University of Cincinnati

Date: 11/28/2011

I, Michael R Ciresi , hereby submit this original work as part of the requirements for the degree of Master of Science in Nutrition.

It is entitled:

Relationship between Dietary Intake of Fatty Acids and Disease Activity in Pediatric Inflammatory Bowel Disease Patients

Student's name: **Michael R Ciresi**

This work and its defense approved by:

Committee chair: Graciela Falciglia, PhD

Committee member: Abigail Peairs, PhD

2187

**Relationship between Dietary Intake of Fatty Acids and Disease Activity in Pediatric
Inflammatory Bowel Disease Patients**

A thesis submitted to the
Graduate School of the University of Cincinnati
in partial fulfillment of the requirements for the degree of

Master of Science
in the Department of Nutrition
of the College of Allied Health Sciences

by

Michael R. Ciresi

B.S. The Ohio State University

June 2008

Committee Chair: Grace Falciglia, Ph.D.

Abstract

Background. Crohn's disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel disease (IBD), are chronic illnesses that affect predominately the gastrointestinal tract. The pathogenesis and etiology remain unclear but the importance of environmental factors, in particular diet, is evidenced by the increased incidence rates of the recent decades that genetic inheritance cannot account for. In particular, the quantity of fatty acid consumption has been consistently linked with IBD risk. While several studies have investigated the connections between diet, etiology, signs and symptoms associated with IBD, very few have explored the relationship between disease state and specific fatty acid intake in the pediatric IBD population. *Methods.* In this cross-sectional study, 100 pediatric patients from Cincinnati Children's Hospital and the Hospital for Sick Children in Toronto with diagnosed IBD (73 with Crohn's disease (CD) and 27 with ulcerative colitis (UC)) were included. Three-day diet records were collected from the patients for the assessment of their dietary intake. The abbreviated Pediatric Crohn's Disease Activity Index (PCDAI), the abbreviated Ulcerative Colitis Activity Index (PUCAI), and markers of inflammation (lipopolysaccharide binding protein (LBP) and S100A12) were used to assess disease severity. A logistic regression analysis was carried out to correlate disease severity with the dietary intake of specific fatty acids and total dietary intake. *Results.* Total caloric, saturated fat (SFA), and monounsaturated fat (MUFA) intake were negatively associated ($p < 0.05$) with PCDAI scores in CD alone. The individual SFAs butyric, caproic, caprylic, capric, lauric, myristic, palmitic, margaric, and stearic also were also negatively associated with disease activity scores in CD group. However, no significant associations were

observed between the major types of fatty acids and markers of inflammation. Margaric acid was the only fatty acid significantly associated ($p < 0.05$) with the markers of inflammation, as it was positively correlated with S100A12. *Discussion.* Our analysis indicates that both total fatty acid intake and total caloric intake were inversely associated with disease activity. A change in habitual dietary intake is the most likely explanation for this negatively associated trend. Relapsed patients consumed significantly lesser amounts of fatty acids and calories than patients who were in remission. The importance of this relationship should not be disregarded since pediatric IBD patients are at a high risk for growth failure, delayed puberty, anemia, osteoporosis, and other medical conditions. This study adds reason for the importance of follow-ups with nutrition professionals and gastroenterologists during remission and active states in order for pediatric IBD patients to maintain a healthy nutritional status.

Table of Contents

	Page
Introduction	
Significance of the Study	1
Literature Review	2-4
Purpose of the Study	5-6
Research Design and Methods	
Subjects	6
Assessment of Dietary Intake	7
Anthropometric Measurements	7
Biochemical Markers of Inflammation	7-8
Statistical Analysis	9-10
Results.	10-14
Discussion	14-19
Conclusion	19
References	20-22

List of Tables

Table	Page
1. Demographic and Clinical Characteristics	10
2. Average Intake of Nutrients	11
3. Disease Activity Scores and Levels of Markers of Inflammation	12
4. Logistic Regression (Fatty acid intake and disease scores)	12
5. Logistic Regression (Saturated Fatty acid intake and disease scores)	13
6. Logistic Regression (Fatty Acid Intake and Markers of Inflammation)	13
7. Logistic Regression (Saturated Fatty acid intake and Markers of Inflammation)	14

Introduction:

Crohn's disease (CD) and ulcerative colitis (UC), the two major clinical subtypes of inflammatory bowel disease (IBD), are chronic, autoimmune illnesses that affect predominately the gastrointestinal tract. CD and UC are both characterized by unpredictable periods of relapse and remission and are associated with multiple symptoms such as nausea, pain, and diarrhea which are due to the disruption of the gastrointestinal tract by inflammation. Although the two diseases have many similarities, there are important differences between them. For example, inflammation affects only the colon in UC while it may affect any part of the gastrointestinal (GI) tract in CD. Also, the pattern of inflammation that each form of IBD takes in the gastrointestinal tract is very distinct. Inflammation associated with UC tends to be continuous throughout the inflamed areas while the inflammation of CD is likely to occur in patches throughout the GI tract.

The burden of IBD is highest among adults in Western Europe and North America and seems to be on the rise in developing countries [1]. Of particular importance is the growing burden of IBD in children, as is estimated that one in every four diagnoses of IBD are made before the age of 20 years and this rate has continued to increase during the past ten years [2]. Childhood-onset IBD patients usually have a more aggressive form of the disease and growth failure is a critical concern in this population. It has also been shown that UC is associated with an increased risk of colorectal cancer and quality of life is severely impaired due to complications associated with this illness [3]. Although it is difficult to estimate the prevalence of IBD in children, it is believed

that about 100,000 children in the United States are suffering from these diseases at any given time [1]. A nationwide study in Canada revealed that the incidence rate of CD in the years 1998-2008 among children less than 20 years of age was approaching the incidence rate of CD in adults.

