Effects of High versus Low Fatty Acid Levels During Hyperinsulinemia by Measuring Relative Intromycellular and Extramyocellular Triglyceride Levels with Proton Magnetic Resonance Spectroscopy in Normal Subjects

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Introduction

Although insulin resistance is a key component in the pathogenesis of type 2 diabetes, the complex mechanisms which cause insulin resistance are not fully understood. Recent muscle biopsy studies (1) have shown a relation between fatty acid metabolism and insulin resistance. Proton magnetic resonace spectroscopy (MRS) studies (2,3) have also demonstrated a correlation between increased intramyocellular lipid levels (IMCL) measured in soleus muscle and insulin resistance measured by euglyemic hyperinsulinemic clamp.

The aim of this proton MRS study was to examine the dynamic response of the IMCL content in two groups (N=7 in each group, 4 males, 3 females) of young healthy non-obese volunteers. Spectra were acquired both before and after either a lipid and heparin or saline infusion under eugleycemic hyperinsulinemic conditions for comparison.

Methods

Each subject was admitted to the Temple University General Clinical Research Center before 5 p.m. on the evening prior to the study and fasted for 12 hours before the first MR spectra were acquired. After the first MR spectra was obtained, subjects underwent four hours of hyperinsulinemic euglycemic clamp, insulin clamped at 450 pmol/l, glucose at 5 mmol/l . One group received an infusion of lipid and heparin, while the other group received saline. Free fatty acid levels were also measured. Another MR spectra was obtained ~6 hours after the start of the hyperinsulemic euglycemic clamp. For MRS, subjects were brought to the Temple University Hospital Outpatient Imaging Center and placed in the MRI scanner (Signa 1.5T, GE Medical Systems, Waukashsaw, WI) in the supine position. Each subject's calf was placed on padding in a transmit/receive extremity coil. The superior-inferior center was chosen at the largest diameter of the calf. Care was taken to ensure that the calf was not angled in the coil to avoid the magic angle variation in the IMCL peak. The calf was marked with an ink cross at the position of the aligning crosshairs from the scanner to ensure that the repeat scan after the intervention was performed in the same position. Anatomical axial T1-weighted spin-echo images (TR=400ms, TE=10 ms, FOV=16cm, 256x256) were acquired with high contrast between muscle tissue and the hypointense facial planes. The soleus muscle was easily distinguished on these images.

The voxel for MR spectrum acquisition was placed in the slice that showed the largest diameter of the soleus muscle, typically this slice was at isocenter. The voxel was either ~8 cc or ~1 cc depending the homogeneity of the tissue in the soleus muscle. Voxels were placed in homogeneous muscle tissue avoiding vessels and facial planes in all three directions. The 3D reformatting capabilities at the scanner console were used to check for homogeneity in all three planes. The spectra were acquired using a standard clinical PROBE pulse sequence with water suppression, TR=1174ms, TE=35ms, NEX=8, scantime=1:47, FOV=16cm, 1024 spectral points.

The SAGE spectroscopy software package (GE Medical Systems, Fremont CA) was used to analyze the spectroscopy data sets. Raw data files were reconstructed using the PROBE quantification algorythim. The following steps are performed: pure water subtraction, convolution filtering, apodization, zero-filling, and fast Fourier transform. After reconstruction, the resulting spectra were phase-corrected with a zero-order phase correction and baseline subtracted.

Peak areas were measured for 0.1 ppm width areas centered on the following peaks: creatine, EMCL methylene, and IMCL methylene peaks. A width of 0.1ppm was used to measure the ratio of the peak areas pre- and post-infusion because the spectral resolution was inadequate to resolve the methyl and methylene EMCL and IMCL peaks in some subjects. The normalization of two different spectra taken at the different times was carried out by using the ratio of the creatine peak at 3.04ppm as a standard.

Results

All data reported in these results had at least 94% of the water signal suppressed and full width half maximum of the spectra were equal to or less than 11 Hz to resolve the methylene EMCL and IMCL peaks. Typically the creatine at 3.0 ppm, methylene EMCL at 1.5 ppm and IMCL peak at 1.3 ppn were well resolved (see Figure 1) while in the spectra from some subjects the methyl EMCL at 1.1 ppm and IMCL at 0.9 ppm peaks were also visible. In the group infused with lipid and heparin the IMCL content increased (mean of post/pre integral areas +/- s.e.m. = 1.12 +/- 0.05), EMCL remained constant (1.00 +/- 0.36) and free fatty acids levels remained between 500-700 umol/l. In the group infused with saline, the both IMCL and EMCL levels remained constant (0.95 +/- 0.03 s.e. and 1.07 +/- 0.39 s.e. respectively) while free fatty acids dropped from 500 to 50 umol/l.

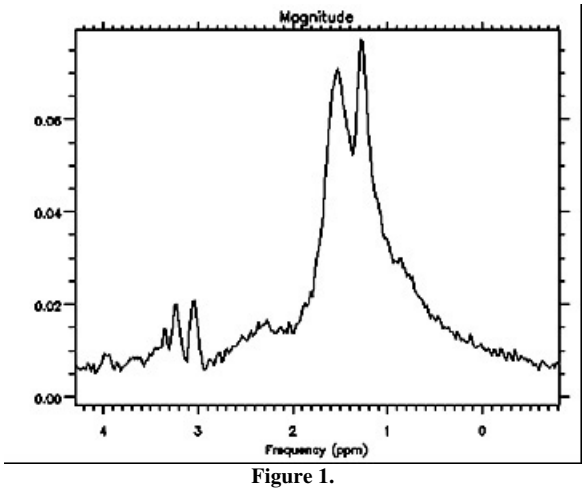

Figure 1.

Discussion

These results showed that under conditions of high fatty acid and hyperinsulinemia there was an 12% increase in IMCL-TG content and a 40% increase in insulin resistance. When fatty acids were low there was a trend toward a decrease in IMCL-TG and normal insulin sensitivity. These data support the hypothesis that increased intracellular triglyceride levels are related to insulin resistance.

References

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