THESIS

RELATIONSHIPS BETWEEN OBJECTIVELY MEASURED PHYSICAL ACTIVITY AND STOOL METABOLOME IN INDIVIDUALS AT HIGH RISK FOR COLORECTAL CANCER

Submitted by

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ABSTRACT

RELATIONSHIPS BETWEEN OBJECTIVELY MEASURED PHYSICAL ACTIVITY AND STOOL METABOLOME IN INDIVIDUALS AT HIGH RISK FOR COLORECTAL CANCER

Physical activity is known to reduce the risk of colorectal cancer and adenomatous polyp recurrence. Targeted metabolomic profiling of the stool metabolome may provide insight to the mechanistic links between physical activity and colorectal cancer. This study utilized baseline physical activity and metabolomics data from a dietary fiber intervention for individuals at high risk for colorectal cancer. Participants (*N*=21), 59±9 years, BMI 28.1±3.35 wore an activPAL™ accelerometer for 7 consecutive days, provided a stool sample, and a 3-day dietary log. Stool bile acids and short chain fatty acids were quantified with gas chromatography mass spectroscopy and ultra-high performance liquid-chromatography tandem mass spectrometry. Linear regression models examined relationships between light intensity physical activity minutes, moderate to vigorous physical activity minutes, MET-hours per day, and sedentary minutes, with abundances of short chain fatty acids and bile acids while accounting for dietary fiber intake. Light intensity PA minutes predicted butyrate and propionate abundance when accounting for dietary fiber. MVPA predicted deoxycholic acid and ursodeoxycholic acid abundance. These data suggest that both low intensity PA and MVPA may both have implications for colorectal cancer primary and secondary prevention through different mechanisms.

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1. INTRODUCTION

With 135,000 new cases of colorectal cancer and over 50,000 colorectal cancer related deaths in 2017, colorectal cancer is the third most common, and the second leading cause of death from cancer in the United States.[1].[2] Colorectal cancer is a cancer of the large intestine or rectum that typically originates from adenomatous polyps in 90-95% of all large bowel tumors. Adenomatous polyps form from uncontrolled crypt cell division, and appear as circumscribed lumps of epithelial dysplasia.[3] Polyps can be discovered with a colonoscopy, and the American Cancer Society (ACS), recommends that asymptomatic individuals should undergo a colonoscopy at age 45, and every 10 years thereafter should no polyps be found, or every 3 years thereafter if polyps are found.[4] Colorectal cancer is found in nearly all races and both sexes, but there is a marked difference among geographic location. Adenomatous polyps are rare in Africa and some Asian countries, but are very common in Western countries. Colorectal cancer rates generally correlate with the adoption of a western lifestyle, but mortality is much lower in higher income countries with access to screening techniques.[5]

The majority of colorectal cancer incidence is linked to modifiable lifestyle factors. Only 5-10% of incidences of colorectal cancer are due to hereditary conditions, such as Lynch Syndrome and Familial Adenomatous Polyposis.[6] According to the World Cancer Research Fund, 30-40% of all cancers can be prevented by appropriate diet and exercise, and this figure may be as high as 70% for colorectal cancer.[7] Physical activity is known to be protective against a multitude of cancer types including colon and rectal cancers,[8-10] and may also protect against adenomatous polyp recurrence.[10,11] Physical activity is known to induce many systemic effects,[12,13] and several proposed mechanisms exist by which physical activity may prevent colorectal cancer. Physical activity can reduce systemic inflammatory status, as well as increase anti-inflammatory cytokines, catecholamines, and reduce adipokine and pro-inflammatory cytokine production.[14] Further, physical activity reduces bowel transit time,[15] and increases bowel motility,[14] which are both protective against colorectal cancer progression. Additionally, hyperinsulinemia may play a role in epithelial cell growth[16] and physical activity is known to increase insulin sensitivity. However, recent

work suggests that both microbial and innate metabolism may play a pivotal role in the colon and rectal microenvironment and affect colorectal cancer development.[17,18] Thus, targeted metabolomics is a method that could help elucidate the relationship between physical activity and colorectal cancer prevention.