The exact pathogenesis and etiology of IBD has yet to be elucidated due its complexity. It is postulated that IBD represents the interplay of three essential cofactors; genetic susceptibility, environment and host immune response [5]. The importance of environmental factors is evidenced by the increasing rates of IBD diagnoses in the recent decades that genetic inheritance cannot account for. Of the many possible environmental factors, the 'Western diet', which consists mainly of foods containing high amounts of fats and calories and low levels of fruits and vegetables, is thought to have a strong connection with IBD [6]. There is growing epidemiological evidence for the effect of environmental factors, such as diet, on IBD. The incidence of IBD has increased dramatically in developing populations that have inherited a 'Western' diet [7]. Recent epidemiologic studies have assessed the dietary intake of adult patients with newly diagnosed IBD to explore potential dietary causes of disease onset. Saturated fat [8-10], refined carbohydrates [11-13], and sugar intake [8-9] have shown significant positive associations while fruits & vegetables [14,15] have shown significant negative correlations with IBD risk.

In relation to disease, saturated fat has been shown to have pro-inflammatory effects in the gastrointestinal tract in clinical and animal studies. When evaluating the dietary intake of

saturated fatty acids (SFA), it is a common practice in research to combine all of the individual fatty acids into one group. However, it should be noted that fatty acids behave independently in vivo and should also be investigated on an individual basis. For example, long chain SFA such as palmitic, myristic, and lauric acid have been shown in vitro to contribute to the activation of macrophages and production of pro-inflammatory cytokines, while short chain SFA such as butyric and caproic acid have provoked little influence in inflammation [38].

The breakdown of immunological tolerance towards the microflora in genetically susceptible individuals is believed to be the major event in the pathogenesis of IBD [39]. Though the role SFA play in this event is unclear, it is thought that SFA may induce neutrophil influx and increase antigen presentation in the gastrointestinal tract, create a change in prostaglandin balance, and alter the microflora [16]. Studies have showed that SFA stimulate tissue inflammation in vitro by a process that involves the activation of Toll-like receptors on dendritic cells that falsely recognize commensal bacteria and induce pro-inflammatory immune response directed normally at pathogens [22,38,39]. Therefore, dietary fat has emerged as a leading factor in increasing intestinal permeability of disease-causing bacteria and toxins, which in turn causes elevated susceptibility to autoimmune attack.

Case-control trials have been conducted to study the relationship between FA intake and IBD risk in humans. For example, a study conducted in Canada that included children under the age of 20 years noted a positive but non-significant association for saturated fatty acid intake (OR 1.81, $p=0.40$) and CD [4]. Further, a recent case-control study of a Japanese population aged 15-

34 years revealed that high dietary fat intake (>65.5 g) in adults was associated with an increased risk of developing IBD (OR 2.86, $p<0.002$) [17]. Finally, a study in central Israel showed that adults with high saturated fat intake was associated with an increased risk for UC (OR 2.98, $p=0.07$) [18]. Epidemiological evidence also supports the theory that polyunsaturated fats, in particular omega-3 fatty acids, possess potent immunomodulatory activities in autoimmune diseases that prevent IBD development [36]. The relationships between gastrointestinal inflammation and monounsaturated and trans fat are less understood [18] but were included in this study to identify any trends. While most of these studies have examined the effect dietary factors have on disease incidence, very few studies, if any, have performed a dietary assessment after onset of illness in the pediatric population to evaluate associations between dietary factors and disease course.

The effects between diet and IBD have been shown in animal models. Studies have shown that a high fat diet increases the disease severity and the occurrence of IBD relapse in rats [19-20]. One study in particular by Suzuki et al revealed that a diet high in fat significantly increased small bowel inflammation when compared to a standard chow for rats [20]. It was hypothesized that this effect was due to the high intake of n-6 polyunsaturated fatty acids and saturated fat. However, a study showed that when mice with IBD were treated with omega-3 fatty acids, in particular eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA), it significantly reduced inflammation. These animal models suggest that the different types of dietary fat have different effects on disease state and inflammation.

Physical evaluations by gastroenterologists and measurements of markers of inflammation are common ways to accurately assess disease activity in patients. The acute-phase protein lipopolysaccharide binding protein (LBP) is a marker of inflammation is commonly measured in patients with UC and CD to assess disease activity. LBP plays key roles in promoting innate immunity against Gram-negative bacteria by transferring lipopolysaccharide to a binding site of membrane bound CD14, which results in activation of endothelial and epithelial cells [37]. While this marker gives insight to the degree of inflammation in a patient's system, it is not specific to intestinal inflammation. However, the S100A12 protein, a neutrophil-derived protein, is a fecal marker that is highly specific to the intestinal tract. It has recently been shown to be elevated in the feces of children with IBD and represents a sensitive and specific disease marker [21]. S100A12 is linked to the pathogenesis of IBD due its function of activating pro-inflammatory receptors in the gastrointestinal tract [23]. Numerous S100A12 expressing phagocytes have been detected as pro-inflammatory cells at sites of intestinal inflammation in patients with CD or UC [23].

The study of the relationships between dietary intake and IBD is also important due to IBD's associations with nutritional deficiencies, which is often a result of disease activity and poor oral intake [12,24]. IBD-associated malnutrition was prevalent in a study that included Canadian adults, ranging from 23% in outpatients to 85% in clinically admitted patients [24]. The presence of deficiencies can influence clinical outcomes which could affect body growth, the immune system, and tissue repair [24].

