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Alterations in Lipid, Amino Acid, and Energy Metabolism Distinguish Crohn's Disease from Ulcerative Colitis and Control Subjects by Serum Metabolomic Profiling

Elizabeth A. Scoville, MD, MSCI[1], **Margaret M. Allaman, BS**[1], **Caroline T. Brown, MA**[1], **Amy K. Motley, BS**[1], **Sara N. Horst, MD**[1], **Christopher S. Williams, MD, PhD**[1,3,6], **Tatsuki Koyama, PhD**[5], **Zhiguo Zhao, MS**[5], **Dawn W. Adams, MD, MS**[1,6], **Dawn B. Beaulieu, MD**[1], **David A. Schwartz, MD**[1], **Keith T. Wilson, MD**[1,2,3,4,6], and **Lori A. Coburn, MD**[1,4,6]

[1]Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

[2]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA

[3]Department of Cancer Biology, Vanderbilt University Medical Center, Nashville, TN, USA

[4]Vanderbilt Center for Mucosal Inflammation and Cancer

[5]Department of Biostatistics, Vanderbilt University Medical Center, Nashville, TN, USA

[6]Veterans Affairs Tennessee Valley Healthcare System, Nashville, TN, USA

Abstract

Introduction—Biomarkers are needed in inflammatory bowel disease (IBD) to help define disease activity and identify underlying pathogenic mechanisms. We hypothesized that serum metabolomics, which produces unique metabolite profiles, can aid in this search.

Objectives—The aim of this study was to characterize serum metabolomic profiles in patients with IBD, and to assess for differences between patients with ulcerative colitis (UC), Crohn's disease (CD), and non- IBD subjects.

Methods—Serum samples from 20 UC, 20 CD, and 20 non-IBD control subjects were obtained along with patient characteristics, including medication use and clinical disease activity. Non-targeted metabolomic profiling was performed using ultra-high performance liquid chromatography/mass spectrometry (UPLC-MS/MS) optimized for basic or acidic species and hydrophilic interaction liquid chromatography (HILIC/UPLC-MS/MS).

*Correspondence: Dr. Lori A Coburn, Vanderbilt University Medical Center, Division of Gastroenterology, 2215B Garland Ave., 1030C MRB IV, Nashville, TN, USA 37232, Phone: 615-875-4222; Fax: 615-343-6229, lori.coburn@vanderbilt.edu.

Conflicts of Interest: David A. Schwartz has consultancy agreements with Abbvie, UCB, Janssen, Takeda, and Tigenix. Dawn B. Beaulieu has a consultancy agreement with Abbvie. Sara N. Horst has consultancy agreements with UCB and Salix. However, these agreements and grants had no relationship to the current research study. Keith T. Wilson has had a consulting agreement with Immune Pharmaceuticals. However, this agreement had no relationship to the current research study and is no longer active. The remainder of the authors declare that they have no conflict of interest.

Results—In total, 671 metabolites were identified. Comparing IBD and control subjects revealed 173 significantly altered metabolites (27 increased and 146 decreased). The majority of the alterations occurred in lipid-, amino acid-, and energy-related metabolites. Comparing only CD and control subjects revealed 286 significantly altered metabolites (54 increased and 232 decreased), whereas comparing UC and control subjects revealed only 5 significantly altered metabolites (all decreased). Hierarchal clustering using significant metabolites separated CD from UC and control subjects.

Conclusions—We demonstrate that a number of lipid-, amino acid-, and tricarboxylic acid (TCA) cycle- related metabolites were significantly altered in IBD patients, more specifically in CD. Therefore, alterations in lipid and amino acid metabolism and energy homeostasis may play a key role in the pathogenesis of CD.

Keywords

Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Metabolomics

INTRODUCTION

The inflammatory bowel diseases (IBD), including the two major forms, Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing inflammatory conditions associated with significant morbidity and affect more than 1.6 million people in the United States (Shivashankar et al. 2016). While multiple predisposing genetic, environmental, and immunologic factors have been identified, the etiology and exact pathogenesis remains unknown (Podolsky 2002; Xavier and Podolsky 2007; Hendriksen et al. 1985; Gregor et al. 1997). The diagnosis of both CD and UC involves a multifactorial approach combining clinical, endoscopic, histopathologic, and radiologic features. Even with this invasive, multidisciplinary approach, the diagnosis of IBD can be challenging and diagnostic delays can have significant clinical impact (Vavricka et al. 2012; Schoepfer et al. 2013; Ricart et al. 2008). In addition, differentiation between UC and CD can be difficult (Geboes et al. 2008). Non- or semi-invasive diagnostics with adequate sensitivity and specificity are not available in clinical practice (Vermeire et al. 2006; Zhou et al. 2016). New biomarkers for diagnosis, as well as disease monitoring, are needed.

Metabolomics has the potential to elucidate disease mechanisms and to identify biomarkers for diagnosis and monitoring disease activity (Lin et al. 2011). Metabolomic profiling has shown promise for distinguishing IBD from control, as well as CD from UC, using a variety of specimen types including urine (Williams et al. 2009; Stephens et al. 2013; Kolho et al. 2017), serum (Dawiskiba et al. 2014; Ooi et al. 2011; Schicho et al. 2012; Williams et al. 2012; Zhang et al. 2013; Bjerrum et al. 2017), and stool (Marchesi et al. 2007; De Preter et al. 2015; Bjerrum et al. 2015; Kolho et al. 2017). The majority of existing studies have used nuclear magnetic resonance (NMR) based analysis and have identified alterations in only a small number of metabolites. Advances in methodology and mass spectroscopy (MS) based methods have allowed a wider detection range (Lin et al. 2011).

