HR-MAS Spectroscopy of Human Testicular Biopsy Tissue Demonstrates Phosphocholine as a Biomarker of Male Fertility

R. Iman[1], M. Swanson[1], P. J. Turek[2], D. Aaronson[2], H. Gurascier[1], D. Vigneron[1], S. Nelson[1], and J. Kurhanewicz[1]

[1]Radiology, University of California San Francisco, San Francisco, CA, United States, [2]Urology, University of California San Francisco, San Francisco, CA, United States

Introduction

The clinical evaluation of male infertility is challenging due to the wide biological variation observed in semen analyses, hormone levels, and testicular biopsies. Previous ^{31}P MRS studies have demonstrated higher levels of phosphomonoesters (e.g, phosphocholine) in normal versus azoospermatic men (1). Phosphocholine (PC) is a major precursor in phospholipid membrane synthesis and could serve as a specific marker for spermatogenesis. With recent technical advances in water and lipid suppression, high spatial resolution ^{1}H MRSI mapping of the in vivo human testes is now clinically possible. To determine metabolic biomarkers for *in vivo* ^{1}H MRSI studies of infertile men, *ex vivo* ^{1}H high resolution magic angle spinning (HR-MAS) spectroscopy was performed on human testicular biopsy tissues.

Methods

Fourteen testicular biopsies were obtained from 14 different patients with normal (N=4), maturation arrested (N=5), or azoospermatic (N=5) diagnoses. Samples were weighed (mean 15.77 ± 6.62 mg) and placed into custom designed 20 or 35 µl leak proof zirconium rotors containing 3.0 µl D_2O + 0.75% TSP. ^{1}H HR-MAS data were acquired at 11.7T, 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4 mm gHX nanoprobe. Quantitative 1D spectra were acquired with 2s relaxation, 2s presaturation, 2s acquisition (TR = 6s), 40,000 points, 20,000 Hz spectral width, and 256 transients. The Electronic Reference To access In vivo Concentrations (ERETIC) (2) method was used as a quantitation standard. Spectra were fit using a custom version of QUEST (3) adapted for analysis of short-echo time HR-MAS spectra containing 40,000 points. Basis set spectra of 19 metabolites in solution and known macromolecular peaks were incorporated into the QUEST fitting routine. The custom version of QUEST estimated the macromolecule signals using an HLSVD algorithm and iterated between fitting the metabolites and modeling the macromolecules 12 times. Finally, concentrations were calculated relative to the peak area of the ERETIC signal. A one-way ANOVA was performed with three classes to determine which metabolites were the best predictors of male infertility.

Results

Figure 1 shows representative 1D presat HR-MAS spectra of A) normal and B) azoospermatic testicular tissue. Note the much larger PC signal observed in normal vs azoospermatic tissue. The concentrations of phosphocholine (PC) in normal, maturation arrested, and azoospermatic testes are shown in Figure 2. The concentration of PC in azoospermatic tissue (mean = 1.52 ± 0.27 mmol/kg) was significantly lower than in normal (mean = 5.35 ± 1.37 mmol/kg, p= 0.0113) testicular tissue. Moreover, the concentrations of PC in azoospermatic biopsies were very tightly grouped (1.26–1.89mmol/kg) and there was no overlap with PC concentrations in normal biopsies. PC concentrations in the maturation arrested biopsies fell between these two groups, with some overlapping the normal and others overlapping the azoospermatic groups.

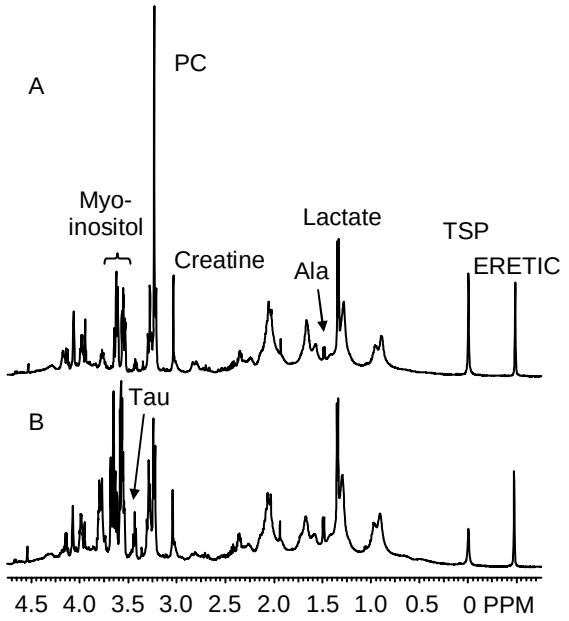

Figure 1. ^{1}H HR-MAS spectra of A) normal and B) azoospermatic human testicular tissue.

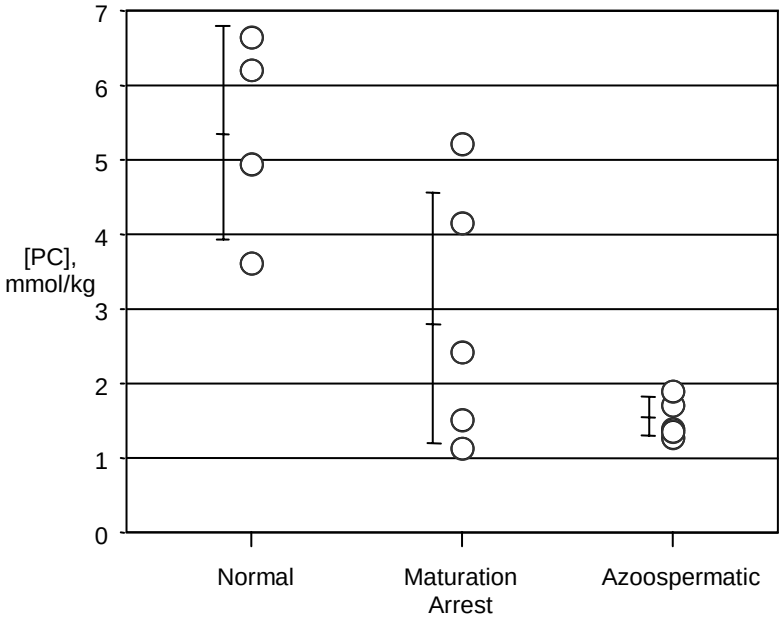

Figure 2. PC concentrations (mmol/kg) in normal, maturation arrested, and azoospermatic human testicular tissue.

Discussion and Conclusions

The observation that PC concentrations were significantly higher in normal testes and did not overlap with azoospermatic testes is consistent with the markedly higher metabolic activity resulting from spermatogenesis in which ~1000 sperm/sec are produced compared to the azoospermatic testis in which there is very reduced sperm production. The normal PC concentrations (3.61–6.64mmol/kg) on the other hand were much more spread out consistent with the natural variability of sperm production in fertile men. The lack of overlap of the normal and azoospermatic testis suggests that PC concentration is a very good biomarker of spermatogenesis and fertility in individual men. The maturation arrested group of men demonstrated varying levels of sperm production clinically, and this is reflected in PC concentrations that overlap both normal and azoospermatic testis. Although there is a clear need for a larger patient cohort study to validate these findings, the concentration of PC measured by MRS provides a sensitive quantitative assessment of male infertility.

References

1) Chew WM, et al. (1990) Radiology 177:743-747; 2) Ziarelli F and Caldarelli S. (2006) Solid State Nucl Magn Reson 29:214-218; 3) Ratiney H, et al. (2005) NMR Biomed 18(1):1-13.