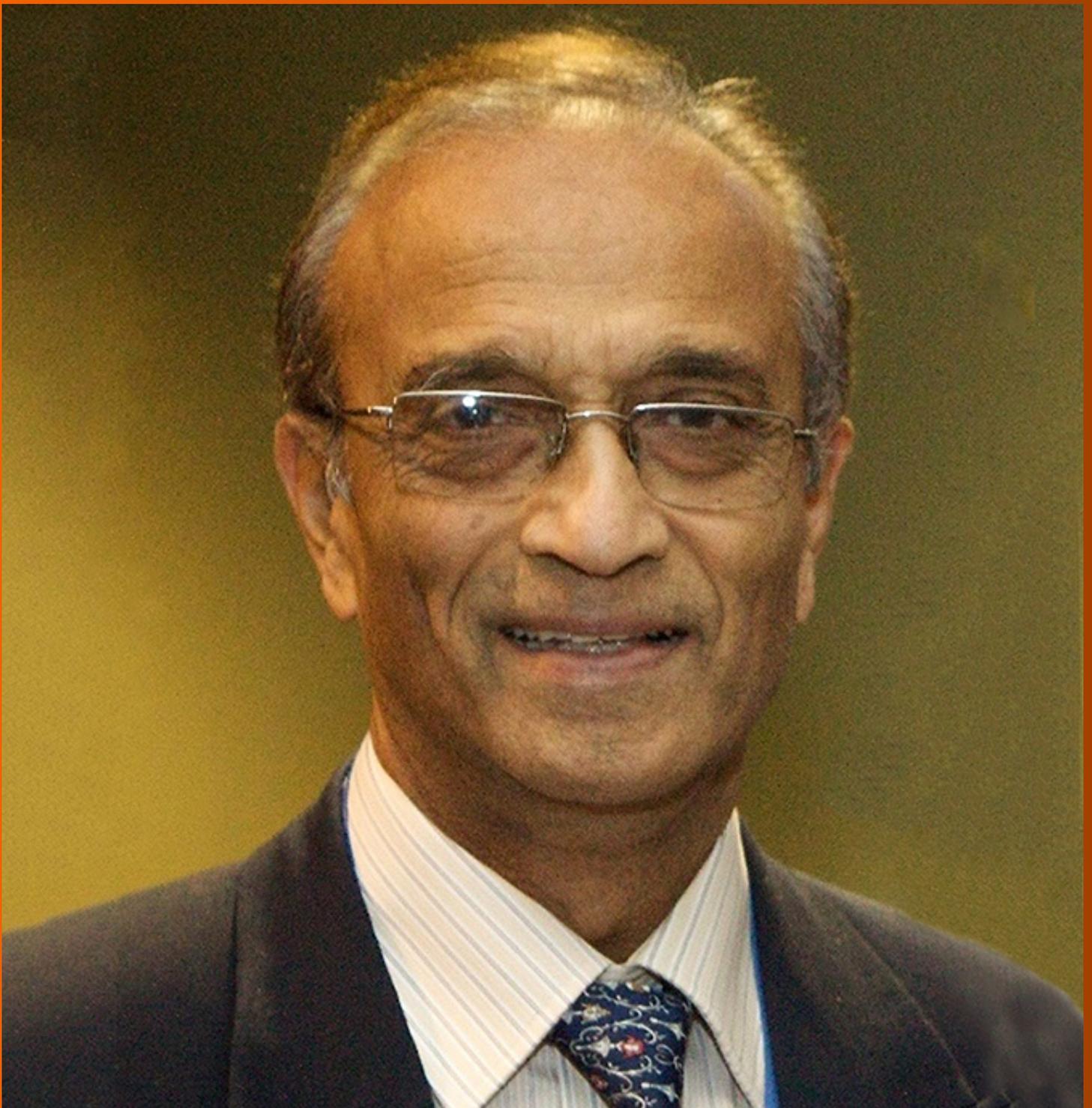


World Journal of *Gastroenterology*

World J Gastroenterol 2020 January 21; 26(3): 266-374



MINIREVIEWS

- 266 Abdominal compartment syndrome: Often overlooked conditions in medical intensive care units
Rajasurya V, Surani S
- 279 Fibroblast growth factor signaling in non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Paving the way to hepatocellular carcinoma
Ocker M

ORIGINAL ARTICLE**Basic Study**

- 291 *Bacteroides fragilis* enterotoxin upregulates heme oxygenase-1 in dendritic cells *via* reactive oxygen species-, mitogen-activated protein kinase-, and Nrf2-dependent pathway
Ko SH, Jeon JI, Woo HA, Kim JM

Case Control Study

- 307 Nucleotide excision repair pathway gene polymorphisms are associated with risk and prognosis of colorectal cancer
Li YK, Xu Q, Sun LP, Gong YH, Jing JJ, Xing CZ, Yuan Y

Retrospective Study

- 324 Idarubicin *vs* doxorubicin in transarterial chemoembolization of intermediate stage hepatocellular carcinoma
Roth GS, Teyssier Y, Abousalihac M, Seigneurin A, Ghelfi J, Sengel C, Decaens T

Observational Study

- 335 Metabolite profile comparisons between ascending and descending colon tissue in healthy adults
Baxter BA, Parker KD, Nosler MJ, Rao S, Craig R, Seiler C, Ryan EP

SYSTEMATIC REVIEWS

- 353 Programmed cell death-1 inhibitor-related sclerosing cholangitis: A systematic review
Onoyama T, Takeda Y, Yamashita T, Hamamoto W, Sakamoto Y, Koda H, Kawata S, Matsumoto K, Isomoto H

CASE REPORT

- 366 Unexpected metastasis of intraductal papillary neoplasm of the bile duct without an invasive component to the brain and lungs: A case report
Nam NH, Taura K, Kanai M, Fukuyama K, Uza N, Maeda H, Yutaka Y, Chen-Yoshikawa TF, Muto M, Uemoto S

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Observational Study

Metabolite profile comparisons between ascending and descending colon tissue in healthy adults

Bridget A Baxter, Kristopher D Parker, Michael J Nosler, Sangeeta Rao, Rebecca Craig, Catherine Seiler, Elizabeth P Ryan

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Abstract**BACKGROUND**

Obesity is a risk factor for colorectal cancer, yet metabolic distinctions between healthy right and left colon tissue, before cancer is diagnosed, remains largely unknown. This study compared right-ascending and left-descending colon tissue metabolomes to identify differences from the stool metabolome in normal weight, overweight, and obese adults.

AIM

To examine right and left colon tissue metabolites according to body mass index that may serve as mechanistic targets for interventions and biomarkers for colon cancer risk.

METHODS

Global, non-targeted metabolomics was applied to assess right-ascending and left-descending colon tissue collected from healthy adults undergoing screening colonoscopies to test the hypothesis that BMI differentially impacts colon tissue metabolite profiles. The colon tissue and stool metabolome of healthy adults ($n = 24$) was analyzed for metabolite signatures and metabolic pathway networks implicated in progression of colorectal cancer.

Conflict-of-interest statement:

Authors have nothing to disclose.

Data sharing statement:

Metataxonomics sequence data are available *via* NCBI SRA BioProject Accession PRJNA594611 and on this project's GitHub repository github.com/kdprkr/ConjurersBre.

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**RESULTS**

Ascending and descending colon contained 504 host, food, and microbiota-derived metabolites from normal weight, overweight and obese adults grouped according to body mass index. Amino acids, lipids, and nucleotides were among the chemical types that further differentiated from the stool metabolite profiles. Normal weight adults had 46 significantly different metabolites between ascending and descending colon tissue locations, whereas there were 37 metabolite differences in overweight and 28 metabolite differences for obese adults ($P < 0.05$). Obese adults had trimethylamine N-oxide, endocannabinoids and monoacylglycerols with different relative abundances identified between ascending and descending colon. Primary and secondary bile acids, vitamins, and fatty acids also showed marked relative abundance differences in colon tissue from overweight/obese adults.

CONCLUSION

There were metabolite profile differences between right-ascending and left-descending colon tissue in healthy adults. Colon lipids and other metabolites in obese and overweight adults were distinguished from normal weight participants and associated with gut inflammation, nutrient absorption, and products of microbiota metabolism.