Therefore evaluating the dietary intake of diagnosed IBD patients is of extreme importance due to the pro-inflammatory nature of certain fats, the relapsing tendency of IBD, and the possibility of malnourishment. Inducing and prolonging remission are the primary goals for gastroenterologists and this study could give further insight into the management of the typical disease course of IBD. This study was designed to explore the relationship between disease activity and dietary intake of FA in children with already clinically diagnosed IBD.

Materials and Methods

Subjects

Data for this cross-sectional study was extracted at baseline from a large, ongoing prospective cohort study which enrolled male and female subjects in the United States and Canada between the ages of 5 and 16 years with diagnosed IBD. The objective of the prospective cohort study was to track linear growth of pediatric IBD patients. Subjects were recruited from clinics at the Cincinnati Children's Hospital and the Hospital for Sick Children in Toronto during their routine IBD follow-up doctor's visit. Patients were eligible to participate in the study if they have been clinically diagnosed with either CD or UC and were between the ages of 2 and 18 years. Of the 308 subjects that were enrolled in the original study, 100 were included in this cross-sectional study. 208 subjects had to be excluded from the study since they did not submit either diet records or fecal samples. The Institutional Review Board of each participating hospital provided approval to carry out this study. Patients were also required to give informed consent before participating in the study.

Dietary Intake

Patients who consented to participate were given a 3-day diet record to provide information regarding their usual dietary intake. They were instructed by their gastroenterologist to keep a record of their diet for two consecutive days and one contiguous weekend day. The diet diary provided the following information: the time of consumption, the food item, the amount consumed of each item, and the time and consistency of bowel movements. Food records were analyzed by a nutrition professional using the Nutrient Data System for Research (NDSR) developed by the Nutrition Coordinating Center (NCC) at the University of Minnesota for data entry and nutrient analysis.

Anthropometric measurements

Height was measured with a sliding bar attached to a scale to the nearest 0.5 cm. Weight was measured on a calibrated Filizola® scale to the nearest 0.1 kg, with the subject barefoot and wearing minimal clothing. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m²).

Biochemical Markers of Inflammation

Non-fasted serum samples were drawn for the collection of lipopolysaccharide-binding protein (LBP). LBP was determined by a solid-phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle (Hycult Biotechnology, Uden, Netherlands). Samples and standards were captured by a solid bound specific antibody (IgG). Biotinylated tracer antibody was bound

to capture human LBP. Streptavidin-peroxidase conjugate was added to bind to the biotinylated tracer antibody. Streptavidin-peroxidase conjugate was then reacted with the substrate, tetramethylbenzidine (TMB). The enzyme reaction was stopped by the addition of citric acid. The absorbance at 450 nm was then measured with a spectrophotometer. A standard curve was obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human LBP standards (log). This protocol was performed by the Denson Gastroenterology, Hepatology and Nutrition Lab at Cincinnati Children's Hospital.

Stool samples were collected for the determination of the concentration of the protein S100A12. Flat-bottom 96-well microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated at 50 µL/well with 5 mg/mL of anti-A12 IgG in 0.05 M sodium carbonate buffer, pH 9.6; incubated for 2 h at 37°C; blocked with 0.1% ovalbumin (Sigma) in wash buffer for 1 h at 37°C. Plates were washed four times before samples were added. Samples diluted in RPMI 1640 containing 0.1% BSA (100 µL/well) were incubated overnight at 4°C. Recombinant A12 (0.0625–2.5 nM) diluted in RPMI 1640 and 0.1% BSA was included as the standard. After four washes, biotinylated anti-A12 IgG (4 mg/mL, 100 µL/well) was added, incubated for 1 h at 37°C, and washed four times with wash buffer. Plates were incubated with streptavidin-horseradish peroxidase conjugate (1:1,000 dilution; Amersham, Buckinghamshire, England) for 1 h at 37°C. After washing, plates were incubated with ABTS [2,29-azinobis(3-ethylbenzthiazoline sulfonic acid)] and H₂O₂ (Kirkegaard & Perry, Gaithersburg, MD) for 1 h at room temperature, the reaction was stopped with 1% SDS, and absorbency at 405 nm was measured (Titertec

Multiscan MCC/340; Labssystem, Helsinki, Finland) [40]. This protocol was carried out by the Department of Immunology at the University of Muenster, Germany.

Statistical Analysis

Information on the daily consumption of specific food groups was abstracted from the 3-day diet records. Dietary intake of nutrients was adjusted by subject's body weight for this analysis. Logistic regression analyses were carried out to analyze the association between the dietary intake of fatty acids and the disease activity of the subjects. Subjects were divided into two groups, remission and active, based on their abbreviated Pediatric Crohn's Disease Activity Index (PCDAI) score or their abbreviated Pediatric Ulcerative Colitis Activity Index (PUCAI) score. Remission state was defined as having a PCDAI or PUCAI score of 0-10 or 0-9, respectively and an active state was defined as having a PCDAI or PUCAI score of >10 or >9, respectively [45]. An additional logistic regression analysis was carried out to analyze the association between the dietary intake of fatty acids and the levels of markers of inflammation, lipopolysaccharide-binding protein (LBP) and S100A12 protein. Subjects were divided into either a high or low level group based on the mean values of LBP and S100A12 values. Subjects who had levels less than the mean were placed in the low group while those with a level higher than the average were placed in the high level group. All logistic regression analyses performed grouped each disease separately, and also together. Wald confidence intervals were computed for 95% probability.

