m^2, duration of diabetes = 7.4±4.7 years, HbAlc=7.4±1.3%) were recruited. T_1-weighted TFE images (flip angle=15°, resolution = 1.76x1.76x15mm^3, with 60 slices in the transverse plane and 10 sagittal slices) were acquired to allow positioning of the volume within the right lobe of the liver and for calculation of total liver volume. ^{1}H MR spectra were then acquired using a PRESS sequence with the following parameters: TE/TR = 40/5000ms, VOI = 30x30x30mm, N_{ave} = 8, BW = 2000Hz, 1024 samples.

Calf lipid reproducibility: Ten healthy subjects (mean ± SD: age = 52±10 years, BMI = 27.6±3.5 kg/m^2) and seven type II diabetic subjects (age = 66±5 years, BMI = 30.6±3.0 kg/m^2, duration of diabetes = 10.0±5.9 years) were recruited. T_1-weighted TFE images were acquired, as for the liver, to allow positioning of the volume within the soleus muscle. Two ^{1}H MR spectra were then acquired; water-suppressed for measurement of IMCL/water (N_{ave}=16) and non water-suppressed for total calf lipid/water measurement (N_{ave}=8). PRESS localization was used with the following parameters: TE/TR = 40/7000ms, VOI = 20x20x20mm, BW = 2000Hz, 1024 samples. All spectra were post-processed using jMRUI and peak areas were calculated using the AMARES algorithm, fitting to Gaussian lineshapes, and an in-house written Matlab program.

Same day consecutive measurements: 4 of each of the above measurements were also performed in a single session, repositioning the voxels between scans, on four diabetics and two healthy subjects to establish the effect on the measurement accuracy of scanner variation and repositioning.

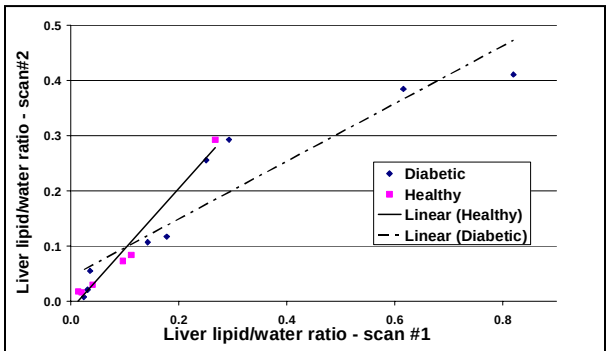

Figure 1: An example plot showing correlation between liver lipid/water measurements on visits 1 and 2.

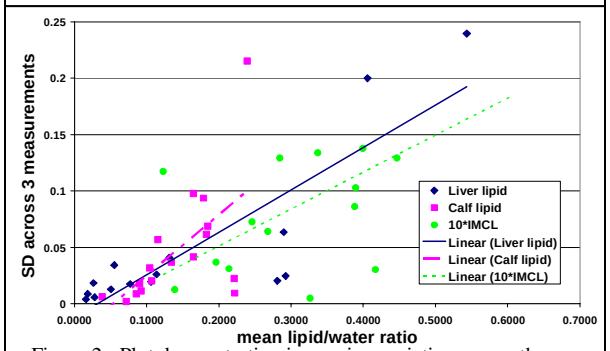

Figure 2: Plot demonstrating increasing variation across the three measured values with increasing mean value for liver and calf lipid/water ratio and IMCL/water ratio.

Results and Discussion: Percent coefficients of variation values (%CV) from same day reproducibility studies showed relatively small intra-subject variation in liver lipid levels which were not substantially different between the diabetic and healthy subjects (9.2% and 13.3% respectively). The %CVs calculated from repeat measurements over a month were much (3-fold) larger (30.1% and 34.6%) when compared with the same day repeat %CVs. This implies a biological (probably dietary) influence on liver lipid content which is supported by Pearson correlation coefficients (table 1 and fig. 1) whereby correlation between measurements decreases with increasing time between measurements. %CVs for calf total lipid/water for same day repeatability measurements were similar for both the diabetic and healthy groups (14.7% and 19.0% respectively) and are much smaller than those found when comparing data recorded over a month (28.9% and 29.6%). Similarly the %CVs for IMCL/water ratios were also smaller when measured on a single day for diabetic (19.4%) and healthy (25.5%) subjects compared with those taken over a month (24.5% and 31.8% respectively). Values calculated over the period of a month are comparable with previous measurements in overweight subjects (31.3%) in the tibialis anterior (TA) muscle from Shen et al[1]. Pearson correlation coefficients calculated for both total calf lipid and IMCL (table 1) showed poor correlation although this may be due to smaller numbers of pairs of values compared with %CVs which were calculated from all subjects with more than two liver lipid measurements. For liver and calf total lipid/water and IMCL/water the SD of the three measurements was found to be significantly correlated with the mean measured value in each individual subject (R = 0.86 (P<0.001), R=0.603 (p=0.01) and R=0.633 (p=0.01) respectively, Fig 2). This explains the similar coefficients of variation despite different absolute lipid contents for the groups.

Liver Lipid/water Correlation R (p)		
time between scans (days)	Diabetic	Healthy
5-8	0.94 (p<0.001)	0.97 (p<0.001)
13-23	0.90 (p=0.001)	0.97 (p<0.001)
21-28	0.79 (p=0.01)	0.95 (p=0.004)
Calf Lipid/water Correlation R (p)		
5-8	0.99 (p=0.02)	0.67 (p=0.1)
13-23	0.48 (p=0.4)	0.24 (p=0.6)
21-28	0.22 (p=0.7)	0.47 (p=0.2)
Calf IMCL/water Correlation R (p)		
5-8	0.65 (p=0.5)	0.32 (p=0.5)
13-23	(-)0.91 (p=0.09)	0.53 (p=0.3)
21-28	0.51 (p=0.5)	0.90 (p=0.006)

Table 1: Pearson Correlation coefficients (R) between three measurements.

Conclusion: Measurements of hepatic and skeletal muscle lipid level variations over a month in diabetic and healthy subjects are similar and larger than would be expected from inaccuracies due to instrumental variation and repositioning errors alone, indicating substantial biological changes in basal concentrations. In each case, the extent of these changes scales with the mean value of the lipid/water ratio.

References: 1. McGarry, J. D, Diabetes (2002) 51(1), pp7. **2.** Shen, W. et al: NMR Biomed (2007)