Key words: Colon; Ascending; Descending; Metabolomics; Obesity; Stool

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Core tip: This study identified metabolite profile differences between right-ascending and left-descending colon from normal, overweight or obese adults. We also show that stool metabolite composition does not accurately reflect the right-ascending colon. There is limited knowledge of human colon small molecules and metabolite signatures that may impact colon cancer risk. Colon cancer of the right-ascending colon has a poorer prognosis and reduced survival outcome when compared to colon cancer on the left-descending colon. Diet and lifestyle are additional factors of overweight and obesity that may influence colon tissue metabolite composition with respect to inflammation. Right and left colon metabolite profiles may be helpful to evaluate after interventions that seek to prevent or mitigate cancer risk.

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INTRODUCTION

Body mass index (BMI) of 30% or greater is an established risk factor for colon cancer in men and women^[1,2]. Obesity is a complex lipid-storage disease with metabolic aberrations locally in the gut and systemically in the host that increase risk for multiple chronic diseases^[3]. Similar relationships occur for obesity and the incidence of larger (*vs* smaller) colon adenomas^[3,4]. Weight gain from early to middle adulthood increases risk^[5], whereby middle-aged obese adults had a 60% increase risk of right-side colon cancer compared to the left-side^[6]. We and others have previously shown that stool reveals changes in microbial communities^[7], and modulation by diet^[8], yet this may not accurately reflect metabolic differences between the right and left side colon tissue^[9].

Right-cancer patients have a worse prognosis with a median survival of 76.6 mo while left-sided have median survival of 101 mo^[10] and right-sided tumors are significantly larger in size with a higher tumor grade when compared to left side colon cancer^[11,12]. African American and non-Hispanic blacks have 24% greater odds of right-sided colon cancer^[13]. Physical inactivity, excess body weight, alcohol, smoking, and a central deposition of adiposity are consistent risk factors for colorectal cancer.

High consumption of red meat more than 3 times/wk has been associated with 2-fold increased risk for colon cancer and these food components merit attention in the tissue of healthy adults^[14].

Metabolomics is a high-throughput screening methodology that is sensitive for detection of exogenous and endogenous (microbial, host and food) products of metabolism^[15] and can aid in identification of disease risk biomarkers^[16]. Metabolite profiling analysis of ascending and descending colon tissue was conducted herein to assess metabolic differences between colon locations that differ from stool. This study utilized normal weight, overweight and obese adults for investigation of colonic compounds that may impact colon cancer risk^[17]. Metataxonomics of colon tissue by location has varied results^[18,19] and provided rationale for using metabolomics. The major objective of this study was to identify metabolic pathways that distinguished ascending and descending colon tissue and to reveal metabolites altered by overweight and obesity that may pose elevated risks for developing cancer. We hypothesized that lipids (*e.g.*, fatty acids, bile acids, phospholipids, monoacylglycerol, and endocannabinoids) are distinct in type and abundance between the ascending and descending colon, and that colon tissue metabolomes will differ according to BMI when compared to stool in overweight and obese adults.

MATERIALS AND METHODS

Study design- participant recruitment

Ninety-three healthy adults were contacted prior to a scheduled colonoscopy in Fort Collins CO. Forty adult males and females provided written informed consent to collect a stool sample and an ascending and descending colon tissue biopsy. Twenty-four individuals (colon and stool) were assessed for non-targeted metabolomics. Eligible participants were provided a stool kit and study instructions. The gastroenterology clinical nurses and research staff confirmed study code number assignments and ensured the completed de-identification at the site of colonoscopy procedure. Colorado State University study personnel were contacted by clinic staff for sample retrieval immediately following procedure. Three study groups were BMI 20-24.9 for normal weight ($n = 9$), BMI 25-29.9 for overweight ($n = 9$) and BMI 30+ for obese ($n = 6$) adults. One normal weight female (BMI 24) had the right and left colon and stool sample applied for metataxonomic analysis (16S rRNA gene sequencing). Participant's inclusion criteria for this study were at least 18 years of age, a scheduled routine colonoscopy, no prior history of colorectal cancer diagnosis, non-smoker, and not having taken antibiotics for at least one month prior to the standard of care, routine screening colonoscopy.

The colon tissue collected for this study was visually determined by the gastroenterologist performing the procedure to be normal, healthy tissue without polyps. Each participant had an about 5 mm biopsy of ascending (right) and descending (left) colon tissue and a self-collected stool sample prior to bowel preparation. Colonoscopy was completed by Centers for Gastroenterology-Fort Collins and University of Colorado-Health North Gastroenterology Clinic (Fort Collins, CO, United States). Samples were de-identified for personal information and study ID coded before storage and metabolite processing at Colorado State University. The number of polyps removed by the doctor with the respective location was provided following the procedure. This study received IRB approval, and include protocol number; Colorado State University IRB No. 15-6051, and University of Colorado Health IRB No. 0010144. Participants in this study had no history of diseases related to the liver or biliary tract and they did not have previous procedures such as cholecystectomy or ileal resections. This study did not collect information regarding the family history of colorectal cancer and did not perform hereditary genetic or epigenetic screening history on the patients. **Table 1** shows the study participant characteristics.

Colon tissue and stool sample collection

Stool samples were self-collected by participants in a pre-labeled study coded container and frozen at -80 °C. Approximately 5 mm of normal healthy colon tissue were stored immediately at -80 °C following collection. Samples were shipped on dry ice to Metabolon, Inc. (Durham, NC, United States) and a single participant sample underwent DNA extraction for metataxonomics.

Sample accessioning and preparation

Tissue and stool metabolite extraction was completed using 80% methanol as previously described^[7], prior to ultrahigh performance liquid chromatography-

Table 1 Characteristics of the study participants (*n* = 24)

Characteristics	Total (<i>n</i> = 24)	Normal (<i>n</i> = 9)	Overweight (<i>n</i> = 9)	Obese (<i>n</i> = 6)
Sex				
Males	5	0	4	1
Females	19	9	5	5
BMI (mean ± SD, kg/m ²)	27.6 ± 5.8	22 ± 1	26.7 ± 1.3	35 ± 5
Total number of people with polyps removed	14	6	4	4
Total number polyps removed	-	14	13	22
Polyp Location				
Cecum	-	1	0	3
Ascending	-	6	2	10
Transverse	-	2	1	1
Sigmoid	-	2	4	6
Rectum	-	0	6	0
Descending	-	3	0	2
History of hypertension				
Yes	6	2	3	2
No	18	7	6	4
History of type 2 diabetes				
Yes	3	0	2	1
No	21	9	7	5
Taking dietary supplements				
Yes	14	4	8	2
No	10	5	1	4
Smoking history				
Past smoker	9	2	6	1
Never	15	6	4	5

No participants had cancer detected from the screening exam. BMI: Body mass index.

tandem mass spectroscopy (UPLC-MS/MS) as completed by Metabolon, Inc. Positive and negative ion modes were chosen to provide broad, non-targeted detection of metabolites.

Samples were extracted using the automated MicroLab STAR® system from Hamilton Company. A set of recovery standards were added prior to the first step in the extraction process for quality control purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

UPLC-MS/MS analysis

The UPLC-MS/MS portion of the platform was based on a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35000 mass resolution. The protocol has been previously described by our lab^[7].

Data extraction and compound identification

Raw data were extracted, peak-identified, and processed using Metabolon's hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities as previously described^[7].

Statistical analysis

Healthy colon tissue or stool metabolite profiles were semi-quantified in terms of relative abundance and median scaled to 1. Fold differences were calculated for normal weight, overweight and obese (colon tissue and stool) and for colon tissue between ascending and descending sites. A matched-pairs 2-way ANOVA was completed using the scaled relative abundance of each metabolite, experimental groups in ArrayStudio on log transformed data, were used for normal weight, overweight and obese. Metabolite profile distinctions between ascending and descending colon tissue were evaluated using $P < 0.05$ for statistical significance with matched pair *t*-test. An estimate of the false discovery rate (*Q* value) was calculated to account the multiple comparisons across metabolites that are typical of metabolomics-based studies with a *Q* value ≥ 0.01 . A linear regression analyses for colon metabolites were performed to compare the groups with polyp removal to no polyp removal, after adjusting for the effect of weight category of the subjects.

