

## Simultaneous measurement of fat content and phosphorous compounds of liver in middle aged to elderly healthy subjects

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### Introduction:

Type 2 diabetes (T2D) is the most common chronic metabolic disease in the elderly, affecting approximately 30 million people over 65 years old in developed countries. Insulin resistance is the best predictor of the development of T2D and associates with increased ectopic and visceral fat, and altered mitochondrial activity as assessed with MRI, <sup>1</sup>H and <sup>31</sup>P MRS [1-5]. While this suggests a potential role of muscular mitochondrial function in the decline of insulin sensitivity during aging [4], no data on <sup>1</sup>H and <sup>31</sup>P MRS in the liver have been reported with respect to age. Of note, we have recently reported that ATP and Pi content are altered in the livers of T2D, but the relative roles of age versus body mass are not entirely clear [1]. Thus, this study aimed to simultaneously measure liver fat content and ATP/Pi ratios using <sup>1</sup>H and <sup>31</sup>P MRS and to test their relationships with age and body mass.

### Materials and methods:

**Subjects:** All subjects were locally recruited and consented to a research protocol which was approved by the local review board of human studies. Based on the aims of the study, 51 subjects with detectable liver fat above 0.5% were included in this study (healthy volunteers age ranging 40-69 years old, age: 60.0 ± 8.2 years old, body mass index (BMI): 26.1 ± 2.6 kg/m<sup>2</sup>).

**MRI and <sup>31</sup>P MRS:** MR data were acquired on a 3 Tesla MR scanner (Philips Achieva X-series, the Netherlands) using a 14 cm circular <sup>31</sup>P receiver/transmitter RF-coil for <sup>31</sup>P MRS, a 16 channel SENSE receive coil for liver <sup>1</sup>H MRS. The body coil was used for transmission for imaging, NOE and proton decoupling. Transverse and coronal images were employed to properly localize a 60x60x60 mm<sup>3</sup> voxel within the liver. The acquisition sequence parameters were TR/NS/pulse sequence = 4 sec/192/ISIS followed by an adiabatic excitation pulse using proton decoupling and NOE, SW=3000 Hz, data points=2K. Parameters for proton-decoupling and NOE were employed similar to a previous publication [6].

jMRUI (MRUI for Java, Magnetic Resonance User Interface, EC Human Capital and Mobility Networks, France) was used for processing of <sup>31</sup>P MRS using the AMARES algorithm with *a priori* knowledge. Post processing consisted of 15 Hz Gaussian line broadening, phasing and shifting the  $\gamma$ -ATP signal set at -2.48 ppm. *A priori* knowledge with some constraints was applied to analyze spectra, such as, Gaussian shape fitting; fixed relative phases; soft constraints on the expected resonance frequency; equal linewidth of both PME and PDE signals; linewidth of NADPH and UDPG constrained to  $\alpha$ -ATP. The linewidth of PEP was also constrained to PDE, thus a broad resonance could not overlay and distort the PDE signal. After testing 4 combinations of Gaussian and Lorentzian shapes in apodization and line fitting, Gaussian apodization and line fitting were chosen based on minimal residuals after subtraction of simulated peaks from the original spectra.

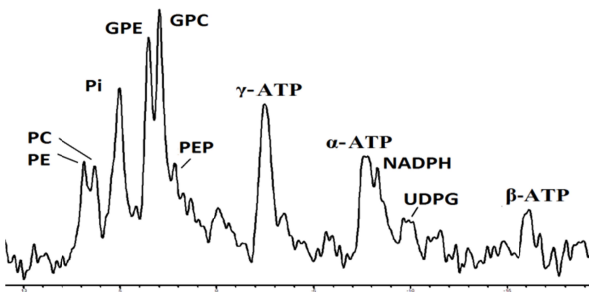
**<sup>1</sup>H MRS of the liver:** A STEAM sequence was utilized with the following acquisition parameters: VOI:3x3x2 cm<sup>3</sup>; TR=4 sec; TE/TM=10/16 msec, NS=32 with and without water suppression. <sup>1</sup>H MRS spectra were processed with NUTS software (Acom NMR, CA, US). Liver fat content was calculated relative to water after correcting T2 values [7].

**Statistics:** All evaluations for correlations were performed using Pearson product moment, SigmaStat® (Systat Software, Inc).

P values <0.05 were considered statistically significant.

### Results and Discussion:

A typical <sup>31</sup>P-31 MRS spectrum is shown in the below. The absence of the phosphocreatine signal at ~0 ppm indicates an excellent localization within the liver without infiltration of overlaying muscles. Eleven peaks can be identified in <sup>31</sup>P MRS of liver. The mean values of  $\gamma$ -ATP/P<sub>i</sub> ratio and liver fat content were 1.25±0.29 and 4.5±6%. The  $\gamma$ -ATP/P<sub>i</sub> correlated with the liver fat content ( $r=0.29$ ,  $p<0.05$ ). This may reflect a decreased P<sub>i</sub> or an increased  $\gamma$ -ATP content in the subjects with higher liver fat. In a group of insulin resistant T2D subjects, it was reported that both P<sub>i</sub> and  $\gamma$ -ATP content decreased, but P<sub>i</sub> decreased to a greater extent [1]. Thus the current results are consistent with the previous publications [1,2]. BMI correlated with liver fat content ( $r=0.30$ ,  $p<0.05$ ) but not with ATP/P<sub>i</sub>. In conclusion, increased liver fat content implies an increase in insulin resistance during aging, with altered ATP and Pi content, especially after middle age.



### References

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