A logistic regression analysis was chosen as the statistical model for this study because of the uneven distribution of disease scores and concentrations of markers of inflammation. The uneven distribution of the dependent variables is evidenced by their means and standard deviations. This type of model allows for the patients to be grouped into two categories, in this case, active and remission disease states, thereby increasing the power of the analyses.

Results

A total of 184 subjects enrolled in the study between August 2007 and August 2009. Eighty-four subjects (46.7%) were excluded from the study since they did not fill out the 3-day diet diary. Of the 100 participants included, 21 were from Cincinnati, while the remaining 79 were from Toronto. Of the total 100 participating subjects, 36 were considered to be in an active disease state while 64 were in remission based on their disease activity scores. Demographic and clinical characteristics of the patients are shown in Table 1.

Table 1. Demographic and Clinical Characteristics			
	Total	CD	UC
Number of Subjects	100	73	27
Male	67	50	17
Female	33	23	10
Age at enrollment (S.D)	11.45 (2.44)	11.77 (2.14)	11.21 (2.82)
BMI (z-score)	17.28 (-0.4048)	17.29 (-0.4244)	17.26 (-0.3516)
Location			
Toronto	79	58	21
Cincinnati	21	15	6
Disease Activity			
Remission	64	51	13
Active	36	22	14

The average intake of macronutrients of the subjects is summarized in Table 2. The means and standard deviations of disease activity scores and the markers of inflammation (LBP & S100A12) are shown in Tables 3. The dietary caloric intake was not statistically different between patients from Cincinnati and Toronto as evidenced by a student's T-test ($p=0.20$), thus patients were combined for subsequent analyses.

Table 2. Average Intake of Nutrients				
	CD Patients		UC Patients	
Nutrient Type	Average Total Intake	Average Adjusted Intake ¹ (S.D)	Average Total Intake	Average Adjusted Intake ¹ (S.D)
Kilocalories	1960.64 (524.76)	56.74 (20.29)	1794.99 (773.43)	57.43 (22.70)
Protein (g)	72.62 (32.27)	2.03 (0.78)	68.37 (20.64)	2.12 (0.81)
Carbohydrates (g)	260.39 (82.79)	7.60 (2.71)	258.56 (93.73)	8.22 (4.22)
Saturated Fat (% of total caloric intake)	11.47 (3.24)	n/a	10.51 (2.32)	n/a
MUFAs ² (g)	26.16 (11.27)	0.77 (0.38)	23.21 (12.19)	0.68 (0.26)
PUFAs ³ (g)	13.91 (5.44)	0.43 (0.26)	14.80 (10.50)	0.40 (0.18)
Omega-3 Fats (g)	1.41 (0.80)	0.04 (0.03)	1.32 (0.58)	0.04 (0.02)
Saturated Fats (g)	25.54 (11.67)	0.74 (0.24)	21.12 (10.25)	0.65 (0.20)
Butyric Acid (g)	0.64 (0.54)	0.018 (0.015)	0.54 (0.29)	0.017 (0.011)
Caproic Acid (g)	0.32 (0.25)	0.009 (0.007)	0.26 (0.15)	0.008 (0.005)
Caprylic Acid (g)	0.28 (0.25)	0.008 (0.008)	0.22 (0.14)	0.007 (0.004)
Capric Acid (g)	0.51 (0.39)	0.015 (0.012)	0.42 (0.23)	0.013 (0.008)
Lauric Acid (g)	1.04 (1.02)	0.032 (0.044)	0.85 (0.71)	0.027 (0.024)
Myristic Acid (g)	2.36 (1.66)	0.067 (0.048)	1.95 (0.91)	0.060 (0.031)
Palmitic Acid (g)	13.29 (6.33)	0.384 (0.188)	11.08 (3.73)	0.339 (0.116)
Margaric Acid (g)	0.10 (0.06)	0.002 (0.001)	0.07 (0.06)	0.002 (0.002)
Stearic Acid (g)	6.35 (2.77)	0.184 (0.085)	5.27 (1.96)	0.162 (0.066)
Arachadic Acid (g)	0.13 (0.08)	0.004 (0.003)	0.10 (0.07)	0.003 (0.002)
Behenic Acid (g)	0.11 (0.12)	0.003 (0.003)	0.10 (0.11)	0.003 (0.003)
Trans Fats (g)	4.38 (2.16)	0.130 (0.07)	3.69 (1.76)	0.116 (0.064)
¹ Average intake adjusted by grams of nutrient/kg of body weight/day, ² Monounsaturated Fatty Acid, ³ Polyunsaturated Fatty Acid				

Table 3. Disease Activity Scores and Levels of Markers of Inflammation			
Type of Patient	Mean Disease Activity Score (S.D.)	LBP Mean $\mu\text{g/ml}$ (S.D.)	S100A12 Mean $\mu\text{g/kg}$ (S.D.)
All	12.1 (16.64)	29.40 (12.1)	802 (868.65)
UC	18.7 (23.15)	26.25 (6.51)	1114.00 (846.54)
CD	9.6 (12.82)	30.48 (13.35)	716 (851.46)

Negative associations ($P < 0.05$) of energy intake, total fat, saturated fat, % saturated fat of energy intake, and monounsaturated fatty acids (MUFA) with disease activity were detected by logistic regression analysis when the two disease groups were combined (Table 4). Only CD patients had a significant negative association ($P < 0.05$) between saturated fat intake as a percentage of energy and disease activity. Negative associations between polyunsaturated fatty acids (PUFA), trans fat, and omega-3 fats and PCDAI were also present but did not reach statistical significance (Table 4). Similar associations with individual SFA and disease activity scores were seen such as butyric, caproic, caprylic, capric, lauric, myristic, palmitic, margaric, and stearic acid all had negative associations with disease activity scores ($P < 0.05$) in the combined disease group (Table 5).