Principal component analysis and hierarchical clustering were applied to understand the similarities and differences between samples and/or groups of samples in a complex dataset. Unsupervised clustering was performed using the ward D2 method^[20]. Random forest (RF) analysis, a supervised classification technique, was applied for identifying candidate biomarkers. To determine which variables (biochemicals) make the largest contribution to the classification of BMI, a “variable importance” measure was computed. We used the “Mean Decrease Accuracy” as this metric prediction accuracy^[21].

Metataxonomics: Sample handling and DNA extraction, sequence read processing, and feature table analyses

DNA was extracted from colon tissue and stool with the MoBio PowerSoil Kit according to manufacturer protocols. Amplification of the V4 region of the 16S rRNA gene and amplicon sequencing followed the standards outlined by the Earth Microbiome Project. Raw FASTQ-formatted forward reads were imported into the Quantitative Insights Into Microbial Ecology 2 (QIIME 2) platform^[22]. A feature table comprised of amplicon sequence variants (ASVs) was inferred from reads using the DADA2 algorithm^[23]. Taxonomy was assigned to each representative ASV sequence using Naïve Bayes classifiers trained against 99% OTU reference collections from Greengenes 13_8 or SILVA 132. The raw feature table, representative sequences, and taxonomy tables were exported from QIIME 2 for further processing using R^[24]. Following import, a master table comprised of ASV IDs with corresponding representative sequences, full and truncated Green-genes and SILVA taxonomic lineages, and absolute abundances for all ASVs within each sample was constructed. This master table served as the entry point for all downstream processing and analysis. Comparisons of microbiota composition proceeded from the compositional data analysis paradigm with count zero multiplicative replacement prior to applying the centred log-ratio (clr) transformation^[25]. Taxon abundance are depicted as proportions (*i.e.*, relative abundances). Supplemental Methods for additional details regarding amplification conditions, library preparation, sequencing, and a comprehensive account of analytical approaches.

Data availability

Metataxonomics sequence data supporting the conclusions of this manuscript are available via NCBI SRA BioProject Accession PRJNA594611 and on this project’s GitHub repository located at github.com/kdprkr/ConjurersBrew, along with each of the materials needed to reproduce the analysis.

RESULTS

Colon tissue and stool metabolomes of normal weight, overweight and obese adults

The stool metabolome of healthy adults classified according to BMI as normal weight, overweight or obese had a total of 842 named compounds (Supplemental Table 1). The 842 stool metabolites consisted of 175 amino acids, 26 peptides, 33 carbohydrates, 11 energy, 345 lipids, 46 nucleotides, 47 cofactors and vitamins, and 159 were classified as exogenous and referred to as xenobiotics. There were 98 stool metabolites that significantly differ according to BMI were 22 amino acids, 1 peptide, 8 nucleotides, 2 cofactors and vitamins, 9 xenobiotics and 56 lipids. Principal component analyses (PCA) for the stool metabolome (Supplemental Figure 1A) and hierarchal clustering (Supplemental Figure 1B) did not clearly separate participants by BMI groups. The 504 colon metabolites with known identity and 44 unnamed/unknown compounds from 24 male and female participants are provided

in **Supplemental Table 2**. The colon metabolome contained 93 amino acids, 13 peptides, 35 carbohydrates, 10 metabolites were classified under TCA cycle and oxidative phosphorylation and there were 20 cofactors and vitamins. The largest portion of the colon tissue metabolome were lipids (about 50%, 262 lipid metabolites) that span 40 sub-metabolic pathways. Other notable small molecules from colon were 38 nucleotides and 32 exogenous, xenobiotic metabolites. Fourteen out of twenty-four healthy participants had 1-14 polyps removed during screening colonoscopy. A regression analysis for colon metabolites adjusting for the effect of weight was done, revealing 17 colon metabolites that had lower expression correlated with polyps removed (**Supplemental Table 3**). **Figure 1A**, shows 2-arachidonoylglycerol, 3-phosphoglycerate, and 6-phosphogluconate had a lower relative abundance in ascending and descending tissue from participants with polyps removed. **Figure 1B-C**, shows 1-dihomo-linolenylglycerol, aspartate, and glycerophosphorycholine (GPC) with lower expression in ascending colon tissue, while glutarate (C5-DC), and 2-hydroxyarachidate had lower expression in descending tissue from participants with polyps removed (**Figures 1B and C**).

Random forest analysis of colon tissue comparing normal weight *vs* overweight/obese yielded 30 metabolites with a predictive accuracy of 56% for the overweight/obese phenotype (**Supplemental Figure 2A**). BMI associated colon metabolites were bile acids and cofactors/vitamins (*e.g.*, biliverdin, alpha-tocopherol, and pyridoxate). The predictive accuracy for metabolites in ascending versus descending colon was 73%, and 24 of the top 30 metabolites were classified as lipids (**Supplemental Figure 2B**). **Table 2** further shows the 12 metabolites with statistically significant fold difference identified in right/left colon tissue and in stool by BMI comparisons. **Figure 2** shows lipids that are significantly different between weight groups for colon tissue and stool ($P < 0.05$). The phospholipid, trimethylamine N-oxide (TMAO), was 2.80-fold difference from ascending in obese adults, and 6.23-fold difference from stool in overweight adults when compared to normal weight adults (**Figure 2A**). Endocannabinoids, linoleylethanolamide (2.11-fold) and oleylethanolamide (1.60-fold) difference from ascending in normal weight adults and decrease in stool of obese adults (**Figure 2B**). Furthermore, the median scaled relative abundance of primary and secondary bile acids (chenodeoxycholate 2.89-fold and 0.41-fold, cholate 0.55-fold, and taurodeoxycholate 1.49-fold) had significant differences by colon location and stool (**Figure 2C**). The primary bile acid, chenodeoxycholate is 2.89-fold difference from ascending in normal weight and 0.41-fold difference from descending in overweight adults, while 21.80-fold difference in stool of overweight adults when compared to normal weight. Cholate is 0.55-fold difference from obese descending colon and 61.06-fold difference from overweight stool when compared to normal weight (**Figure 2C**). The secondary bile acid, taurodeoxycholate, had 1.49-fold difference from ascending colon in overweight adults and 9.32-fold difference in stool of overweight adults compared to normal weight (**Table 2** and **Figure 2C**).

BMI differentiates ascending and descending colon lipids

We next utilized metabolomics to distinguish ascending and descending colon tissue. There were 87 metabolites with statistically significant fold differences between ascending *vs* descending colon by BMI. These included, 46 metabolites in normal weight, 37 metabolites in overweight, and 28 metabolites in obese. Notably, the number of metabolites distinguishing ascending and descending colon decreased as BMI increased. We found that 62% of the metabolites distinguishing ascending and descending colon were lipids. **Table 3** shows 54 colon tissue lipids with statistically significant fold difference between ascending and descending colon. In normal weight adults there were 29 colon lipids, 24 of which were fatty acids and lysophospholipid (1.48-fold – 2.16-fold difference) from ascending, and 5 that were from descending colon. Overweight adults had 24 significantly different colon lipids; 11 metabolites from ascending, (8 derived from monoacylglycerols 1.67-fold – 2.61-fold), and 13 metabolites with higher abundance from descending tissue (**Table 3**). Obese adults had the fewest significant differences in colon lipids between ascending and descending tissue (15 identified metabolites). **Table 3** shows 4 metabolites increased in ascending tissue and 11 increased in descending tissue that were primarily fatty acids. **Figure 3** shows median scaled relative abundance of right and left colon tissue lipids, including those that are food derived long chain fatty acids and microbiome-products. The long chain fatty acids; palmitate 1.35-fold, arachidate 1.35-fold, and stearate 1.39-fold from ascending tissue in normal weight, and palmitate 0.73-fold, arachidate 0.69-fold, and stearate 0.68-fold from descending tissue in obese adults (**Figure 3A**). **Figure 3B** shows microbiome-influenced metabolites that show significant fold difference in colon; 15-HETE from ascending tissue is 1.69-fold in normal weight and 1.93-fold in obese while, 3-hydroxybutyrate is 0.48-fold from descending in normal weight, and 2-