It remains unknown what metabolic alterations can be detected in serum of patients with UC and CD using a highly sensitive, metabolomic platform. The aim of our study was to use

ultra-high performance liquid chromatography/tandem mass spectroscopy to identify alterations in metabolic pathways in the serum of UC and CD patients compared with non-IBD subjects, and to evaluate this method as a novel tool for distinguishing between UC, CD, and non-IBD subjects.

MATERIALS AND METHODS

Ethical Considerations

The study protocol was approved by the Vanderbilt University Institutional Review Board. Written informed consent was obtained from control and UC subjects for analyses of demographics and medical histories as well as for serum collection as a part of the clinical trial "Effects of L-Arginine in Colitis and Colon Cancer", identifier NCT01091558 (clinicaltrials.gov). De-identified serum samples and written informed consent from patients with CD were obtained under a separate IRB protocol.

Study Subjects

Serum was obtained from previously collected cohorts of confirmed UC or CD subjects and non-IBD controls. Patients with confirmed UC (n = 20) were recruited from either the outpatient endoscopy unit or IBD clinic at Vanderbilt University Medical Center (Coburn et al. 2013; Wiese et al. 2016). Patients with confirmed CD (n = 20) were recruited from the IBD clinic at Vanderbilt University Medical Center. IBD diagnosis was determined by accepted clinical and histologic criteria by an IBD specialist (D.A.S.; D.B.B.; S.N.H.). Non-IBD control subjects (n = 20) were recruited from patients undergoing colonoscopy for colorectal cancer screening or other non-IBD related indications (Coburn et al. 2013; Wiese et al. 2016). Individuals who were pregnant, had known coagulopathy or bleeding disorders, known renal or hepatic impairment, prior organ transplant, or were unable to give informed consent were excluded.

For all participants, demographics including age, gender, medical history, and medication use were determined from participant reporting and review of the medical record on the day of serum collection. Participants were not required to be fasted, however, all but 4 subjects (all CD) were fasted at least 4 hours prior to blood draw. The blood was allowed to clot for at least 30 minutes, and serum was collected within 1 hour of blood draw after centrifugation (2000 × g for 10 minutes at 4° C). Serum was snap frozen with dry ice and then stored at –80°C until utilized. The Mayo Disease Activity Index (DAI) was determined for UC subjects by standard measures. Endoscopic severity and the physician's global assessment portions of the DAI were determined by IBD specialists (D.A.S.; D.B.B.; S.N.H.) as follows: normal, mild disease (erythema, decreased vascular pattern, mild friability), moderate disease (marked erythema, lack of vascular pattern, friability, erosions), or severe disease (spontaneous bleeding, ulceration) (Schroeder et al. 1987). A DAI ≥ 3 was defined as active disease for UC participants. The Harvey Bradshaw index (HBI) was determined for CD subjects by standard measures with a score of ≥ 5 defined as active disease for CD participants (Harvey and Bradshaw 1980). General well-being, abdominal pain, and the number of liquid stools per day were graded by the participant.

Analysis of Human Serum Metabolites

All metabolite measurements were performed by Metabolon® (Research Triangle Park, NC) using ultra-high performance liquid chromatography and gas chromatography separation coupled with tandem mass spectrometry (Evans et al. 2009). A detailed description of procedures and metabolite identification steps can be found in Supplemental Material 1. Briefly, serum samples underwent ethanol extraction and were split into aliquots for analysis by ultra-high performance liquid chromatography/tandem mass spectroscopy (UPLC-MS/MS). One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. A second aliquot was analyzed using acidic positive ion conditions but was chromatographically optimized for more hydrophobic compounds. The third aliquot was analyzed using basic negative ion optimized conditions. The fourth aliquot was analyzed via negative ionization following elution from a hydrophilic interaction chromatography column. Metabolites were identified by comparison to a reference library of purified standards (or recurrent unknown entities) based on retention time/index, mass to charge ratio (*m/z*), and chromatographic data (including MS/MS spectral data) using software developed at Metabolon® (Dehaven et al. 2010). Biochemical identification was based on retention index within a narrow window, accurate mass match to the library ± 10 parts per million, and the MS/MS forward and reverse scores between the experimental data and authentic standards. Peaks were quantified using area under the curve. To minimize confounding from differences in medications or dietary intake between groups, metabolites classified as xenobiotics (n = 107) were removed *a priori*.

Statistical Analysis

Demographic data was summarized by disease status (UC, CD, or control). For continuous variables, median and interquartile range were reported. Data with more than two groups were first analyzed by the Kruskal-Wallis H test and if $p < 0.05$, then pairwise comparisons using the Mann-Whitney U test was used to compare continuous variables. For categorical variables, frequency with percentage was reported and compared using the Pearson's χ^2 test. For metabolomic analysis, we imputed missing data with the smallest observed values for that metabolite. Given the large range of ion intensities, each metabolite was scaled so that the median for each metabolite was set equal to one and log base 2 transformed. Linear regression models including disease categorization (UC, CD, or control) were fit for each metabolite and adjusted for potential confounders. Models comparing IBD with control and UC or CD with control were adjusted for age. The model comparing CD with UC was adjusted for age and biologic medication use. To control for false discovery rate (FDR), the resultant p values were then adjusted for multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg 1995). A false discovery rate of 10%, or FDR adjusted $p < 0.1$, was considered significant for metabolites (Goedert et al. 2014; Playdon et al. 2016). For hierarchical clustering, average linkage and Pearson correlation distance were used. Statistical analysis was performed using "R" software version 3.3 (http://cran.r-project.org/).