Table 4. Logistic Regression (Fatty acid intake and disease scores)						
	Ulcerative colitis, N=27		Crohn's Disease, N=73		All Subjects, N=100	
Variable*	PUCAI**	p	PCDAI***	p	PUCAI/PCDAI	p
Kilocalories	-0.00112	0.09	-0.00079	0.05	-0.0009	<0.05
Total Fat (g)	-0.0143	0.38	-0.0161	0.07	-0.0163	<0.05
Saturated Fat (g)	-0.0532	0.25	-0.0578	<0.05	-0.0582	<0.05
% Saturated Fat	-0.0902	0.47	-0.2593	<0.05	-0.2112	<0.05
MUFA ¹ (g)	-0.0353	0.41	-0.0419	0.09	-0.0424	<0.05
PUFA ² (g)	-0.0100	0.85	-0.0040	0.91	-0.0071	0.82
Trans Fat (g)	-0.0382	0.86	-0.1147	0.32	-0.1036	0.29
Omega-3 (g)	-0.2282	0.68	-0.1312	0.70	-0.1640	0.57
*variables adjusted to weight of subjects, **Abbreviated Pediatric Ulcerative Colitis Activity Index,						
***Abbreviated Pediatric Crohn's Disease Activity Index,† $p < 0.05$, ¹ Monounsaturated Fatty Acids, ² Polyunsaturated Fatty Acids						

No statistical significant correlations were evident between the intake of total fat, monounsaturated fat, polyunsaturated fat, trans fat, and omega-3 fat and markers of inflammation (Table 6). The only individual fatty acid that had a significant correlation with the markers of inflammation was the long chain SFA , which had a positive association ($p < 0.05$) with S100A12 in the combined disease group (Table 7).

Table 5. Logistic Regression (Saturated Fatty acid intake and disease scores)						
	Ulcerative colitis, n=27		Crohn's Disease, n=73		All Subjects, n=100	
Variable*	PUCAI**	p	PCDAI***	p	PUCAI/PCDAI	p
Butyric Acid	-1.0815	0.38	-1.1616	0.07	-1.1621	<.05
Caproic Acid	-2.8344	0.25	-2.3882	0.05	-2.5313	<.05
Caprylic Acid	-4.3460	0.16	-3.1057	<.05	-3.4419	<.05
Capric Acid	-2.1806	0.20	-1.8557	<.05	-1.9598	<.05
Lauric Acid	-0.7721	0.23	-0.7305	0.07	-0.7581	<.05
Myristic Acid	-0.4676	0.24	-0.4772	<.05	-0.4848	<.05
Palmitic Acid	-0.0897	0.32	-0.1019	<.05	-0.1025	<.05
Margaric Acid	-11.4271	0.14	-12.5904	<.05	-12.4686	<.05
Stearic Acid	-0.1731	0.33	-0.2345	<.05	-0.2258	<.05
Arachidic Acid	-5.7602	0.31	-2.6623	0.45	-4.0645	0.17
Behenic Acid	-3.2179	0.42	-1.0429	0.61	-1.7682	0.33
*variables adjusted to weight of subjects (g/kg body weight), **Pediatric Ulcerative Colitis Activity Index						

Table 6. Logistic Regression (Fatty Acid Intake and Markers of Inflammation)												
	UC				CD				All Subjects			
Variable*	LBP** (n=22)	p	S100A12 (n=13)	p	LBP (n=68)	p	S100A12 (n=43)	p	LBP (n=90)	p	S100A12 (n=56)	p
Kilocalories	-0.0004	0.54	0.0009	0.26	0.00004	0.90	0.0006	0.12	0.0002	0.47	0.0006	0.06
Total Fat (g)	-0.0206	0.23	0.0045	0.81	-0.0050	0.49	0.0033	0.71	0.0021	0.75	0.0028	0.72
Saturated Fat (g)	-0.0347	0.44	0.0245	0.63	-0.0022	0.91	0.0109	0.63	0.0146	0.39	0.0107	0.60
Saturated Fat (%)	-0.0472	0.70	0.0266	0.85	-0.0163	0.78	-0.0569	0.49	0.0259	0.63	-0.0396	0.57
MUFA ¹ (g)	-0.0447	0.32	0.0264	0.60	-0.0149	0.44	0.0084	0.72	0.0083	0.63	0.0093	0.65
PUFA ² (g)	-0.1288	0.11	-0.0475	0.52	-0.0426	0.22	0.0001	1.00	-0.0286	0.31	-0.0141	0.70
Trans Fat (g)	-0.3581	0.17	-0.1155	0.67	-0.0349	0.71	0.0779	0.49	-0.0784	0.37	0.0435	0.68
Omega-3 (g)	-0.5695	0.34	0.0187	0.98	-0.3452	0.30	-0.1458	0.74	-0.0553	0.84	-0.1006	0.78
*variables adjusted to weight of subjects, **Lipopolysaccharide Binding Protein, ¹ Monounsaturated Fatty Acids, ² Polyunsaturated Fatty Acid												