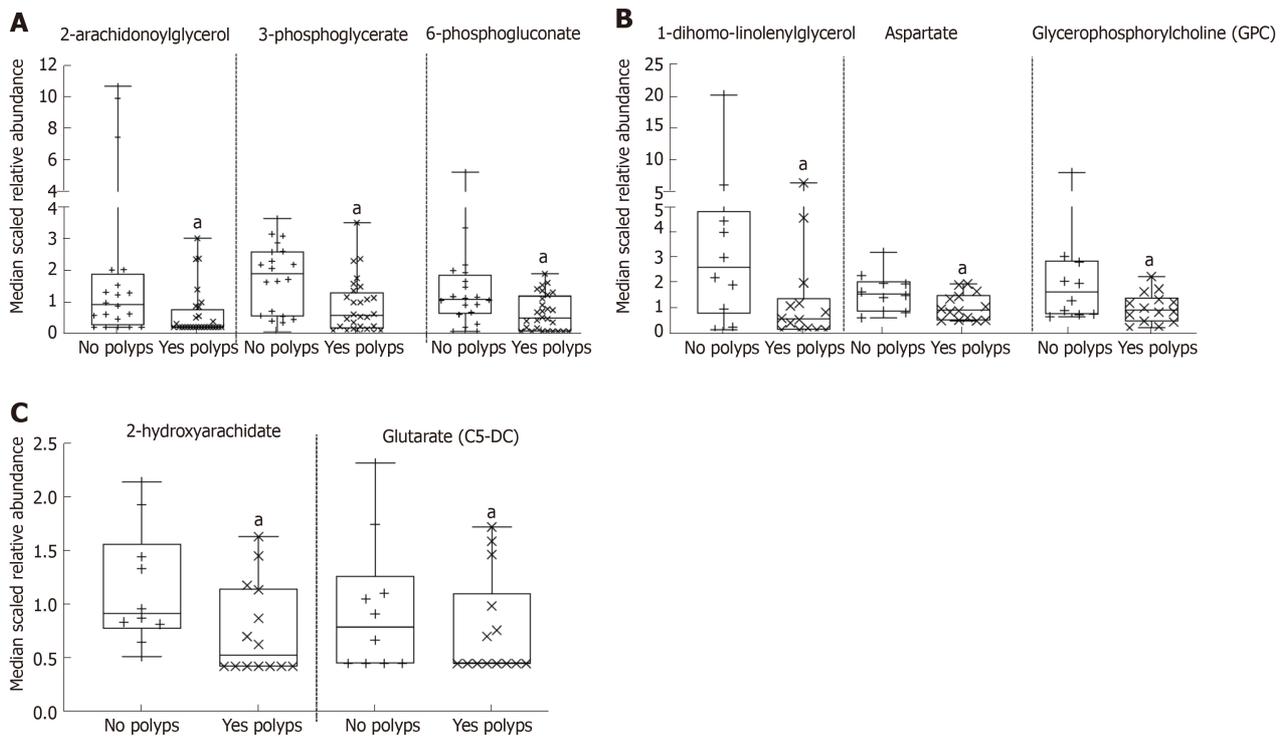


Figure 1 Comparison of colon tissue metabolites between adults with and without polyps. A: Colon metabolite abundance differences from both ascending and descending colon; B: Metabolite abundance in ascending colon; C: Metabolite abundance in descending colon. Statistical significance was examined using linear regression analysis ($^*P < 0.05$).

archidonoylglycerol is 1.68-fold from ascending tissue in overweight adults (Figure 3B). This study suggests as BMI increases lipid diversity decrease in the colon and primarily in the ascending colon.

Ascending and descending colon tissue and stool microbiota composition

To explore associations of taxonomic groups with ascending colon, descending colon, and stool in a healthy weight adult female, we constructed a compositional PCA biplot from centred log-ratio transformed relative abundances. We observed marked separation across all three sample types at the phylum-, family-, and genus-levels. (Figure 4, Supplementary Table 4). Differences between ascending and descending colon were driven by increased abundance for several taxa in the Firmicutes phylum, including *Anaerostipes*, *Blautia*, *Dorea*, and *Fusicatenibacter* (all members of the *Lachnospiraceae* family), as well as *Streptococcus* and *Romboutsia* (members of the *Streptococcaceae* and *Peptostreptococcaceae* families, respectively). Stool samples were also differentiated by the genus *Bifidobacterium* (a member of the phylum Actinobacteria). Comparisons of the composition between colon samples (ascending versus descending) indicated enrichments for *Bacteroides*, *Ruminiclostridium* 9, *Ruminococcus gnavus*, and *Tyzzellerella* in the ascending colon, while the descending colon harbored more *Barnesiella*, *Faecalibacterium*, *Parabacteroides*, *Parasutterella*, and *Roseburia* (Figure 4C and Supplemental Table 4).

DISCUSSION

This study demonstrated colon tissue metabolite profile differences between normal weight, overweight and obese adults, and metabolic distinctions between ascending and descending colon within each of the BMI groups. A healthy human colon tissue metabolome had not previously been established across multiple metabolic pathways and revealed 504 known metabolites in both ascending and descending colon locations. Metabolomics has been widely employed for understanding changes that may result from colon metabolism, but the actual metabolite measurements for association with gut health have been from plasma^[26], urine^[27], stool^[7,9], and from cancerous tissue^[28] or other digestive disease conditions^[29].

Our findings support that a healthy normal weight colon tissue metabolome involves complex lipid metabolism and that differences in lipid metabolite abundance between the right and left colon is associated with regulation of body weight.

Table 2 Fold-differences of colon and stool metabolites

Sub pathway	Biochemical name	Fold difference colon ascending/descending					Fold difference stool BMI comparisons						
		NW	P value	OW	P value	OB	P value	Ow NW	P value	OB NW	P value	OB OW	P value
Methionine, Cysteine, SAM and taurine metabolism	N-acetylglutamine	1.47	0.027	1.15	0.412	1.13	0.534	1.20	0.711	3.44	0.029	0.35	0.096
Endocannabinoid	Oleoylethanolamide	1.6	0.038	1.06	0.790	1.55	0.109	0.23	0.261	1.94	0.174	0.12	0.026
	Linoleoylethanolamide	2.11	0.005	1.26	0.351	1.52	0.171	0.21	0.172	2.49	0.146	0.08	0.012
Phospholipid metabolism	Trimethylamine N-oxide	0.92	0.832	1.02	0.969	2.8	0.049	1.47	0.494	6.23	0.025	0.24	0.149
Phosphatidylethanolamine	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	0.77	0.145	0.66	0.029	0.7	0.113	2.63	0.044	2.57	0.244	1.02	0.322
Lysophospholipid	2-palmitoyl-GPC (16:0) ¹	1.52	0.019	1.18	0.317	0.93	0.709	0.84	0.667	2.81	0.061	0.30	0.039
	1-linoleoyl-GPG (18:2) ¹	1.55	0.030	1.26	0.239	1.05	0.819	1.25	0.335	3.45	0.001	0.36	0.010
Primary bile acid metabolism	Cholate	1.12	0.612	0.84	0.447	0.55	0.041	3.13	0.512	61.06	0.019	0.05	0.114
	Chenodeoxycholate	2.89	0.049	0.41	0.094	0.9	0.872	0.76	0.697	21.80	0.005	0.03	0.004
Secondary bile acid metabolism	Taurodeoxycholate	1.13	0.482	1.49	0.027	0.78	0.249	0.43	0.434	9.32	0.080	0.05	0.024
Pyrimidine metabolism, cytidine containing	Cytidine	1.93	0.036	1.46	0.216	1.12	0.748	1.25	0.469	2.06	0.047	0.61	0.249

¹Not officially confirmed, but confident. Values presented are fold-change of the mean relative abundance within ascending versus descending colon tissue and BMI comparisons from stool. Bold indicates *P* value < 0.05. NW: Normal weight; OW: overweight; OB: Obese; BMI: Body mass index.

