Validating the potential of ^{1}H MRS in assessing the effects of dietary fatty acids on the modulation of inflammation in an animal model of inflammatory bowel disease (IBD)

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Introduction: Our previous work has shown that ^{1}H MRS is a sensitive tool for the detection of inflammation in IBD (1). We have also shown that it can detect the effects of various dietary fatty acids on normal colon (2). A large body of research has shown that ω-3 fatty acids have anti-inflammatory properties, whereas ω-6 fatty acids and saturated fats are pro-inflammatory (3). As dietary composition of fatty acids is translated into the colonic metabolic composition, these dietary fats can modulate colonic inflammation. The objective of the present study was to assess the potential of ^{1}H MRS in detecting the effects of dietary fat on inflammation in an animal model of IBD.

Material & methods: Twenty male Sprague Dawley rats were fed 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. Corn oil served as ω-6, flax seed oil as ω-3 and beef tallow as saturated fatty acids sources. The low fat corn oil diet contained 5% fat whereas the high fat diets contained an additional 7% of corn oil, flax seed oil or beef tallow by weight. All the animals were also fed 2% carrageenan in their diets to induce colonic inflammation. At the end of 2 weeks, all the animals were sacrificed and their colons were excised. The colonic mucosa was stripped from the deeper layers using a glass slide and cut into 5-7 mm pieces. ^{1}H MRS was performed on these samples using Bruker 360 MHz spectrometer at 25°C with presaturation of the water signal. This resulted in a total of 119 spectra. The acquisition parameters included: 90° RF pulse, NS = 256, SW = 4990.02 Hz, RD = 3 sec and TD = 8K. The MR spectra were analyzed using a multivariate analysis method and the following preprocessing steps: normalization to the total spectral area and alignment to the TSP 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 (4). The regions selected most often by the genetic algorithm were used to develop the final classifier: LDA with coefficients optimized using a bootstrapping method (5). Immediately after ^{1}H MRS, the mucosal samples were fixed in formalin and processed for histological analysis using haematoxylin-eosin stain.

Table 1: Multivariate analysis of control vs. dietary groups

Class	N	Accuracy	Regions (ppm)	Metabolites
Low fat corn oil Vs. High fat corn oil				
Control	24	96.6%	**2.69-2.79**	=HC-CH_2-CH=
			2.05-2.11	-H_2C-C**H=CH**-
High fat corn oil	35		**1.35-1.4**	**(CH_2) Chain**
			1.1-1.22	Unassigned
Low fat corn oil Vs. High fat flax seed oil				
Control	24	98.2%	**3.0-3.08**	**Creatine**
			2.69-2.83	=HC-CH_2-CH=
High fat flax oil	32		**1.15-1.22**	Unassigned
			0.87-0.94	**-CH_3**
Low fat corn oil Vs. High fat beef tallow				
Control	24	100%	**3.0-3.05**	**Creatine**
			2.76-2.9	=HC-CH_2-CH=
High fat beef tallow	28		**2.04-2.08**	**-H_2C-CH=CH-**

Table 2: Comparison of dietary groups amongst each other

Class	N	Accuracy	Regions (ppm)	Metabolites
High fat corn oil vs. Flax seed oil				
High fat corn oil	35	97%	**2.90-3.10**	**Creatine**
			2.58-2.78	=HC-CH_2-CH=
Flax seed oil	32		**1.95-1.98**	-H_2C-C**H=CH**-
			0.96 – 0.98	**-CH_3**
High fat corn oil vs. Beef tallow				
High fat corn oil	35	98.4%	**2.99-3.04**	**Creatine**
			2.69-2.77	=HC-CH_2-CH=
Beef tallow	28		**1.94-2.00**	-H_2C-C**H=CH**-
Flax seed oil vs. Beef tallow				
Flax seed oil	32	100%	**2.88-3.05**	**Creatine**
			2.57-2.68	=HC-CH_2-CH=
Beef tallow	28		**1.59-1.61**	**-H_2C**- CH_2-COO

Results & Discussion: The potential of ^{1}H MRS to analyse the effects of the proportion of dietary fat on IBD is indicated by the results of the multivariate analysis shown in table 1. A comparison is made here between the results of the high-fat diet groups and the control (low fat corn coil) group. Moreover, the 3 specialized diets were also compared amongst each other to identify whether ^{1}H MRS can differentiate between the effects of different types of fatty acids on inflamed colon. These results are shown in table 2. The metabolites found to be discriminatory are also shown in these tables. In the histological assessment, 21 out of 23 blocks analyzed from flax seed oil, and 20 out of 21 blocks from low fat corn oil group, had none to mild inflammation. On the other hand, all of the blocks from beef tallow (n=20) and high fat corn oil (n=23) group showed moderate to severe inflammation. We observed a tight concordance between histology results and ^{1}H MRS analysis.

Conclusion: Our results suggest that ^{1}H MRS can identify the effects of dietary fatty acids on inflammation in an animal model of IBD with accuracy ranging between 97 – 100%. In our study, low fat corn oil and high fat flax seed oil groups showed minimal inflammation suggesting that both the type and amount of fat are important to modulate colonic inflammation. The strong correlation between ^{1}H MRS spectral characteristics and histological analysis suggest that ^{1}H MRS can serve as a tool in further studies to analyze chronologically the role of diet in IBD and its progression to colon cancer.

References

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