Evaluation of Human Prostate Tissue Metabolites with HRMAS 1HNMR After Three-Year Storage at -80ºC

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Introduction: The assumption that tissue sample storage in the –80ºC condition prevents metabolite degradation or alteration is widely accepted, but has never been tested. Considering the up and coming prominence of metabolite studies for biomedical utilities, and taking into consideration that many of the samples for these studies come from tissue banks where long term storage is implicit, we felt the effect on metabolite concentrations, if any, of extended storage time should be examined. In this study human prostate tissues, removed from various zones of five prosatectomy patients, that had been stored at –80ºC for 32 months were analyzed with high resolution magic angle spinning (HRMAS) MR spectroscopy, and compared to the original measurements of the same specimens from 2002 when snap frozen for less than 24 hours.

Materials and Methods: In 2002 we conducted a study that evaluated the tissue freeze-thawing process in the presentation of HRMAS spectroscopy measured metabolite concentrations. The 11 excess tissue samples from the 2002 study were maintained at -80ºC for three years. In 2005 four samples with extra material were duplicated and a total of 15 samples were run via the same experimental protocol as the 2002 study. Briefly, samples were carried out on a Bruker (Billerica, MA) ADVANCE spectrometer operating at 600 MHz (14.1T); spectra were collected at 3ºC for optimal tissue preservation with spinning speeds of 600 and 700 Hz with or without rotor synchronized DANTE sequence of 1000 DANTE pulses of 1.5 µs (8.4º flip angle). Spectra measured at 600 Hz spinning without DANTE were used to quantify the total metabolite signal intensities. Spectroscopic data were processed with Nuts software (Acorn NMR Inc. Livermore, CA). Following spectroscopic analyses all samples were formalin fixed, paraffin embedded, cut at 5µm, and H&E stained. Serial sections at 100 µm apart were read by a pathologist and volume percentages of histological benign epithelia and stromal cells were quantified.

Results and Discussion: Figure 1 compares two proton HRMAS spectra acquired from the same prostate specimen from (a) the 2005 and (b) the 2002 study, illustrating that metabolic alterations after storage at –80ºC are as minimal as visual evaluation can dissimilate. While other samples in the study produced visually dissimilar spectra, histopathology revealed that dissimilarities in spectra are more critically influenced by tissue heterogeneity then freeze storage effects. To confirm this hypothesis we examined the paired t-tests for 21 common metabolite resonance peaks measured from HRMAS spectra; significant metabolite differences exist (after Bonferroni corrections) between the 2002 and 2005 samples *only* if the vol% epithelial difference is greater that 20%. When rigorous controls on pathological compositions are applied we found that no statistically significant metabolic differences exist when absolute epithelial differences are less than 10% or relative differences are less than 20% (**Table 1**).

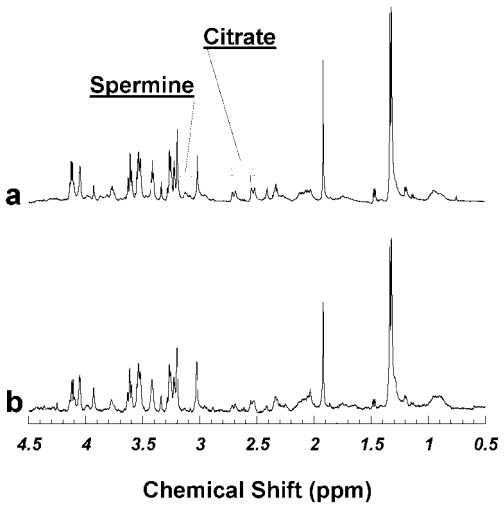

Figure 1: Human prostate tissue HRMAS proton spectra from the same surgical specimen of a cancerous prostate measured (a) in 2005 after stored at –80ºC for 32 months, and (b) in 2002 after snap freezing.

Table 1: The concentration (mM) of 21 prostate metabolites for seven sample pairs with relative differences of epithelial volume percentages less than 20% within each pair; mean, standard deviation, and paired t-tests are compared.

	2005		2002		Paired t-Test
	Mean	SD	Mean	SD	
Lac(4.10-4.14) [a]	14.29	3.98	12.87	6.68	0.38
MI(4.05)	10.30	2.77	12.57	5.39	0.31
3.60-3.63 [b]	16.98	6.78	20.17	13.57	0.56
3.34	5.18	2.08	5.49	1.95	0.82
3.29	1.11	0.63	2.53	2.13	0.13
3.27	8.79	2.38	11.17	5.60	0.30
3.25-3.26	12.22	3.32	13.95	5.85	0.26
Pch(3.22)	1.05	0.28	1.44	1.05	0.38
Chol(3.20)	1.53	0.22	1.70	0.91	0.64
Spm(3.05-3.14)	1.75	2.69	2.21	1.72	0.52
Cr(3.03)	2.66	0.92	5.19	3.69	0.08
Cit(2.70-2.73)	4.87	1.86	3.44	1.66	0.19
2.31-2.37	5.60	2.13	10.17	3.32	0.06
2.01-2.14	29.67	14.92	42.51	6.99	0.13
Acet(1.92)	1.36	3.02	1.19	2.54	0.44
1.68-1.78	10.89	7.11	10.98	7.34	0.98
Ala(1.47-1.49)	1.00	0.42	1.52	1.13	0.18
Lac(1.32-1.34)	17.45	4.18	20.53	9.80	0.37
1.19-1.20	2.70	2.30	2.13	2.67	0.35
1.04-1.05	0.51	0.32	1.59	2.21	0.25
Lipid(0.90) [c]	22.87	5.11	14.65	6.55	0.03

Conclusion: Analyzing samples that have been preserved at –80ºC for 32 months, and comparing their spectroscopic results to those from the same specimens after they were snap frozen for less than 24 hours, led us to conclude that the effect of freeze storage on metabolite concentration is negligible. Such alterations, even if in existence, are much less critical to the interpretation of tissue HRMAS spectroscopy for disease management purposes than the influence of tissue heterogeneities. These results are prostate tissue specific, however, and cannot be directly extended to other tissue types without further study.