Differences were identified for right and left colon metabolites from the endocannabinoid pathway that may signify control over energy metabolism, which regulates appetite, lipolysis, and energy expenditure. The endocannabinoid pathway is implicated in both homeostatic and hedonic food intakes that result in increased hunger^[30]. Specific endocannabinoids, such as the monounsaturated oleoylethanolamide, saturated palmitoylethanolamide and polyunsaturated linoleoylethanolamide showed higher relative abundance in normal weight adults from ascending colon compared to descending colon, and relative higher abundance from stool in overweight adult compared to normal weight. These lipids are also important for regulating metabolism in immune and neuronal cells^[31]. Oleoylethanolamide levels in the mucosal layer of the proximal small intestine was shown to increase with nutrient availability and may be another factor in the

Table 3 Colon lipid metabolites with fold-differences by colon location and body mass index

Sub pathway	Biochemical name	Fold difference ascending/descending					
		NW	P value	OW	P value	OB	P value
Long chain fatty acids	Palmitate (16:0)	1.3	0.02	1.0	0.75	0.7	0.04
		5	1	4	4	3	4
	Margarate (17:0)	1.2	0.13	0.8	0.33	0.7	0.03
		1	1	8		1	7
	Stearate (18:0)	1.3	0.01	0.9	0.61	0.6	0.01
		9	3	4	5	8	6
	Oleate/vaccenate (18:1)	1.5	0.04	1.2	0.36	0.9	0.80
			8		5	4	0
	Nonadecanoate (19:0)	1.1	0.29	0.8	0.26	0.6	0.01
		2		8	5	9	1
Arachidate (20:0)	1.3	0.02	0.9	0.56	0.6	0.02	
	5	5	3	4	9	5	
Eicosenoate (20:1)	1.4	0.04	1.1	0.56	1.0	0.88	
	8	1	1	2	3	3	
Erucate (22:1n9)	1.5	0.03	1.0	0.81	1.1	0.47	
	2	1	4	1	8	2	
Polyunsaturated fatty acid	Dihomo-linolenate (20:3n3 or n6)	1.5	0.04	1.2	0.28	1.1	0.56
		9	1	6	5	6	7
Dihomo-linoleate (20:2n6)	1.6	0.01	1.1	0.41	1.2	0.46	
	8	7	8	7		3	
Fatty acid, dicarboxylate	Dodecadienoate (12:2) ¹	0.9	0.64	0.8	0.26	0.7	0.03
		5	5	7	1	1	2
Ketone bodies	3-hydroxybutyrate (BHBA)	0.4	0.00	0.9	0.90	1.0	0.82
		8	5	7	4	7	5
Fatty acid, metabolism (Acyl choline)	Linoleoylcholine ¹	1.2	0.34	1.1	0.62	2.0	0.02
		6	4	3		6	1
Fatty acid, monohydroxy	2-hydroxystearate	1.3	0.04	0.9	0.67	0.7	0.13
		2		5	7	9	5
Eicosanoid	15-HETE	1.6	0.04	1.1	0.51	1.9	0.03
		9	5	8	5	3	9
Endocannabinoid	Oleoyl ethanolamide	1.6	0.03	2.2	0.79	1.5	0.10
		8		3		5	9
	Palmitoyl ethanolamide	1.3	0.02	1.0	0.56	1.1	0.42
		7	4	8	1	4	1
Linoleoyl ethanolamide	2.1	0.00	1.2	0.35	1.5	0.17	
	1	5	6	1	2	1	
Phosphatidylethanolamine	1-stearoyl-2-oleoyl-GPE (18:0/18:1)	0.8	0.28	0.6	0.01	0.7	0.15
		1	4		6		6
	1-stearoyl-2-linoleoyl-GPE (18:0/18:2) ¹	0.9	0.87	0.6	0.04	0.6	0.09
		7	5	5	3	5	7
	1,2-dioleoyl-GPE (18:1/18:1)	0.6	0.07	0.6	0.03	0.7	0.19
9			5	8	3		
1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6) ¹	0.6	0.01	0.7	0.08	0.4	0.00	
	2		3	1	3	1	
Lysophospholipid	1-palmitoleoyl-GPC (16:1) ¹	1.5	0.03	1.3	0.20	1.2	0.33
		9	6	1	2	8	3
	1-linoleoyl-GPC (18:2)	1.6	0.00	1.5	0.01	1.4	0.08
		8		7	1	3	5
	1-arachidonoyl-GPC (20:4n6) ¹	1.74	0.035	1.55	0.092	1.39	0.285
1-linoleoyl-GPS (18:2) ¹	1.8	0.00	1.4	0.07	1.2	0.31	
	9	2	1	7	6	4	

Plasmalogen	1-palmitoyl-GPG (16:0) ¹	2.1	0.00	1.3	0.18	0.8	0.51	
		1	5	8	3	3	5	
	1-stearoyl-GPG (18:0)	2.1	0.00	1.3	0.16	0.8	0.66	
		6	2	6		9	5	
	1-oleoyl-GPG (18:1) ¹	1.6	0.01	1.0	0.66	1.1	0.47	
				7	8	6	6	
	1-palmitoyl-GPI (16:0)	1.5	0.04	1.1	0.55	0.7	0.31	
		8	1	3	4	7	8	
	1-stearoyl-GPI (18:0)	1.4	0.03	1.0	0.66	0.8	0.50	
		9	4	8	3	7	7	
	1-oleoyl-GPI (18:1)	1.6	0.02	1.2	0.24	0.9	0.81	
		4	7	8	7	4	8	
	1-linoleoyl-GPI (18:2) ¹	1.4	0.01	1.1	0.48	1.2	0.22	
		8	7	1	8	6	5	
Plasmalogen	1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1) ¹	0.7	0.08	0.6	0.02	0.6	0.13	
		1	9	2	2	9	8	
	1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1) ¹	0.7	0.10	0.6	0.01	0.7	0.16	
	4	5	3	9	3			
Monoacylglycerol	1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	0.7	0.23	0.6	0.03	0.7	0.31	
		9	4	4	3	8	1	
	1-palmitoylglycerol (16:0)	1.3	0.25	2.6	0.00	1.2	0.53	
		5	3	1	1	2	7	
	1-palmitoleoylglycerol (16:1) ¹	1.6	0.09	2.2	0.00	1.1	0.62	
			2	3	7	8	3	
	1-oleoylglycerol (18:1)	1.6	0.20	2.2	0.03	0.7	0.50	
			5	5	6	4	7	
	1-linolenoylglycerol (18:3)	1.5	0.07	2.0	0.00	1.0	0.82	
		4	4	7	5	7	3	
	1-dihomo-linolenoylglycerol (20:3)	1.5	0.25	2.2	0.04	0.7	0.57	
		5	1	6		7	4	
	1-arachidonoylglycerol (20:4)	1.5	0.21	2.3	0.01	1.1	0.78	
		1	8	4	7	2	3	
Diacylglycerol	2-palmitoleoylglycerol (16:1) ¹	1.3	0.25	1.7	0.03	0.9	0.89	
		4	7	7	4	6	8	
	2-arachidonoylglycerol (20:4)	1.3	0.25	1.6	0.03	0.9	0.75	
		1	3	8	5	1	2	
	Linoleoyl-linolenoyl-glycerol (18:2/18:3) (2) ¹	1.3	0.51	1.6	0.29	3.3	0.03	
		4	7	1	6	2	9	
	Ceramides	N-palmitoyl-sphingosine (d18:1/16:0)	0.9	0.73	0.6	0.00	0.7	0.04
			6	1	9	8	3	9
		N-stearoyl-sphingosine (d18:1/18:0) ¹	1.0	0.57	0.7	0.03	0.8	0.33
			9	8		1	3	9
		N-palmitoyl-heptadecasphingosine (d17:1/16:0) ¹	0.9	0.95	0.7	0.04	0.8	0.20
			9	7	4	2		0
	Hexosylceramides (HCER)	Glycosyl-N-palmitoyl-sphingosine (d18:1/16:0)	0.7	0.04	0.6	0.00	0.6	0.02
		4	8	2	3	7	9	
Primary bile acid	Glycochenodeoxycholate	0.9	0.75	1.8	0.00	0.8	0.54	
			8	6	3	7	0	
	Taurochenodeoxycholate	1.0	0.98	1.6	0.45	0.8	1	
		4	7	2	6	6		
Secondary bile acid	Glycolithocholate sulfate	0.9	0.70	1.4	0.01	1	1	
		5	3		2			
	Deoxycholate	0.9	0.79	0.6	0.12	0.7	0.00	
		7	8	3	3	9		

¹Not officially confirmed, but confident. Values presented are fold-difference of the mean relative abundance between ascending/descending colon, by

weight class. Bold indicates *P* value < 0.05. BMI: Body mass index; NW: Normal weight; OW: Overweight; OB: Obese.