Table 7. Logistic Regression (Saturated Fatty acid intake and markers of inflammation)												
	UC				CD				All Subjects			
Variable (g)*	LBP** (n=22)	p	S100A12 (n=13)	p	LBP (n=68)	p	S100A12 (n=43)	p	LBP (n=90)	p	S100A12 (n=56)	p
Butyric Acid	-0.6711	0.58	0.4678	0.75	0.3859	0.41	0.0643	0.91	1.0257	0.26	0.0890	0.87
Caproic Acid	-2.2186	0.36	0.4891	0.86	1.0150	0.29	0.3084	0.80	-0.1936	0.83	0.2593	0.81
Caprylic Acid	-3.2222	0.28	-1.0046	0.76	0.0721	0.94	0.2372	0.85	0.3946	0.51	-0.0048	1.00
Capric Acid	-1.2530	0.44	0.2583	0.89	0.3297	0.60	0.2290	0.78	-0.2431	0.26	0.1787	0.80
Lauric Acid	-0.6694	0.29	-0.6684	0.41	-0.0863	0.67	0.1104	0.65	0.1695	0.24	0.0198	0.93
Myristic Acid	-0.0948	0.80	0.2737	0.54	0.0524	0.72	0.0475	0.80	0.0369	0.28	0.0647	0.70
Palmitic Acid	-0.0573	0.52	0.0679	0.50	-0.0059	0.87	0.0179	0.69	-0.6083	0.83	0.0213	0.59
Margaric Acid	-5.4227	0.44	7.9244	0.30	-1.8162	0.57	7.1465	0.06	0.0434	0.53	6.9201	<0.05
Stearic Acid	-0.1424	0.42	0.0920	0.65	-0.0310	0.68	0.0663	0.47	-1.8208	0.48	0.0610	0.45
Arachidic Acid	-20.7691	0.11	-4.5433	0.52	-0.9029	0.78	-1.3142	0.76	0.5751	0.73	-2.5353	0.48
Behenic Acid	-8.8886	0.23	-9.6464	0.39	1.0691	0.57	-4.0142	0.23	0.0006	0.06	-4.9010	0.12
*variables adjusted to weight of subjects, **Lipopolysaccharide Binding Protein												

Discussion

Numerous studies have confirmed the positive relationship between dietary saturated fat intake and risk of developing IBD [4,6,18]. Additional studies have determined that saturated fat intake is positively correlated with disease activity scores in adults [24,35]. However, in this cross-sectional study of an ongoing prospective cohort study we observed an inverse relationship between the dietary intake of SFA in general and disease activity. This observation suggests that an increased intake of these fats was associated with lower disease activity scores. While there was a trend in the UC group, there were no significant associations between fatty acid intake and disease activity which was most likely due to the small number of patients (n=27), compared to higher levels in the CD group (n=73). This shows that the sample size may have an effect on this statistical analysis. When the CD and UC patients were grouped

together statistically, the associations strengthened likely due to significance stemming from the CD group.

Since the inflammatory effect of saturated fat is well documented in vitro but not fully understood, more emphasis was focused on individual saturated fatty acids to examine this relationship in a group with varying levels of disease activity. The intent for the examination of individual fatty acids was to distinguish if any particular fatty acid was more likely to be associated with intestinal inflammation since all fatty acids behave independently. Therefore the dietary intake of the eleven most common SFAs found in food was assessed in this study. The individual saturated fatty acids analyzed in Tables 5 and 7 well represent saturated fat as a whole, including short, medium, and long chain fatty acids. These saturated fatty acids contain carbon chains ranging from 4-22, and are found in foods such as hydrogenated oils, butter, animal fat, cheese, processed meats and whole milk dairy products.

Only one significant association between dietary intake of fats and serum inflammatory markers LBP and S100A12 was evident. Consumption of margaric acid was positively associated with the S100A12 protein. It is a long chain saturated fatty acid that is found in trace amounts in dairy products [41]. Limited evidence has shown that higher consumption of margaric acid from dairy products substantially suppresses oxidative stress and inflammation among overweight and obese adults [43], but the direct effect margaric acid has on intestinal inflammation is unknown. The implications of this relationship are unknown due to lack of literature regarding this fatty acid.

The overall lack of association between levels of markers of inflammation and fatty acid intake is maybe due to the limitations of the selected cross-sectional study design. In order to better understand the concentrations of the markers of inflammation in sera and feces, multiple collections of samples provide a better picture of the relationships between inflammation state and disease status as the normal levels of these markers are likely to differ between subjects.

The inverse correlations between disease severity and nutritional intake of FAs evident in this study are most likely explained by changes in dietary consumption habits that happened as a result of disease. Since relapsed patients experience a variety of discomforting gastrointestinal symptoms, it is highly plausible that they amend their dietary intake by consuming a decreased amount of food overall compared to when in remission. A recent study of Brazilian adults reported that IBD patients are frequently concerned that some foods aggravate their gastrointestinal symptoms and thus, they often modify their diet in an attempt to control their symptoms or to prevent recurrence of the disease [41]. This theory is supported by our data in tables 4 & 5 as total caloric intake was significantly reduced when patients were in an active disease state. These results also suggest that the decreased caloric intake is likely due to the lower consumption of total saturated fat and monounsaturated fatty acids (Table 5). In particular, butyric, caproic, caprylic, capric, lauric, myristic, palmitic, margaric, and stearic acid are the individual fatty acids that were less consumed. Subjects with an active disease state consumed a lesser amount of foods that were cooked in oil which would account for the decreased intake of SFs and MUFAs, which was evidenced by their 3-day diet records. Further,

since gastroenterologists recommend active IBD patients to eat healthier protein-rich foods such as eggs, beans, fish, and chicken, it is likely that they instinctively limit their intake of saturated fat. This means that patients are less likely to eat fast food-type foods that are calorie dense containing high levels of FAs and do not seem to substitute these unhealthy foods with healthier foods since their total caloric intake is significantly reduced. The 3 day diet records collected from the patients support this notion. Patients in remission reported a higher intake of fried foods, animal fat, and desserts, while patients who were active were more likely to report lesser intakes of these foods. A lack of appetite and the reluctance of eating are the best explanations for the decrease in dietary intake since most of the dietary variables included in this study had negative associations with disease activity.