Metabolomics is defined as "the scientific study of the set of metabolites present within an organism, cell, or tissue."[19] It includes lipids, amino acids, carbohydrates, peptides, energy metabolites, cofactors, vitamins, and exogenous compounds. Metabolomics is a unique approach because it allows one to examine both endogenous and exogenous compounds in a specific environment. This is important for understanding the role of the microbiome as well as host metabolism in progression of colorectal cancer. Targeted metabolomics allows for a unique approach to examining specific metabolites in absolute, rather than relative, abundance. Several metabolites in the stool have been associated with colorectal cancer, and they may be modifiable by physical activity.[20,21] Targeted metabolomics is a unique approach that allows for quantification of absolute, rather than relative, abundance of metabolites of interest. Both short chain fatty acids and bile acids are possible mechanistic links between physical activity and colorectal cancer prevention.

Bile acids are biologic detergents synthesized in the liver, stored in the gall bladder, and secreted into the duodenum that facilitate the intestinal absorption of dietary lipids. The majority of bile acids are resorbed in the ileum. However, a small minority are passed to the colon for excretion in stool. Bile acids in the stool may be contraindicated due to possible carcinogenic and inflammatory mechanisms. In the colon, primary bile acids can be conjugated to secondary bile acids by gut microbes[22,23]. Deoxycholic acid (DCA), a secondary bile acid, can activate proteosomal degradation of the tumor suppressor p53, selecting for cells resistant to apoptosis in spite of DNA damage.[24] Further, DCA may cause membrane perturbations leading to the release of arachidonic acid. Arachidonic acid is converted by COX-2 or lipooxygenase to pro-inflammatory and pro-angiogenic prostaglandins and reactive oxygen species. These can damage DNA and inhibit DNA repair enzymes in colonic epithelial cells. Conversely, ursodeoxycholic acid may actually be preventive of colorectal cancer and is used as a supplement in individuals at high risk.[20,25] Bile acids are modifiable by physical activity.[20] Physical activity may help

reduce fecal bile acid concentration through several mechanisms. GI pancreatic hormones such as vasoactive intestinal polypeptide, motilin, and pancreatic polypeptide affect the motility of the large intestine.[24] This can reduce transit time and thus increase bile acid secretion. Additionally, regular aerobic exercise reduces triglycerides, VLDL particle size, and LDL particle size.[26] Caloric restriction has been shown to reduce bile acid synthesis but it is unknown if physical activity has the same effect and/or mechanism.[27]

Short chain fatty acids, in particular propionate, acetate and butyrate, are associated with reduced risk of colorectal cancer and are modifiable by physical activity through intestinal mixing, increased anaerobic fermentation, increased gut motility, and increased expression of butyrate-acetoacetate coA transferase (BCoAT).[21,28,29] Short chain fatty acids are produced by the gut microbiota during fermentation of non-digestible or partially digestible polysaccharides. Some butyrate producing bacteria include *Clostridiales, Lachnospira, Rosebria, f_Lachnospiraceae, and Faecalibacterium*.[21] Butyrate has been studied for its role in nourishing the colon mucosa as well as its ability to help to prevent colon cancer. Butyrate and propionate, but not acetate, inhibit histone deacetylases in immune cells and colonocytes. Some possible mechanisms include promotion of cell differentiation, cell-cycle arrest and apoptosis of transformed colonocytes, inhibition of histone deacetylase, decreased transformation of primary to secondary bile acids because of colonic acidification.[30]

It is well known that total physical activity can help prevent colorectal cancer and polyp recurrence, but it is unknown whether or not stool bile acids and short chain fatty acids provide a possible mechanistic link for this relationship. Further, it is unknown what type or intensity of physical activity has the strongest relationship with these aforementioned metabolites. Metabolic signatures associated with physical activity in populations with high risk for cancer development could be a useful tool to understand disease phenotypes and help to reduce cancer risk[31,32].

Only two previous studies have examined the effects of physical activity on the metabolome[31,32], and none have examined the relationship between the metabolome and physical activity in the context of

colorectal cancer prevention. Thus, the purpose of this study is to examine relationships between physical activity and the stool metabolome in individuals at high risk for colorectal cancer.

2. METHODS

Study Overview

This study utilized baseline data from a pilot, randomized trial "**B**eans/bran **E**nriching **N**utritional **E**ating **F**or **I**ntestinal health and **C**ancer **I**ncluding **A**ctivity for **L**ongevity (BENEFICIAL)". Study visits occurred at the Human Performance Clinical Research Laboratory at Colorado State University. All individuals provided written consent prior to participation in the study, and study protocols were approved by Colorado State University (protocol # 17-7464H).