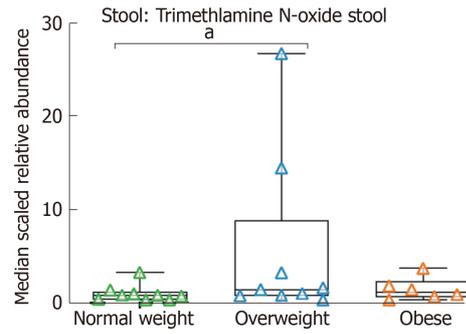
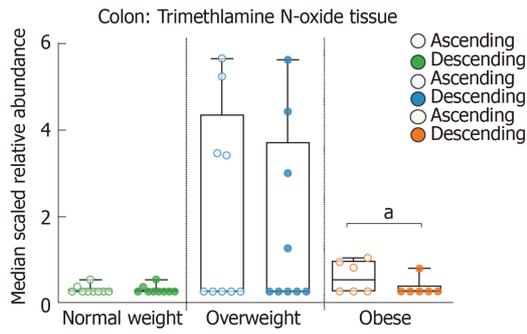
regulation of satiety^[32]. Palmitoylethanolamide has supportive roles for reducing inflammation and eliciting neuroprotective effects^[33], while linoleoylethanolamide has reported anti-inflammatory functions^[34].

Bile acids are important signaling molecules which contribute to regulation of whole-body glucose, lipid metabolism, and body weight^[35]. Primary and secondary bile acids were also identified for distinctions in abundance by colon location and from stool between overweight/obese and normal weight adults. Primary bile acids produced in the liver and increased bile acids in colon tissue may indicate altered reabsorption of bile acid by the liver and that results in subsequent alteration to metabolism by intestinal microbiota^[36]. Primary bile acids, cholic acid and chenodeoxycholic acid, are derived from cholesterol by an enzymatic reaction occurring mainly in the liver^[37]. Chenodeoxycholate has shown to increase colonic transit and improves bowel function^[38]. Dietary cholic acid supplementation in rats caused a significant increase in colon tumors^[39]. Interestingly, this study showed that primary bile acids; glycocholate, glycochenodeoxycholate, and taurochenodeoxycholate had 1.26-fold – 1.86-fold difference in overweight colon and merit attention as a mechanism with alongside other lipid classes to increase cancer risk in people. Concentrations of bile salts was shown to be higher in the proximal colon and bile-acid profiles were hypothesized to increase the risk of proximal cancer^[40].

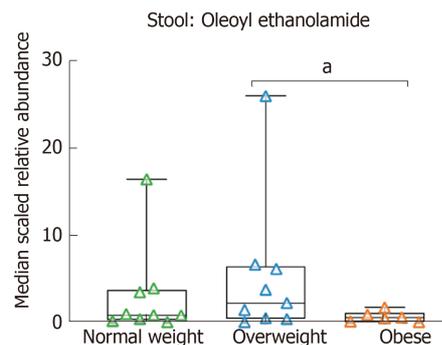
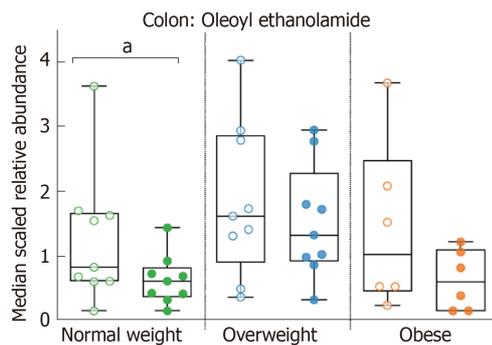
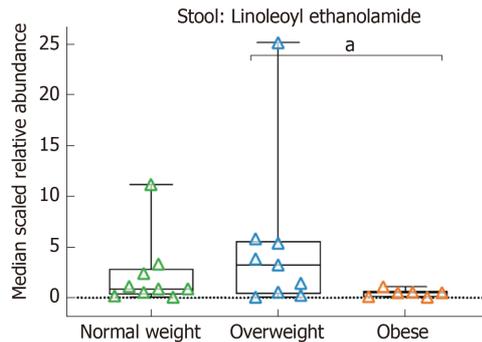
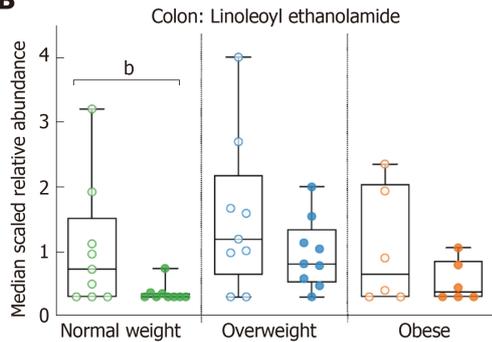
Population- based studies have shown that individuals who consume high-fat and high-beef foods display elevated levels of fecal secondary bile acids, as do patients diagnosed with colonic carcinomas^[3,41]. Secondary bile acid, taurodeoxycholate are generated from primary bile acids and were shown to be increased in obese children plasma with insulin resistance when compared with their non-insulin resistant counterparts, unveiling the influence of the gut microbiota on the host metabolism^[42]. Glycochenodeoxycholate a secondary bile acid produced by microbial flora in the large intestine was associated with colorectal cancer in women^[43], and high levels of deoxycholate in blood, bile feces, and mucosa were increased in colorectal cancer^[7,37]. This study showed elevated glycochenodeoxycholate from descending colon in overweight adults, and elevated deoxycholate from descending colon in obese adults. Impaired bile acid signaling and dysbiosis may contribute to type 2 diabetes and other metabolic disease associated with obesity and colorectal cancer risk^[44]. This study had limitations in the total sample size for each BMI group and did not control for different dietary intake patterns. The lack of gender balance in each BMI subgroup was also a potential source of bias for sex-based differences that may exist in colon tissue metabolite profiles. Future studies that control these variables merit attention because the colon tissue metabolite signatures that emerged herein did demonstrate metabolic relevance to the high risk of overweight and obesity in the progression of proximal and distal colon cancers.

Colonic TMAO abundance in obesity was a major finding from this study with respect to risk for cancer and supports a role for phospholipids from choline metabolism. TMAO was identified herein for increased abundance in ascending colon of obese adults and in stool of overweight adults. Deng *et al*^[26] showed increased plasma levels of TMAO in patients with right sided colon cancer when compared to left sided colon cancer patients. High urine concentration of TMAO also directly correlated to the consumption of a high meat containing diet^[45] and higher total milk and dairy consumption in plasma^[46]. The increased levels in serum and urine were also shown to be associated with predisposition to impaired glucose homeostasis in high fat diet-fed mice^[47]. Links between colorectal cancer and TMAO was detected in a genome-wide systems analysis^[48] and in the development of colorectal cancer^[26]. Our findings also revealed microbiome-influenced metabolites in the colon tissue that were not in the stool, such as the ketone body, 3-hydroxybutyrate, an eicosanoid; 15-Hydroxyeicostetraenoic acid (15-HETE), and the monoacylglycerol; 2-arachidonoylglycerol. Ketone bodies are strongly affected by obesity-related metabolic disorders and are utilized in the body as an energy source^[49]. In visceral adipose tissue from obese subjects, 15-HETE was higher than in healthy subjects^[50]. These aforementioned metabolic changes support the differences in microbiota between stool and colon and between colon locations (ascending *vs* descending). Right and left colon microbiota analysis for differences in healthy adults has been limited^[19]. We observed an increased abundance of *Bacteroides* in the ascending colon and Proteobacteria in the descending colon that were consistent with Flynn *et al*^[19]. Given that stool did not recapitulate the composition of the colonic mucosa-associated microbiota and metabolites, additional investigations with larger cohorts of each BMI group is warranted that will assess impacts of intervention strategies to reduce