The importance of understanding the dietary intake tendencies of pediatric patients with IBD should not be disregarded. Literature has supported the fact that pediatric patients tend to have nutritional deficiencies and malnourishment as a result of increased loss of electrolytes, minerals, trace elements and blood, and poor oral intake [24,42,44]. The presence of inflammatory cytokines, chronic inflammation, and exogenous steroids require extra energy expenditure that is taken away from the body's normal functions. As a result of these dietary problems, children are at higher risk for growth failure, delayed puberty, anemia, osteopenia, and osteoporosis [5].

Nutritional follow-up by nutritionists, gastroenterologists, and clinicians during both the phase of remission and of inflammatory activity should be considered to be of fundamental

importance for IBD patients in order to reduce the risk of nutritional deficiencies and malnourishment. It has been repeatedly reported that enteral feeding with an elemental diet is just as effective as corticosteroids in inducing and maintaining remission, while maintaining a healthy nutritional status [25-34]. This therapy shows that nutritional therapy is efficacious and proves that diet is a major factor in managing IBD. Once consistent results are evident, nutritional suggestions can be given to patients as to how they should alter their dietary habits to support good nutritional statuses while in active disease state and to also maintain remission so that they can rid their reliance of medicinal therapies that can potentially cause malnourishment and nutritional deficiencies which could lead to other medical problems.

There are some limitations to this study. Participants had a relatively mild disease activity and low BMI's. This study did not contain control subjects without diagnosed IBD and included a small number of IBD patients that were recruited from only two clinics. The cross-sectional study design naturally inherits limitations. For instance, since this data only represents a snapshot of information, a temporal relationship cannot be established making it impossible to make causal inferences. Also, advice from a skilled dietitian was not given to the patients or to the patient's parents on how to accurately fill out the 3-day diet diary that was used to assess dietary intake. Another limitation in this study was that the information regarding the type of medications prescribed to active patients was not available as they may influence appetite, metabolism, and inflammation. For example, patients are frequently treated with corticosteroids and this type of therapy has been shown to increase the appetite of patients.

The results of this study are provocative and warrant further investigation. To fill the gaps of this study, a prospective cohort study consisting of at least 1,000 patients from multiple nationwide centers should be followed over time for at least 5 years. Patients should have quarterly visits with their gastroenterologist regardless of their disease status at the time for physical evaluations and collections of dietary records, serum and fecal samples, providing the opportunity to analyze data when individuals are in remission vs. active disease state. Healthy controls without IBD would be used in comparison to better track and relate disease status with dietary consumption. Changes in dietary intake of nutrients can be correlated with disease activity over time and this could give further insight to the pathophysiology and disease course of IBD and provide evidence that consumption of certain dietary components may contribute to disease relapse.

Conclusion

This is one of the first studies comparing the dietary intake of diagnosed IBD pediatric patients with disease activity. While the aim of this study was to explore how the intake of dietary fatty acids is related to disease activity, our results provide helpful information regarding how patients may dietetically respond to the disease. These results need to be confirmed in much larger studies that follow more children over time.

References:

1. Loftus E. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; 126:1504-17.
2. Shikhare G, Kugathasan S. Inflammatory bowel disease in children: current trends. *J Gastroenterol*. 2010. doi: 10.1007/s00535-010-0241-5.
3. Hoivik M, Moum B. Health-related quality of life in patients with UC after a 10-year disease course. *Inflamm Bowel Dis*. 2011. doi: 10.1002/ibd.21863.
4. Amre D, D'Souza S, et al. Imbalances in dietary consumption of fatty acids, vegetables, and fruits are associated with risk for Crohn's disease in children. *Am J Gastroenterol*. 2007; 102:2016-2025.
5. Scholz D. The role of nutrition in the etiology of inflammatory bowel disease. *Curr Probl Pediatr Adolesc Health Care*. 2011. 9:248-53.
6. Shoda R, Matsueda K, Yamato S, Umeda N. Epidemiologic analysis of Crohn's disease in Japan: Increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn's disease in Japan. *Am J Clin Nutr* 1996;63:741-5.
7. Gilat T, Langman M. Environmental factors in inflammatory bowel disease. *Front Gastrointest Res*. 1986. 11: 158-76.
8. Chuah S, Jayanthi V, Lee CN, et al. Dietary fats and inflammatory bowel disease in Asians. *Ital J Gastroenterol* 1992;24:386-8.
9. Persson P, Ahlbom A, Hellers G. Diet and inflammatory bowel disease: A case-control study. *Epidemiology* 1992;3:47-52.
10. Tragnone A, Valpiani D, Miglio F, et al. Dietary habits as risk factors for inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1995;7:47-51.
11. Shoda R, Matsueda K, Yamato S, et al. Epidemiologic analysis of Crohn's disease in Japan: Increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn's disease in Japan. *Am J Clin Nutr* 1996;63:741-5.
12. Reif S, Klein I, Lubin F, et al. Preillness dietary factors in inflammatory bowel disease. *Gut* 1997;40:754-60.
13. Sakamoto N, Kono S, Wakai K, et al. Dietary risk factors for inflammatory bowel disease: A multicenter case-control study in Japan. *Inflamm Bowel Dis* 2005;11:154-63.
14. Kasper H, Sommer H. Dietary fiber and nutrient intake in Crohn's disease. *Am J Clin Nutr* 1979. 31:1898-901.
15. Mayberry J, Rhodes J. Breakfast and dietary aspects of Crohn's disease. *BMJ* 1978. 2:1401
16. De Filippo, Cavaleri D. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA*. 2010. 107:14691-6.
17. Sakamoto N, Kono S. Dietary risk factors for inflammatory bowel disease. *Inflamm Bowel Dis*. 2005; 11:154-163