Participants

Study participants were recruited from the University of Colorado Health System, Centers for Gastroenterology, and via university and community-based email recruitment efforts. Participants were eligible if they met the following criteria: (1) a healthy adult (≥18 years of age) who had one or more adenomatous polyps removed within the previous 3 years , (2) had not received adjuvant treatment such as chemotherapy or radiotherapy with their surgical removal of polyps/tumors, (3) not pregnant or lactating, or planning to become pregnant during the study, (4) no history of food allergies and/or major dietary restrictions, (5) ability to complete study questionnaires in English, and food logs electronically, and (6) willing to provide blood, urine, and stool samples as required by study protocol.

Sample Collection

Stool samples were collected into a sterile urine cup within 24 hours before the lab visit. A sample of raw stool was immediately transferred to a 15mL conical tube and stored at -80°C until further analysis. The remaining stool was lyophilized. The urine cup lid was replaced with autoclaved aluminum foil containing small ventilation holes. Samples were lyophilized for 48-72 hours or until sample mass did not change over a 24-hour period.

Anthropometry

Height, weight, were measured in light clothing without shoes, and used to calculate Body Mass Index (BMI) (kg/m^2). Waist circumference was measured at the umbilicus. Each measurement was taken three times in succession by the same technician, and averaged.

Cholesterol

Twelve-hour fasted blood samples were collected between 7:00am and 11:00am. Blood was drawn through venipuncture into an EDTA tube (Greiner bio-one, Monroe, NC) and immediately stored on ice. Total cholesterol, HDL-Cholesterol, and LDL-Cholesterol were assessed in whole blood with Piccolo® Lipid Panel Plus (Abaxis, Inc, Union City, CA).

Dietary Fiber Quantification

Participants provided a 3-day dietary food log.[33] All foods or beverages consumed and their quantities were recorded. Participants were asked to consume their habitual diet and record two week days and one weekend day. The food logs were analyzed using Nutritionist Pro™ (Axxya Systems, Redmond, WA) and each participants' caloric intake, macronutrient and micronutrient consumption were estimated for each individual.

Physical Activity

Physical activit (PA) was measured using an activPAL accelerometer (PAL Technologies, Glasgow, Scotland). The activPAL quantifies free-living sedentary and ambulatory activities. It has been validated as one of the most accurate wearable activity monitors and has previously been used in healthy adults as well as cancer survivors.[34-36] The activPAL activity monitor is a small device worn on the thigh that uses information about static and dynamic acceleration to 1) distinguish body posture as sitting/lying, standing and stepping and 2) estimate energy expenditure (EE) (expressed as metabolic equivalents (METs)).[37] The activPAL was wrapped in a nitrile sleeve for waterproofing and attached to the anterior aspect of the right thigh with Tegaderm dressing. Participants wore the activPAL for 7 consecutive days, 24 hours per day, for the week immediately following the baseline study visit. Participants were asked to

record times of activPAL removal and sleep/wake-up times. Data were downloaded using the activPAL software (v. 7.2.38) and summarized using the *activpalProcessing* package in R.[38] Days of data collection required at least 600 min (10 h) of wear time to be considered valid. Non-wear periods were excluded from analyses; these were defined using participant logs and verified via visual inspection of the accelerometer data. A cadence based linear regression was used to estimate total MET hours and time spent in sedentary (METs <1.5), light (METs 1.5-2.99), and moderate-to-vigorous (METs≥3) intensity activity per day.[39] Each variable was summed between all valid days and divided by valid days to calculate daily values.