RESULTS

Patient characteristics

In total, serum samples from 20 UC, 20 CD, and 20 non-IBD controls underwent metabolomic analysis. Patients with either UC or CD were significantly younger than control subjects, but there was no significant difference in gender distribution or tobacco use (Table 1). UC and CD patients had modestly lower body mass index (BMI) than controls, though this did not reach statistical significance. Of IBD patients, 95% were on at least one IBD-specific medication, with CD patients more likely to be on an anti-TNF-α agent, while those with UC were more likely to be on a 5-aminosalicylate (5-ASA) therapy. Rates of corticosteroid and immunomodulator use were similar among the UC and CD patients. One CD patient was on vedolizumab. Of the UC patients, 95% had clinically active disease by DAI, while only 40% of the CD patients had clinically active disease by HBI (Table 1). All 20 UC patients had evidence of moderate to severe disease on histology from colonoscopy at the time of blood draw, but histologic information was not available for CD subjects. Nine (45%) CD patients had a history small bowel resection, all of which were ileo-colic resections. No UC or control patient had a history of colon or small bowel surgery. Of CD subjects, 5 (25%) had only ileal disease, 8 (40%) had only other small bowel involvement, 3 (15%) had both colon and small bowel involvement, and 4 (20%) had only colonic disease. Five (25%) CD patients had perianal disease. Eleven (55%) CD patients had stricturing and 5 (25%) had a penetrating phenotype. Of UC patients, 2 (10%) had only rectal involvement, 2 (10%) had left sided colitis, while 16 (80%) had pan-colitis.

Alterations of individual metabolites in IBD

In total, 671 metabolites were identified. Of these 107 were determined to be xenobiotics (i.e. drug metabolites, plant components, or chemicals), which, to avoid the influence of medication or dietary intake, were excluded from further analysis. A complete list of identified metabolites with fold change comparisons and p values between IBD, UC, and CD with controls is available in Supplemental Material 2.

Overall, a large number of metabolites were altered between groups at a FDR adjusted $p < 0.1$ (Fig. 1). Comparing all IBD subjects with controls revealed 173 metabolites that were significantly different (27 increased, 146 decreased) after adjustment for age. Comparing CD with control subjects revealed 286 metabolites that were significantly different after adjustment for age (54 increased, 232 decreased), while comparing UC and control subjects revealed only 5 significantly decreased metabolites.

Using hierarchal clustering of all identified metabolites, CD subjects generally cluster away from UC subjects and non-IBD controls (Supplemental Material 3). UC subjects did not reliably cluster away from controls, and thus could not be confidently differentiated using these cutoff values.

Alterations in individual metabolites differentiate between CD and UC subjects

When comparing CD with UC subjects, 236 metabolites were significantly different after adjusting for age and biologic medication use at FDR adjusted $p < 0.1$. Forty-seven

metabolites were higher in CD than UC, while 189 were lower in CD than UC (Fig. 1). Supplemental Material 4 shows that individuals with CD and UC can be separated using hierarchical clustering of significant metabolites with $p < 0.1$ and at least a 2-fold change. CD subjects with only colon involvement did not consistently cluster together or with UC subjects (Supplemental Material 4). Likewise, CD subjects with history of small bowel resection did not consistently cluster together (Supplemental Material 4).

Lipid metabolism is markedly altered in IBD

Of 302 identified metabolites associated with lipid metabolism, 162 (53.6%) were significantly altered in CD compared with control subjects. Notably, for UC compared with control, all 5 significantly decreased metabolites (deoxycholate, sphingomyelin, glycochenodeoxycholate glucuronide, taurolithocholate 3-sulfate, and dehydroepiandrosterone sulfate) were related to lipid metabolism (Supplemental Material 5). Long chain, polyunsaturated, branched chain, and monohydroxy fatty acids were consistently decreased in all IBD (Fig. 2a) and CD compared with controls (Fig. 2b), as well as in CD compared with UC (Fig. 2c). Glycerol, an indicator of lipolysis and free fatty acid release from adipose tissue, was also significantly reduced in CD (2.3-fold decrease, $p < 0.001$) compared with control. In addition, several essential acylcarnitine metabolites were decreased in CD compared with both control and UC (Fig. 2b and 2c). Acylcarnitine metabolites were also decreased in UC as compared with control, but did not reach significance (Supplemental Material 2).

Bile acid pathways, which are involved in digestion of dietary fats, were markedly altered in IBD (Fig. 2). In CD, both conjugated and unconjugated primary bile acids were elevated compared with control subjects. The secondary bile acids (glycolithocholate, glycoursodeoxycholate, and ursodeoxycholate) were elevated in CD. The notable exception was the sulfated bile acids (glycolithocholate sulfate, taurocholenate sulfate), which were decreased in CD compared with control subjects. In contrast in UC subjects, the primary bile acid glycochenodeoxycholate glucuronide was decreased, and the two most profoundly altered metabolites in UC compared with control subjects were secondary bile acids (Supplemental Material 5).

TCA cycle intermediates were consistently decreased in CD

In addition to lipid metabolites, several other classes of metabolites were consistently altered. There was a significant decrease in tricarboxylic acid (TCA) cycle intermediates, including citrate, aconitate, α-ketoglutarate, succinate, fumarate, and malate in CD vs. control and UC subjects (Fig. 3). In addition, β-hydroxybutyrate, which is synthesized from excess acetyl-CoA, was the most profoundly decreased metabolite in CD at 11-fold lower than controls and 18-fold lower than UC subjects ($p < 0.001$).

Amino acids were decreased in CD

Of 157 identified biochemicals involved in amino acid metabolism, 73 (46.5%) were significantly different in CD vs. control subjects. Essential (leucine, lysine, and valine), semi-essential (arginine and glutamine), and non-essential (serine) amino acids were decreased in CD subjects compared to control subjects (all $p < 0.1$) (Fig. 4). In addition,

leucine, valine, serine, and glutamine were all decreased in CD compared to UC subjects (all $p < 0.05$) (Fig. 4). No differences were seen in serum amino acids in UC compared with control.