18. Reif S, Klein I, et al. Pre-illness dietary factors in inflammatory bowel disease. *Gut*. 1997; 40:754-760.
19. Ma X, Torbensen M. High-fat diet modulates non-CD1d-restricted natural killer T cells and regulatory T cells in mouse colon and exacerbates experimental colitis. *Clinical and Experimental Immunology*, 151: 130–138.
20. Malewska K, Rychlik A. Treatment of inflammatory bowel disease (IBD) in dogs and cats. *Polish J Veterinary Sciences*. 2011. 14: 165-171.
21. Olafsdottir E, Aksnes L, Fluge G, et al. Faecal calprotectin levels in infants with infantile colic, healthy infants, children with inflammatory bowel disease, children with recurrent abdominal pain and healthy children. *Acta Paediatr* 2002; 91:45–50.
22. Schwartz E, Zhang W. Nutrient modification of the innate immune response: a novel mechanism by which saturated fatty acids greatly amplify monocyte inflammation. *Arterioscler Thromb Vasc Biol*. 2010;30:802–808.
23. Foell D, Wittkowski H. Monitoring disease activity by stool analyses: from occult blood to molecular markers of intestinal inflammation and damage. *Gut*. 2009. 58:859-68.
24. Aghdassi E, Wendland B. Adequacy of Nutritional intake in a Canadian population of patients with Crohn's disease. *J Am Diet Assoc*. 2007. 107:1575-1580.
25. Borrelli O, Cordischi L. Polymeric diet alone versus corticosteroids in the treatment of active pediatric Crohn's disease: A randomized controlled open-label trial. *Clinical Gastroenterology and Hepatology*. (2006) 4: 744-753.
26. Canani R, Terrin G. Short- and long-term therapeutic efficacy of nutritional therapy and corticosteroids in pediatric Crohn's disease. *Digestive and Liver Disease*. (2006) 38: 381-387.
27. Johnson T, Macdonald S. Treatment of active Crohn's disease in children using partial enteral nutrition with liquid formula: a randomized controlled trial. *Gut*. (2006) 55: 356-361.
28. Markowitz J, Hyams J. Corticosteroid therapy in the age of Infliximab: Acute and 1-year outcomes in newly diagnosed children with Crohn's disease. *Clinical Gastroenterology and Hepatology*. (2006) 4: 1124-1129.
29. Dziechciarz A, Horvath A. Meta-analysis: enteral nutrition in active Crohn's disease in children. *Alimentary Pharmacology & Therapeutics*. (2007) 26: 795-806.
30. Wilson D, Thomas A. Systematic Review of the Evidence Base for the Medical Treatment of Paediatric Inflammatory Bowel Disease. *JPGN*. (2010) 50: S14-S34.7.
31. Hartman C, Eliakim R. Nutritional status and nutritional therapy in inflammatory bowel diseases. *World Journal of Gastroenterology*. (2009) 21: 2570-2578.
32. Buchanan E, Gaunt W. The use of exclusive enteral nutrition for induction of remission in children with Crohn's disease demonstrates that disease phenotype does not influence clinical remission. *Alimentary Pharmacology & Therapeutics*. (2009) 30: 501-507.
33. Benjamin J, Makharia G. Nutritional status of patients with Crohn's disease. *Indian Journal of Gastroenterology*. (2008) 5: 195-200.
34. Hyams J. Risk/benefit strategies must be employed in the management of pediatric Crohn's Disease. *Digestive Diseases*. (2009) 3: 291-296.

35. Han P, Burke M. Nutrition and inflammatory bowel disease. *Gastroenterol Clin*. 1999. 28:423-436.
36. Simopoulos A. Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr*. 2002. 6:495-505
37. Lakatos P, Kiss L. Serum lipopolysaccharide- binding protein and soluble CD-14 are markers of disease activity in patients with Crohn's disease. *Inflamm Bowel Dis*. 2011. 17:767-77.
38. Harvey K, Walker C. Long chain saturated fatty acids induce pro-inflammatory responses and impact endothelial cell growth. *Clinical Nutrition*. 2010. 29:492-500.
39. Baumgart D, Thomas S. Exaggerated inflammatory response of primary human myeloid dendritic cells to lipopolysaccharide in patients with inflammatory bowel disease. *Clinical and experimental Immunology*. 2009. 157:423-436.
40. Yang Z, Tao T. Proinflammatory properties of the human S100 protein S100A12. *J Leukoc Biol*. 2001. 69:986-994.
41. Wang H, Steffen L. Obesity modifies the relations between serum markers of dairy fats and inflammation and oxidative stress among adolescents. *Nature*. 2011. 10:1-7.
42. Ripoli J, Mischputen S. Nutritional follow-up of patients with ulcerative colitis during periods of intestinal inflammatory activity and remission. *Arq Gastroenterol*. 2010. 47:49-54.
43. Zemel M, Sun X. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. *Am J Clin Nutr*. 2010. 91:16-22.
44. Barot L, Rombeau J. Caloric requirements in patients with inflammatory bowel disease. *Gastroenterol*. 1997. 32:569-71.
45. Shepanski M, Markowitz J. Is an abbreviated pediatric Crohn's disease activity index better than the original? *J Pediatr Gastroenterol Nutr*. 2004. 39:68-72.