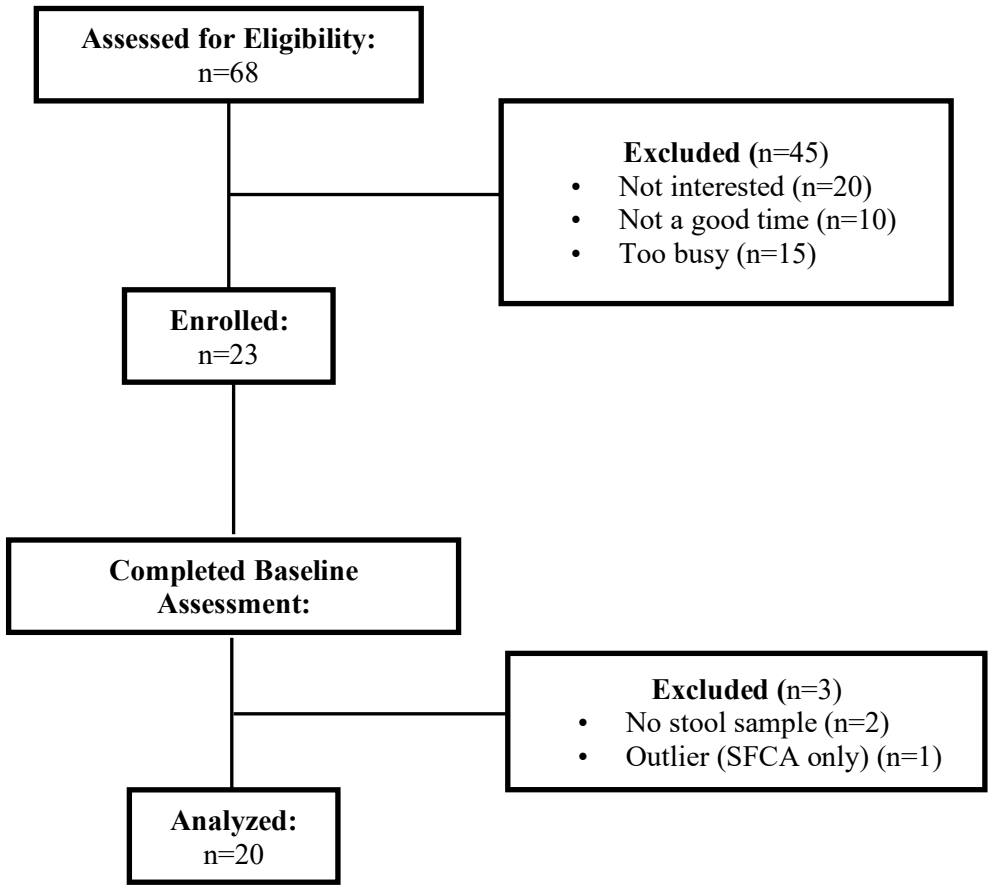

Figure 1: CONSORT Diagram: participants and stool samples used for metabolomic analysis

Targeted Metabolomics

Stool metabolite extraction and targeted metabolic profiling occurred at the Colorado State University Proteomics & Metabolomics Facility using both ultra high-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) and gas chromatography mass spectrometry (GS-MS).

Short chain fatty acids:

Samples arranged in a randomized order were extracted and injected to GCMS along with 7 quality controls (QCs) that were generated from a pooled sample extract. 20 mg frozen stool samples, 340 µL of cold 3 M HCl and 60 µL of internal standard solution containing 1 mg/mL of $^{13}C_2$-acetic acid (Sigmal-Aldrich) and 0.5 mg/mL of $^{13}C_4$-sodium butyrate (Santa Cruz Biotechnology) were added. Samples were vigorously shaken for 30 min, followed by sonication for 10 min in a cold water bath, and then centrifuged at 15,000 *g* for 15 min at 4°C. Supernatants (200 µL) were recovered, and added to 350 µL MTBE, followed by vortexing for 5 s twice. About 60 µL of the top MTBE layer were recovered after centrifugation at 3,000 *g* for 5 min at 4°C, and stored at 4°C until analysis. The MTBE extract (1 µL) of short chain fatty acids were injected into a Thermo Trace 1310 GC coupled to a Thermo ISQ-LT MS, at a 5:1 split ratio. The inlet was held at 240°C. SCFA separation was achieved on a 30m DB-WAXUI column (J&W, 0.25 mm ID, 0.25 µm film thickness). Oven temperature was held at 100°C for 0.5 min, ramped at 10°C/min to 175°C, then ramped to 240°C at 40°C/min, and held at 240°C for 3 min. Helium carrier gas flow was held at 1.2 mL/min. Temperatures of transfer line and ion source were both held at 250°C. SIM mode was used at a rate of 10 scans/sec under electron impact mode. Data were processed using Chromeleon software (version 7.2.8, Thermo). Internal standard, $^{13}C_2$-acetic acid, was used to quantify acetic and propionic acid; $^{13}C_4$-sodium butyrate was used to quantify other acids. Pooled QC samples were injected after every 6 injections. The CV of 6 QCs was 1.0-3.6%. Linearity with R^2 >0.997 was obtained from all the calibration curves. Limit of detection (LOD) and limit of quantification (LOQ)

were calculated using the standard deviation of blanks and the slope of calibration curve.