Alterations in metabolic pathways in IBD

We performed a metabolic pathway enrichment analysis (Fig. 5) of significantly altered metabolites (FDR adjusted $p < 0.1$) to identify pathways that contribute to the major differences between samples of IBD patients compared with non-IBD control subjects. Alterations in amino acid, lipid (glycerolipid, fatty acid, sphingolipid), endocannabinoid, and carnitine pathways were characteristic of IBD subjects compared to controls (Fig. 5a). Similar patterns were seen in CD compared with control subjects in amino acid, fatty acid, and bile acid pathways. In addition, there was enrichment of simple sugar (fructose, mannose, and galactose) and TCA cycle pathways in CD compared with control subjects (Fig. 5b). Fatty acid metabolism was a major effect in differentiating UC and CD (Fig. 5c). In UC compared with control, only 4 pathways contained altered metabolites (Supplementary Material 5) and, given this small number, we did not perform a pathway enrichment analysis for this comparison.

Effects of small bowel surgery on serum metabolic profile

Given the role of the small bowel, especially the terminal ileum, in re-absorption of bile acids and the potential effects on lipid metabolism, we were interested in assessing whether small bowel resection affected the observed metabolomic profiles. Comparing metabolites of CD subjects with history of small bowel resection ($n = 9$) to those without ($n = 11$), 12 metabolites that are all lipid-related, were significantly altered (Supplemental Material 6). The most notably different were the bile acids taurochenodeoxycholate and taurolithocholate 3-sulfate.

Based on these differences, we evaluated whether lipid-related metabolite changes seen in CD compared with control subjects persisted after excluding individuals with small bowel resection ($n = 9$). After comparing the remaining CD subjects with no history of bowel resection to controls, multiple classes of fatty acids remained decreased. Acylcarnitine metabolites including hexanoylcarnitine, octanoylcarnitine, and cis-4-decenoyl carnitine remained notably different (>2-fold decrease, $p < 0.01$), and several sphingomyelin species remained decreased. Interestingly, primary bile acids (cholate, taurocholate, glycocholate, glycochenodeoxycholate, and taurochenodeoxycholate) all remained significantly elevated (all > 2.5-fold increase, $p < 0.1$). Ursodeoxycholate (secondary bile acid) also remained markedly elevated (4.2-fold increase, $p = 0.08$). Overall patterns were unchanged, indicating an additional disease effect independent of surgery on these metabolites.

Despite concerns for alterations in absorption of other nutrients, there were no significant alterations in amino acid metabolites when comparing CD subjects with small bowel surgery compared with those without bowel surgery (Supplemental Material 7).

Effects of clinical disease activity on serum metabolomic profile

To assess if clinically active versus inactive disease had differing effects on the serum metabolomics profile, we performed a subgroup analysis comparing clinically active CD (n = 8) to clinically inactive CD (n = 12). Overall, there were no significant alterations in metabolites between these two groups after adjustment for age and biologic use as well as correction for FDR. Supplemental Material 8 shows the metabolites that reached a $p < 0.05$ in the model containing age and biologic use but without FDR correction when comparing active to inactive CD. Only one UC subject had clinically inactive disease and as such this analysis was not performed in UC.

DISCUSSION

This study provides a detailed characterization of the serum metabolome associated with both UC and CD compared to non-IBD control subjects. We demonstrate herein that serum metabolomic profiles differ substantially in CD, but less so in UC, compared with control subjects. The most notable differences in the untargeted metabolomic profile of IBD subjects occur in lipid metabolism-related pathways. Marked differences occur in fatty acids, acylcarnitine metabolites, sphingolipids, and bile acid metabolism in CD compared with either UC or control subjects. Growing evidence suggests that lipid metabolism and signaling play important roles in inflammation and may have significant implications for the pathogenesis of IBD (Fan et al. 2015; Koutroumpakis et al. 2016). For example, fatty acids, particularly the polyunsaturated fatty acids, have been implicated in the intestinal inflammatory response in IBD via arachidonic acid derived eicosanoids (Sharon and Stenson 1984; Ueda et al. 2008; Esteve-Comas et al. 1992; Marion-Letellier et al. 2013). Similarly, serum fatty acids have been shown to correlate with colonic pro-inflammatory cytokines (Wiese et al. 2016).

To help explore the potential effect of alterations in bowel anatomy on metabolic alterations, we performed a subgroup analysis of subjects who had undergone small bowel resection. As expected, CD subjects with small bowel resection have differences in serum lipid metabolites, particularly the bile acids. The effects of bowel surgery on fecal metabolites are well known, and this effect should also be considered in the design of future serum metabolomic studies (Bjerrum et al. 2015). Excluding subjects with small bowel resection, however, did not markedly alter the significance of lipid-related and bile-related alterations in CD compared with control subjects. The majority of our CD subjects had some degree of small bowel involvement, and although the majority had clinically inactive disease, we cannot exclude the possibility that malabsorption related to small bowel CD could result in these alterations. It is notable, however, that UC subjects also had profound alterations in bile acids. UC subjects do not have small bowel involvement with their disease process and are not confounded by small bowel surgery. Thus, our findings are consistent with prior reports suggesting bile acid metabolism may play a role in IBD independent of altered anatomy and possibly even independent of small bowel disease (Vítek 2015; Lenicek et al. 2011). It has been suggested that malabsorption due to ileal dysfunction in CD may result in compensatory overproduction of primary bile acids measured in the serum and, in addition,

increased bacterial deconjugation in both UC and CD may affect bile acid profiles (Vítek 2015; Lenicek et al. 2011; Gothe et al. 2014; Gnewuch et al. 2009).