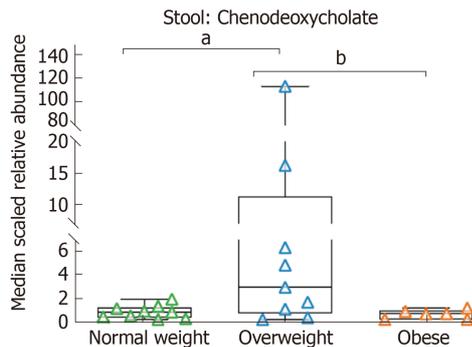
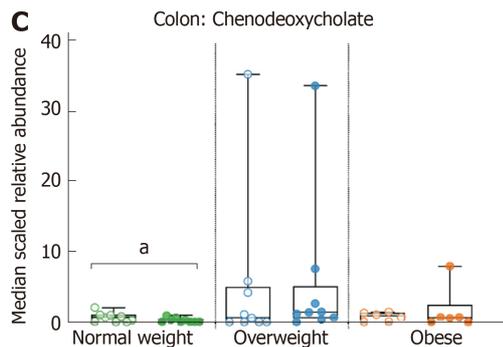
A



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C



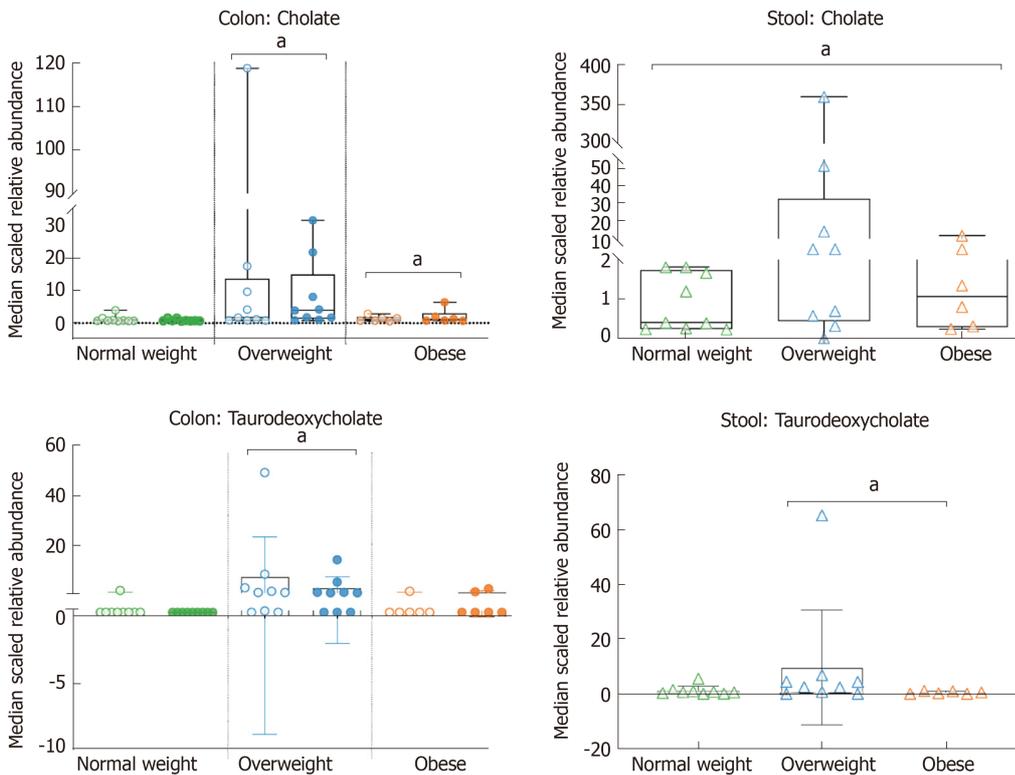


Figure 2 Fold-differences of colon and stool metabolites according to body mass index. Metabolites were A: Phospholipid; Trimethylamine N-oxide; B: Endocannabinoids; linoleoyl ethanolamide, and oleoyl ethanolamide; C: Primary and secondary bile acids; chenodeoxycholate, cholate, and taurodeoxycholate. Left side panel colon, right side panel stool. Open circles represent ascending colon, closed circles represent descending colon, triangles represent stool (^a*P* < 0.05, ^b*P* < 0.01).

disease risk.

Fatty acids were also statistically supported in abundance by right and left location, and alongside BMI. Food-derived long chain fatty acids are found in dairy fat, coconut oil, palm kernel oil, peanut oil and vegetable oils. Monounsaturated long-chain fats such as oleic acid, and palmitoleic acid are found in animal fats, olive, canola and safflower oil. Oleic acid enhances insulin action and inhibits glucose production^[51], but also demonstrates cardiovascular benefits when it replaces heart-damaging saturated fat^[52]. Palmitic acid is the first fatty acid produced during fatty acid synthesis, and is the precursor to longer fatty acids, while excess carbohydrates in the body may also be converted to palmitic acid. This analysis revealed major differences in long chain fatty acids as relevant to normal weight ascending colon and were also significantly different in the opposite direction of obese adults, namely increased in descending colon. Polyunsaturated long chain fats include linoleic acid, alpha-linolenic acid (seeds and nuts), arachidonic acid (meat, eggs, and algae) and eicosapentaenoic acid (fish oil)^[53]. Margarate, also known as heptadecanoic acid is a biomarker of long-term milk fat intake^[54], and was elevated in the obese adults for descending colon. Stearate fed to mice showed 70% reduction of visceral fat^[55], and reduced metastasis tumor burden in a breast cancer mouse model^[56]. Arachidate is necessary for the function of all cells, especially in the nervous system, skeletal muscle and immune systems^[57].

In conclusion, our study identified important metabolic differences between the right and left colon tissue of healthy adults and highlighted a wide range of lipids from normal weight, overweight and obese adults. The magnitude and abundance of a metabolite difference between ascending and descending colon tissue has not been previously evaluated and warrants further investigation for screening risk of proximal versus distal colon cancers.

