Assessing the potential of ^{1}H MRS in analyzing the effects of various dietary fatty acids on normal colon

S. Davda[1,2], R. Bird[3], M. Eskin[2], B. Dolenko[1], and T. Bezabeh[1,2]

[1]Institute for Biodiagnostics, National Research Council, Winnipeg, Manitoba, Canada, [2]Human Nutritional Sciences, Univ. of Manitoba, Winnipeg, Manitoba, Canada, [3]Biology, Univ. of Waterloo, Waterloo, Ontario, Canada

INTRODUCTION: Epidemiological and basic science research has shown that fatty acid composition of dietary lipids affect colon carcinogenesis and inflammation. The omega 3 fatty acids are advocated to be anti inflammatory whereas highly saturated and ω-6 fatty acids may not (1). Omega-3 fatty acids in part could inhibit the formation of leukotrienes, the pro-inflammatory cytokines. Colonic tissue lipid composition reflects fatty acid composition of the dietary lipid. In this study we wanted to investigate if MRS spectra of colonic tissues will be able to differentiate the fatty acid composition of the ingested lipid. Our previous study has shown that ^{1}H MRS is sensitive in assessing inflammatory changes in the colon (2) and thus, may have the potential to assess the differential effects of various fatty acids on colonic mucosa. This is important to guide further studies to identify the association between diet and inflammatory bowel disease or colon cancer. The window between the development of inflammation and colon cancer is wide and if dietary intervention can be provided to modulate the ongoing changes, it might be possible to halt its progression to colon cancer. Our study can provide groundwork for such research, using MRS as a sensitive tool for diagnosing as well as monitoring the efficacy of intervention in inflammatory bowel disease and colon cancer.

MATERIALS AND METHODS: Twenty male Sprague Dawley rats were fed with low fat corn oil (n=5), high fat corn oil (n=5), high fat flax seed oil (n=5) or high fat beef tallow (n=5) for 2 weeks. Low fat corn oil diet contained 5% corn oil based on AIN-76A diet. High fat diets contained 5% corn oil and additional 7% fats from corn oil, flax seed oil or beef tallow. After sacrificing the animals, colon was excised and mucosal layer was stripped off using a glass slide. The samples were frozen in PBS/D_20 at -70^{o}C. For ^{1}H MRS, the samples were thawed and cut into 5 mm pieces along the length. Out of the total samples collected, every alternate piece was subjected to ^{1}H MRS on Bruker 360 MHz spectrometer at 25 ^{o}C with presaturation of the water signal. This resulted in a total of 127 spectra. The acquisition parameters included: 90^o pulse at 9.30μsec, number of scans = 256, spectral width = 4990.02 Hz, relaxation delay =3 sec and TD = 8K. For multivariate analysis, each MRS spectrum was normalized by dividing total data points by the total spectral area and then aligned to the reference peak due to TSP. The region between 0.5 and 4.5 ppm was selected to eliminate the excess water signal at 4.7 ppm. First derivatives were taken, and rank ordering was done on the resulting data to eliminate baseline differences between spectra. For low fat corn oil vs each of the three other diets, random subsets of the data were used to train a genetic algorithm-based subregion selection method (3). The regions selected most often by the genetic algorithm were used to develop the final classifier: LDA with coefficients optimized using a bootstrapping method (4).

RESULTS & DISCUSSION: Table 1 shows that ^{1}H MRS along with multivariate analysis can classify colon specimens subjected to various dietary interventions with accuracy ranging from 92 to 98.4%. This indicates that ^{1}H MRS is sensitive in detecting the subtle alterations in metabolite concentration caused by various fatty acids. Our region selection algorithm identified discriminatory regions that include resonances due to glycerol, unsaturated carbon of monounsaturated fatty acid, polyunsaturated fatty acid and the terminal methyl group in fatty acids. These fatty acids may also cause changes in histological appearance of the colonic mucosa. We are in the process of doing histological assessment using H&E stain on these samples.

Low fat corn oil (control) Vs. High fat corn oil					
Desired class	**N**	**Classification Correct**	**False**	**Accuracy**	**Overall accuracy**
Control	32	31	1	96.9 %	96.8 %
High fat corn	31	30	1	96.8 %	
Low fat corn oil (control) Vs. Flax seed oil					
Control	32	32	0	100 %	98.4 %
Flax oil	30	29	1	96.7 %	
Low fat corn oil (control) Vs. Beef Tallow					
Control	32	30	2	93.8 %	92.4 %
Beef tallow	34	31	3	91.2 %	

Table 1: Results of multivariate analysis

CONCLUSION: Our results suggest that ^{1}H MRS is very sensitive in detecting subtle changes caused by different PUFAs in normal colon. This can mark the beginning of a new perspective for ^{1}H MRS to serve as a tool for studying the effects of dietary fatty acids in inflammatory bowel disease and its progression to colon cancer.

REFERENCES

1. Hegazi R.A.F., et al., Nutrition; 22: 275-82 (2006).
2. Bezabeh T., et al., Am J Gastroenterol.; 96: 442-48 (2001).
3. Nikulin A. E., et al, NMR Biomed.; 11: 209-17 (1998).
4. Lean C.L., et al, Ann Rep NMR Spectrosc.; 48: 71-111 (2002).