In addition, we identified several alterations in energy-related metabolism, some of which overlap with lipid metabolism. Acylcarnitines, which are produced when carnitine is conjugated to a fatty acid for transport into the mitochondria for beta-oxidation, were decreased in CD. A decrease in both carnitine and acylcarnitine species in CD may provide evidence for decreased beta-oxidation of fatty acids in CD. Adding further evidence, beta-hydroxybutyrate, which is synthesized via acetyl-CoA during beta-oxidation of fatty acids, was the most profoundly reduced metabolite in CD compared with controls (Fig. 3). The presence of an active inflammatory state in IBD leads to elevated energy expenditure due to enhanced protein catabolism (Lennie et al. 1995), and these results suggest additional alterations in lipid utilization. Our findings related to fatty acid oxidative pathways, TCA cycle intermediates, and decreased amino acids emphasizes that loss of energy homeostasis may play a significant role in the pathogenesis of CD or are potential markers of disease.

Decreases in serum amino acids have been shown in other cohorts of IBD patients (Hisamatsu et al. 2012; Hong et al. 2010). In addition to the potential utilization of protein as a catabolic energy source in the inflammatory state, there is evidence that some amino acids may attenuate intestinal inflammation. For example, L-arginine, which is a precursor of nitric oxide synthesis via nitric oxide synthase (Wu and Morris 1998), is decreased in the colonic tissue, but not serum, of UC patients and inversely correlates to disease activity (Coburn et al. 2016). In the current study, we show that arginine is decreased significantly in the serum of CD subjects, but not in UC. This difference in serum changes may be related to the higher degree of systemic inflammation in CD. Interestingly, only a subset of our CD subjects had clinically active disease at the time of serum acquisition. We would expect that a study of only active CD patients could show even stronger changes in CD-related metabolites compared with controls, however, in our subgroup analysis of those with clinically active compared with inactive disease there were no significant differences.

Our study has several strengths. First, we have identified a considerably larger number of metabolites than other similar studies by using a high sensitivity platform (Dawiskiba et al. 2014; Zhang et al. 2013; Williams et al. 2009; Schicho et al. 2012). The non-targeted approach gives a broad and detailed view of the serum metabolome. Unlike more targeted approaches or methods that focus on proteomics or lipidomics, we are able to show the relative contribution of each pathway to the overall metabolic alterations. In addition, we have made several attempts to limit the effects of confounders in this non-targeted approach by excluding metabolites known to be associated with medications and dietary intake, as well as adjusting our analyses for age and biologic medication use.

Our study also has some limitations. As an exploratory study, our sample size limits the ability to adjust for all potential confounders. Other potential confounders may exist, such as differences in other medication use, comorbidities, disease patterns, and disease severity. It is not fully known what, if any, effect these parameters have on metabolic profiles. As a cross sectional design, we cannot follow metabolomic profiles longitudinally. A recent study evaluating serial serum metabolomic changes during infliximab induction showed

normalization of some lipid species over time in infliximab responders (Bjerrum et al. 2017). Although we did not see alterations in clinically active CD compared with inactive CD, this suggests that metabolomic profiles of IBD subjects who respond to treatment may become more similar to controls over time. Few alterations in UC met significance after a false discovery rate correction. Future serum studies may require larger sample sizes to show alterations in UC.

CONCLUSIONS

In conclusion, our findings demonstrate that serum lipid-, amino acid-, and energy-related metabolic pathways differ in CD, UC, and control subjects. Lipid metabolism, amino acid deficiency, and loss of energy homeostasis may play a role in the pathogenesis of IBD, particularly Crohn's disease. Individual metabolites may play a role as potential biomarkers for diagnosis or disease monitoring, but these will need to be validated in larger, independent cohorts of IBD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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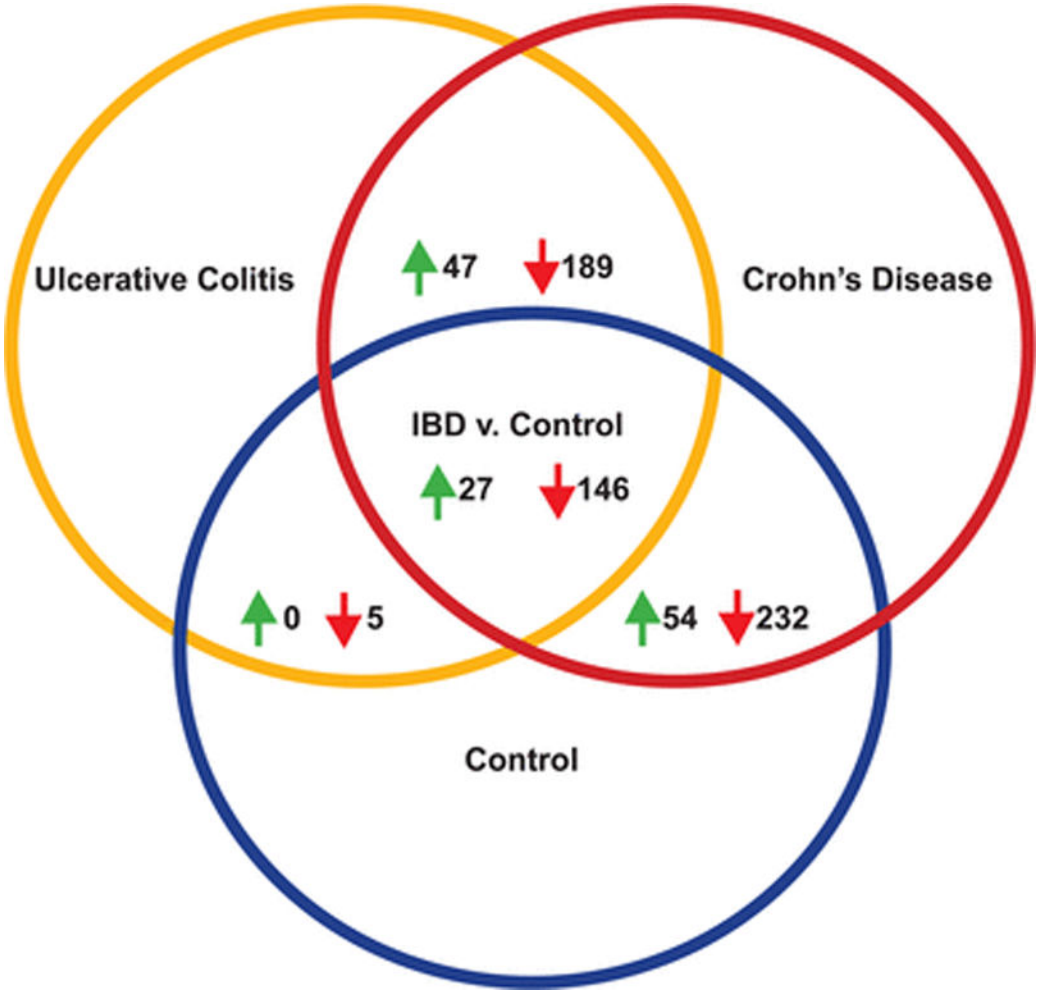