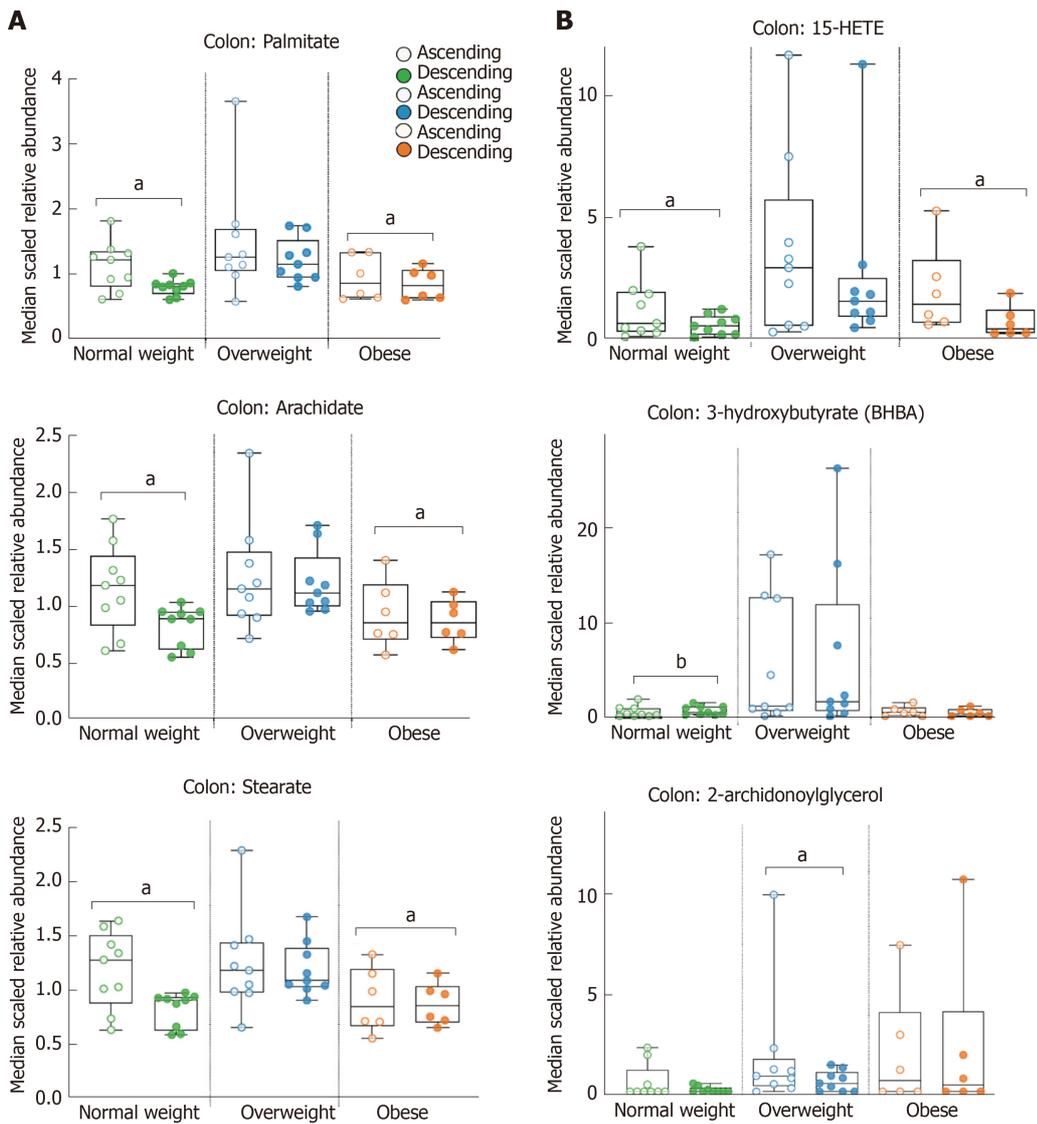


Figure 3 Fold-differences of ascending vs descending lipid metabolites in normal weight, overweight and obese adults. A: Long chain fatty acids; palmitate, arachidate, and stearate; B: Microbiome influenced metabolites; 15-HETE, 3-hydroxybutyrate (BHBA), and 2-archidonoylglycerol. Open circles represent ascending colon, closed circles represent descending colon (^a $P < 0.05$, ^b $P < 0.01$).

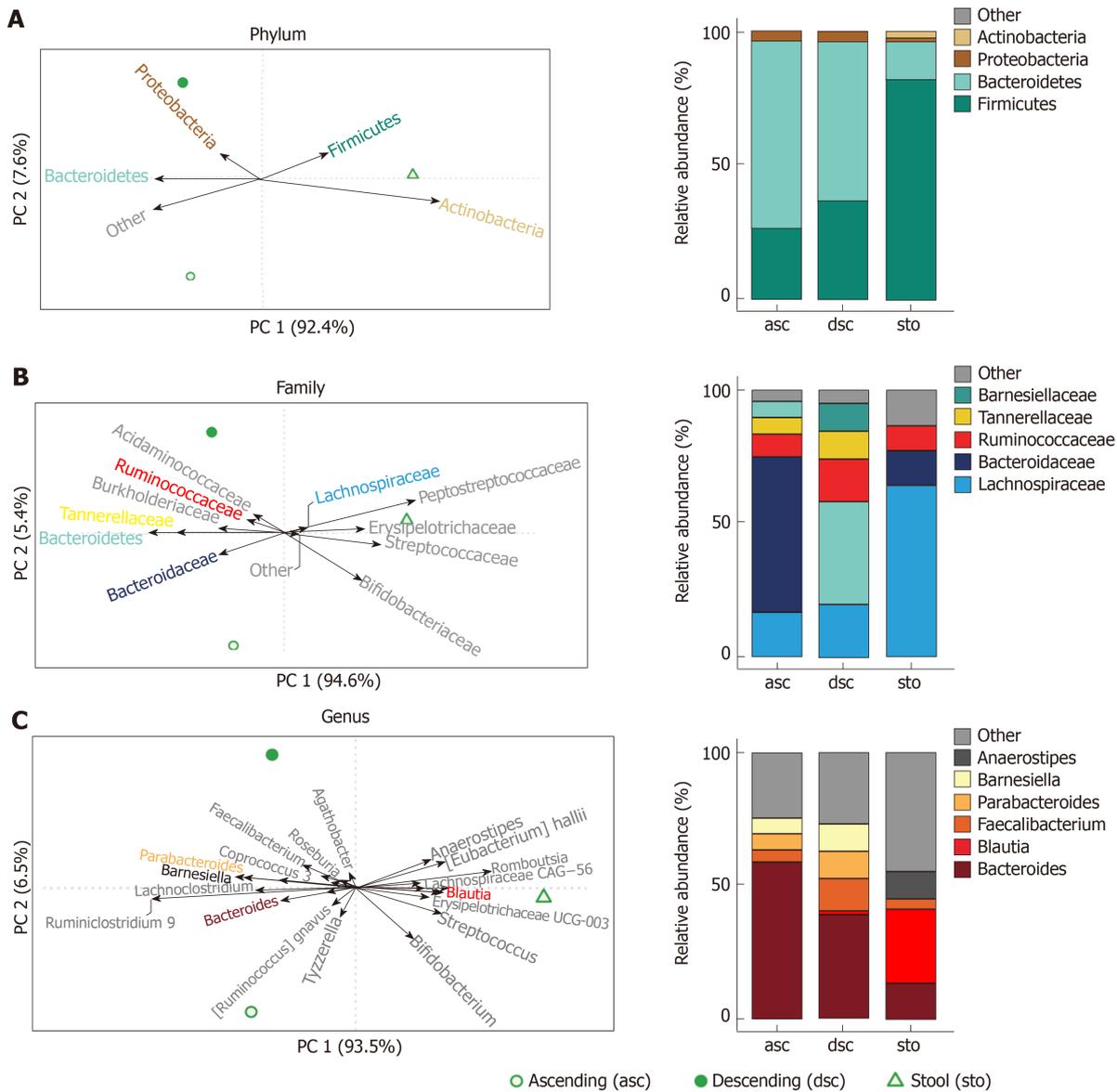


Figure 4 Composition of microbiota from stool, ascending and descending colon in a healthy, normal weight adult. Left side shows principal components analysis biplots of centred log-ratio transformed relative abundances at the phylum; A: Family; B: Genus; C: Levels. Lines with arrows represent individual taxa. Right side shows relative abundance bar charts for the most abundant taxa at the respective taxonomic level across stool and colon location sample types.

ARTICLE HIGHLIGHTS

Research background

Obesity is a risk factor for colorectal cancer, yet metabolic distinctions between healthy right and left colon tissue, before cancer is diagnosed, remains largely unknown.

Research motivation

Colon cancer of the ascending colon has a poorer prognosis and survival when compared to colon cancer on the descending colon. Stool metabolite composition does not accurately reflect proximal/ascending/right sided colon. Development of healthy colon tissue small molecule signatures for ascending and descending colon will aid in our understanding of how to improve gut metabolism and may help prevent or mitigate colorectal cancer risk.

Research objectives

This study compared right-ascending and left-descending colon tissue metabolomes and sought to identify differences from the stool metabolome in normal weight, overweight, and obese adults.

Research methods

Global, non-targeted metabolomics was applied to assess right-ascending and left-descending colon tissue collected from healthy adults undergoing screening colonoscopies to test the

hypothesis that body mass index (BMI) differentially impacts colon tissue metabolite profiles. The colon tissue and stool metabolome of healthy adults was analyzed for metabolite signatures and metabolic pathway networks implicated in progression and prevention of colorectal cancer.

Research results

This is the first report using metabolomics to compare the right-ascending *vs* left--descending colon tissue of healthy adults. Our findings show that BMI was associated with metabolite profile differences between the ascending and descending colon. Disturbances in multiple metabolic pathways of the right and left colon from being overweight/obese may have important implications for increasing colorectal cancer risk.

Research conclusions

There were metabolite profile differences between right-ascending and left-descending colon tissue in healthy adults receiving routine, screening colonoscopies. BMI impacts the number, type and magnitude of metabolite differences between the ascending and descending colon. Colon lipids and other metabolites in obese and overweight adults were distinguished from normal weight participants and associated with gut inflammation, nutrient absorption, and products of microbiota metabolism.

Research perspectives

Right and left colon tissue metabolites that differ in relative abundance between normal weight, overweight, obese adults may be sensitive biomarkers for colon cancer risk. Diet and lifestyle influence right and left sided colon tissue metabolite composition that shape inflammation and cancer risk in overweight and obese adults. Development of healthy colon tissue small molecule signatures for ascending and descending colon will aid in our understanding of how to improve gut metabolism and may help prevent or mitigate colorectal cancer risk.

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