Bile acids:

Hydrolysis Solution: 100 mM NaOH, pH 13

Extraction Solution: 100% Acetonitrile spiked with Internal Standards each at 400 ng/mL

Standard Curve: All target analytes were prepared from dry stock at a concentration of 1 mg/ml in 100% ACN. A master mix of all target analytes was prepared in 100% ACN at a concentration of 0.1 mg/mL. Internal standards (Glycocholic acid-d4, Taurocholic acid-d5, and Deoxycholic acid-d4) were prepared at the same concentrations. Dilution series were made in 30% ACN, 70% 100 mM NaOH spiked with IS-mix at 400 ng/mL. Starting concentration was 5,000 ng/mL. Low point concentration was 1 ng/mL, with an IS only zero point to calculate standard deviation of background signal.

Extraction:

Bile acids were extracted from stool samples in the presence of 0.1 M sodium hydroxide followed by protein precipitation with acetonitrile. Human stool samples were provided homogenized and dried. 10 mg of stool were processed in 0.1 mL of 0.1M Sodium Hydroxide (pH 13) for 1 hour at 60^{O}C. Samples were vortexed intermittently every 20 minutes. 200 microliters of freezer-cold (-20^{O}C) 100% Acetonitrile (spiked with IS mix at 400 ng/mL) was added to each sample prior to vortexing at 4^{O}C for 30 minutes. Samples were left at -80^{O}C overnight. Precipitate was collected via centrifugation the following morning (17,000 x g, 30 min at 4^{O}C). Supernatant (180 uL) was transferred to a 200 uL glass vial insert and vials were stored at -80^{O}C until LC-MS analysis.

LC-MS/MS Analysis:

LC-MS/MS was performed on a Waters Acquity UPLC coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer. Chromatographic separations were carried out on a Waters T3 stationary phase (1 x 100 mm, 1.7 μM) column. Mobile phases were Acetonitrile (B) and water with 0.1% formic acid (A). The

analytical gradient was as follows: time = 0 min, 30% B; time = 1 min, 30% B; time = 11 min, 97% B; time = 12 min, 97% B; time 12.05 min, 30% B; time = 15 min, 30% B. Flow rate was 200 µL/min and injection volume was 2.0 µL. Flow rate was 800 µL/min and injection volume was 0.5 µL. Samples were held at 4°C in the autosampler, and the column was operated at 45° C. The MS was operated in selected reaction monitoring (SRM) mode, where a parent ion is selected by the first quadrupole, fragmented in the collision cell, then a fragment ion selected for by the third quadrupole. Product ions, collision energies, and cone voltages were optimized for each analyte by direct injection of individual synthetic standards. Inter-channel delay was set to 3 ms. The MS was operated in negative ionization modes with the capillary voltage set to 2.1 kV respectively. Source temperature was 150° C and desolvation temperature 500°C. Desolvation gas flow was 1000 L/hr, cone gas flow was 150 L/hr, and collision gas flow was 0.2 mL/min. Nebulizer pressure (nitrogen) was set to 7 Bar. Argon was used as the collision gas. A calibration curve was generated using authentic standards for each compounds and their corresponding stable isotope labeled internal standards in neat solution.

Statistical Analysis

Power was calculated using Lenth's Java Applet for Power and Sample Size[40]. To achieve β=80%, with an R^2 of 0.5 on 2 regressors, a sample size of 21 was required. Post, rather than pre, intervention data was utilized for one female subject (for short chain fatty acids) due to lack of raw stool from baseline. Data was assessed for normality using Q-Q plots. One outlier was detected based on its large Cook's D value for short chain fatty acids, and removed for further analyses. For 9 bile acids, levels below the detectable limit were replaced with 50% of the limit of detection value.

Univariate associations between PA and bile acids and short chain fatty acids were explored using Pearson correlations, separately for participants consuming a high fiber, vs. low fiber diet (based on median split for dietary fiber intake of 23.194 g/day). Specific bile acids (butyrate, and propionate), and short chain fatty acids (DCA, UDCA) were selected based on previous studies suggesting their implication in colorectal cancer prevention, ability to be modified by physical activity.