Fig. 1. Multiple metabolites were significantly altered between UC, CD, and control subjects

Significantly altered metabolites as determined by a FDR corrected $p < 0.1$. The total number of metabolites significantly increased (↑) and decreased (↓) for each comparison group out of a total of 564 identified metabolites

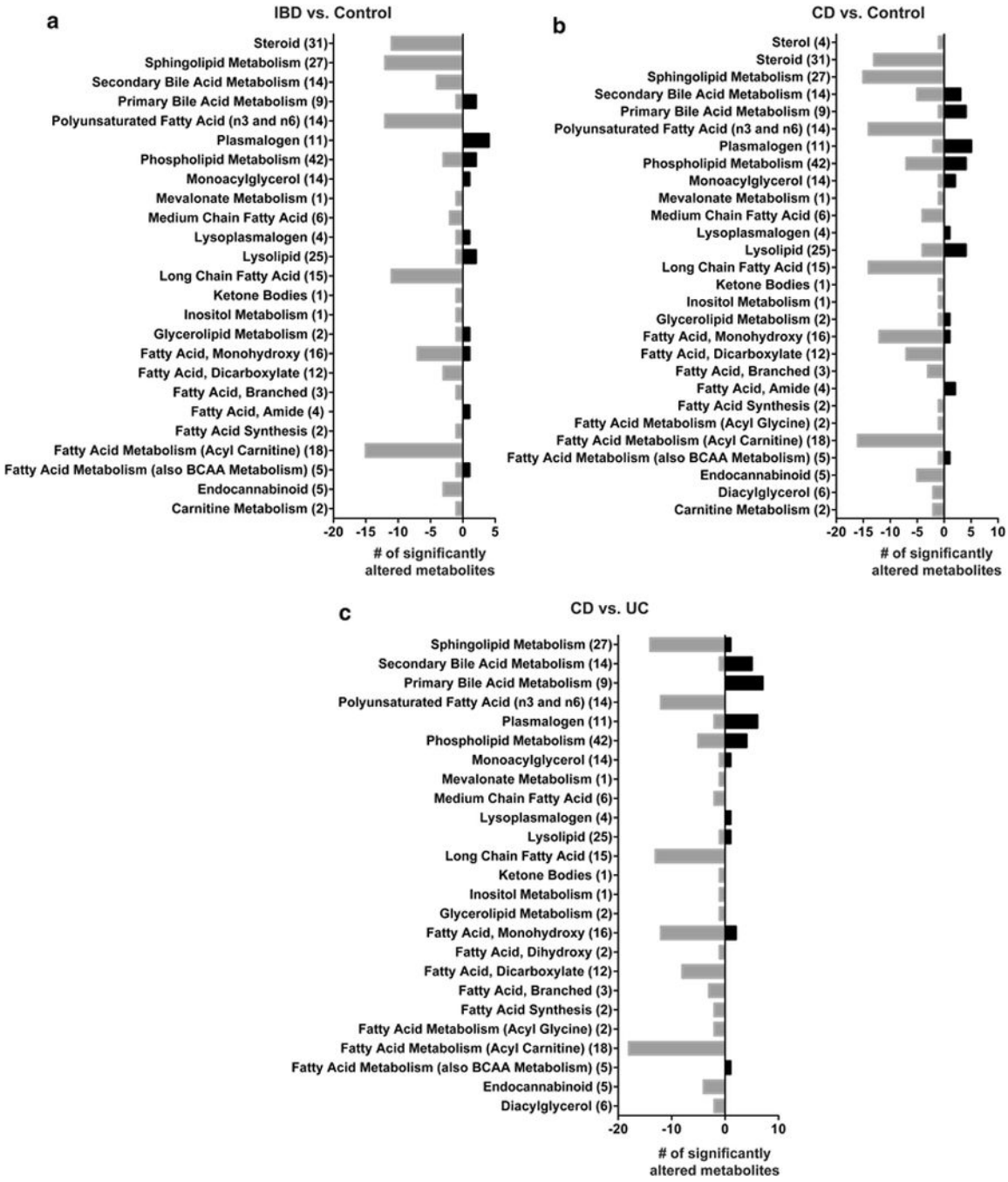

Fig. 2. Lipid related metabolic pathways are altered in IBD and CD compared control subjects as well as CD compared with UC

The bar charts represent the number of significantly altered metabolites in each sub-pathway of lipid metabolism in (a) IBD compared with control, (b) CD compared with control, and (c) CD compared with UC. In a and b, metabolite levels were either increased (black) or decreased (grey) in IBD or CD versus control. In c, metabolite levels were either increased (black) in UC or decreased (grey) in CD. Only sub-pathways with at least one significantly altered metabolite are shown. The number in parenthesis by each pathway name represents the total number of identified metabolites in each sub-pathway

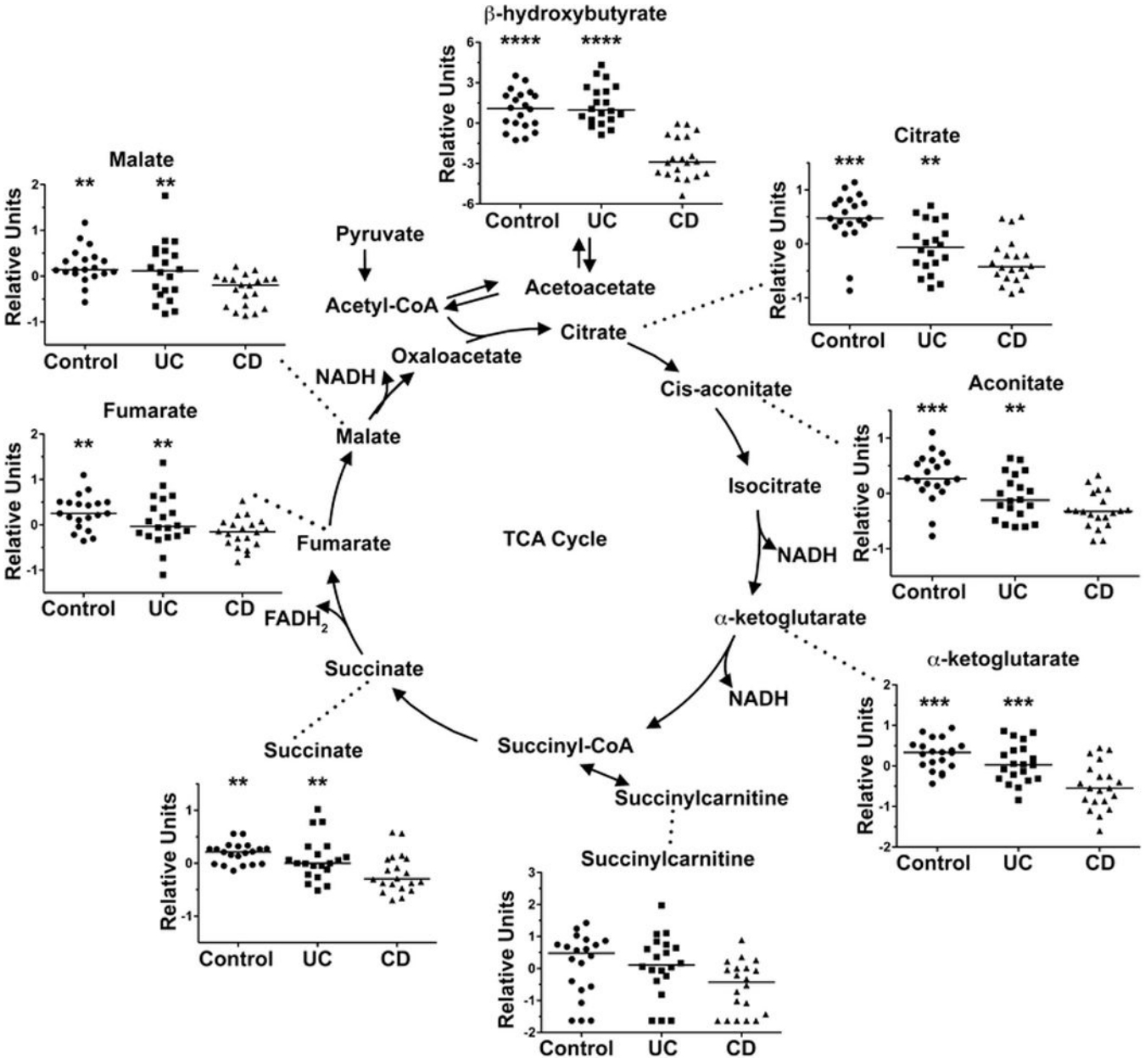

Fig. 3. TCA cycle intermediates are decreased in CD compared with UC and control subjects

Quantities are in log transformed standardized units specific to the internal standards for each quantified metabolite, and normalized to protein concentration. p values shown are from linear regression models with adjustment for age and adjusted for FDR. Lines represent the median. ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$ for regression model including UC or control vs. CD respectively

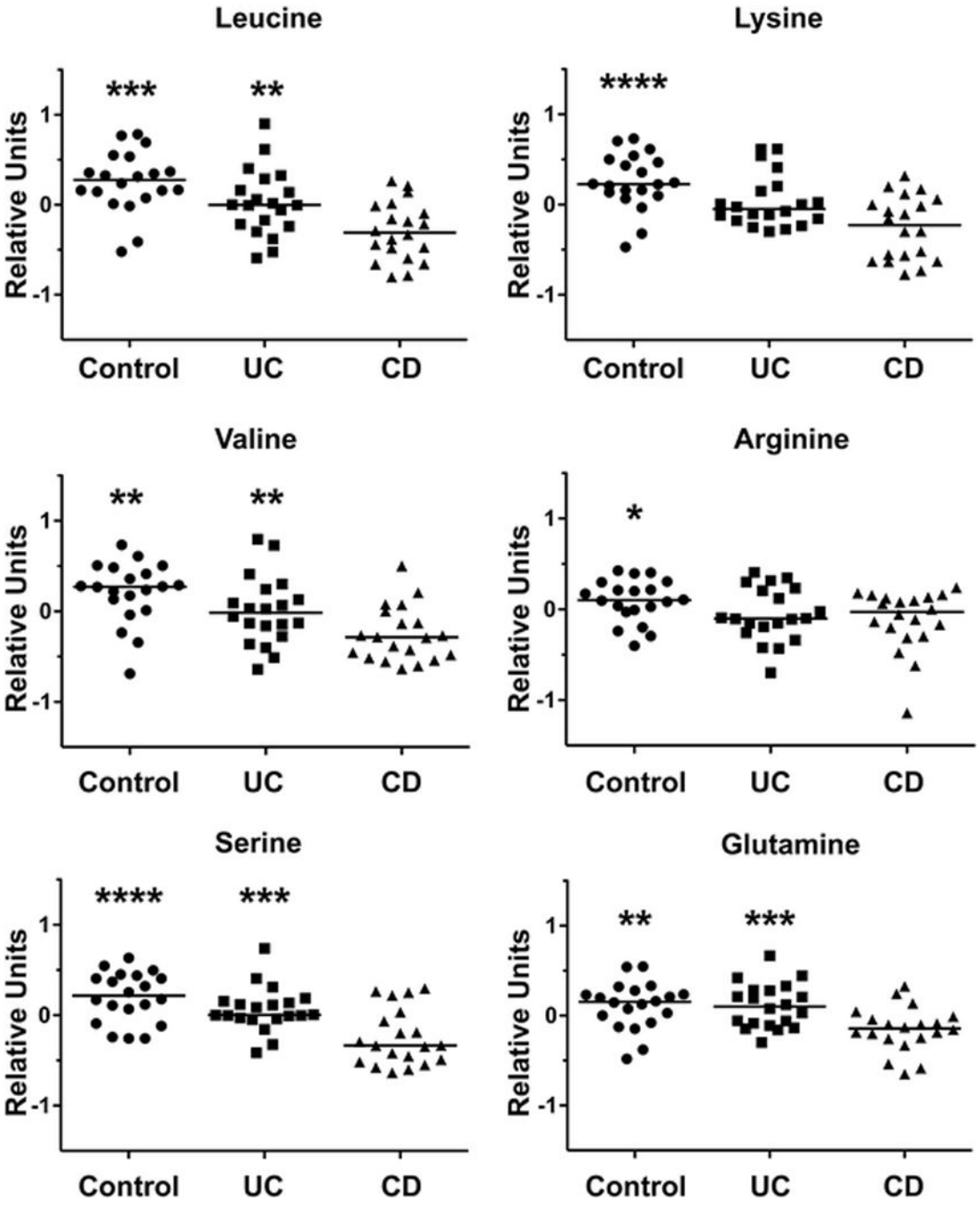

Fig. 4. Amino acids are decreased in CD compared with UC and control subjects

Quantities are in log transformed standardized units specific to the internal standards for each quantified metabolite and normalized to protein concentration. p values shown are from linear regression models adjusted for FDR. Comparisons between CD and control are corrected for age. Comparisons between UC and CD are corrected for age and biologic medication use. Lines represent the median. * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$, **** $p < 0.001$ vs. CD for regression model including UC or control vs. CD respectively

Fig. 5. Metabolite pathway enrichment analysis

Metabolite pathway enrichment analysis identified pathways altered in (a) IBD and (b) CD compared with non-IBD control subjects as well as (c) CD compared with UC subjects. A pathway enrichment analysis was done based on significantly altered metabolites with an FDR adjusted $p < 0.1$. Pathways with less than 2 identified metabolites or pathways with no significant metabolites are not shown. Only pathways with an enrichment value of > 1 are shown in the figure. Enrichment = (# of significant metabolites in the pathway / # of detected metabolites in the pathway) / (total # of significant metabolites overall / total # of

detected metabolites overall). The darker shades of grey indicate pathways with higher enrichment values. A pathway enrichment analysis was not shown for UC compared with control since only 4 pathways had significantly altered metabolites

Table 1

Patient characteristics by diagnosis

Active disease was defined as a DAI ≥ 3 for UC and a HBI ≥ 5 for CD.

	Control (*n* = 20)	UC (*n* = 20)	CD (*n* = 20)
Age, median (25^{th}, 75^{th} percentile)	52.9 (49.5, 61.6)	35.0 (28.6, 41.0)[b]	40.0 (26.3, 46.5)[a]
Male Gender	9 (45%)	12 (60%)	9 (45%)
Body Mass Index, median (25^{th}, 75^{th} percentile)	29.1 (26.1, 33.4)	25.3 (22.5, 31.0)	26.1 (22.8, 32.2)
Tobacco Use	1 (5%)	0	3 (15%)
Any IBD Therapy	–	19 (95%)	19 (95%)
5-ASA	–	13 (65%)	5 (25%) *
Corticosteroids	–	4 (20%)	4 (20%)
Immunomodulators	–	10 (50%)	10 (50%)
Anti-TNF-α	–	6 (30%)	15 (75%) **
Vedolizumab	–	0	1 (5%)
Active Disease	–	19 (95%)	8 (40%) ***
Any Extra-Intestinal Manifestation	–	6 (30%)	10 (50%)
Arthritis	–	5 (25%)	10 (50%)
Uveitis	–	1 (5%)	0
CRP, median (25^{th}, 75^{th} percentile)	–	3.0 (1.6, 7.3)	2.3 (1.1, 11.8)

[a] $p < 0.05$,

[b] $p < 0.001$ vs. control.

* $p < 0.05$,

** $p < 0.01$,

*** $p < 0.001$ vs. UC.

Age and body mass index were assessed by the Kruskal-Wallis test followed by the Mann-Whitney U test. CRP was assessed by the Mann-Whitney U test. Categorical data were analyzed using the Pearson's χ^2 test