Each were plotted with their respective physical activity predictor and dietary fiber (high or low) (GraphPad Prism (Version 8).

Separate multivariable linear regression models were used to examine associations between activPAL variables and short chain fatty acids and bile acids, with dietary fiber as a covariate. Several other variables such as BMI, waist circumference, total cholesterol, HDL-cholesterol, LDL- cholesterol, and dietary fat intake were considered as covariates but we lacked statistical power to use >2 predictors and based on univariate correlations, no significant relationships existed between these parameters with any physical activity outcomes ($p>.05$). Statistical analyses were performed using R software.

3. RESULTS

Of the 68 individuals assessed for eligibility, 21 were eligible and completed the food log, fasted blood draw, stool sample, anthropometry, and 7-days of accelerometer wear. The most common reason for exclusion was disinterest in completing the dietary intervention. Figure 1 shows flow of participants through recruitment, eligibility screening, enrollment, and analyses. Participants were a mean age of 58.95 (SD=8.56) years, mostly Caucasian, and evenly split male/female. Most participants were overweight (50%), and the average BMI was 28.14 ± 3.35 kg/m^2. All participants achieved a minimum of 150 minutes of MVPA per week (i.e., meeting physical activity guidelines).[41] Additional participant characteristics and means and standard deviations for physical activity are shown in Table 1. Means and standard deviations of short chain fatty acids in raw stool and bile acids in lyophilized stool in μg/g are shown in Table 2 .

Table 1: Participant Characteristics (n=21)	Mean (SD) unless otherwise noted
Age	58.95 (8.56)
Sex, female %	48%
Caucasian %	76%
BMI	28.14 (3.35)
Waist Circumference (in)	36.81 (3.62)
Years since polyp removal	1.8 (1.4)
Cancer Stage (n, %)	
0	18 (86%)
I	3 (14%)
Dietary Fiber Intake (g/day)	24.99 (10.83)
MET-hours (per day)	22.67 (2.02)
MVPA (min/day)	71.13 (23.50)
Guideline minutes (min/day)	20.71 (22.63)
Light activity (min/day)	268.24 (76.70)
Sedentary minutes (min/day)	545.99 (85.24)

Table 2: Short Chain Fatty Acid and Bile Acid Abundances	
	Mean, μg/g (SD)
Short Chain Fatty Acids	
Acetic Acid	1972 (1344)
Propionic Acid	556.2 (374.5)
Butyric Acid	439.2 (341.3)
Isobutyric Acid	92.27 (87.29)
Isovaleric Acid	88.33 (92.25)
Valeric Acid	96.20 (91.85)
Primary Bile Acids	
Cholic Acid	69.31 (115.4)
Taurocholic Acid	0.9548 (0.89)
Glycocholic Acid	2.77 (5.30)
Chenodeoxycholic Acid	30.46 (62.07)
Glycochenodeoxycholic Acid*	4.62 (6.13)
Secondary Bile Acids	
Deoxycholic Acid	493.2 (320.7)
Ursodeoxycholic Acid*	11.65 (19.33)
Lithocholic Acid	1567 (1055)
Nutriacholic Acid	275.0 (345.1)
7alpha-Hydroxy-3-oxo-5beta-cholan-24-oic acid*	40.26 (35.93)
Hyodeoxycholic Acid*	15.84 (37.38)
3_Oxocholic Acid*	1.368 (2.160)
3alpha-6beta-7beta-trihydroxycholenoic acid*	2.401 (5.280)
Glycodeoxycholic Acid*	1.680 (4.350)
3beta-hydroxy-5- cholenoic acid*	2.768 (4.018)
Sulfolithocholic Acid*	26.73 (86.18)
Taurodeoxycholic Acid	1.083 (0.8030)
*1 or more values were replaced with ½ LOD value	

Short Chain Fatty Acids and Physical Activity

Univariate analyses found that average daily minutes of light intensity physical activity were positively associated with butyrate (r=0.5791, p=0.007), and propionate (r=0.6818, p=0.0009). Fiber alone was negatively correlated with propionate (r= 0.4926, p=0.027) but unrelated to butyrate (r= 0.3422

, p=0.14). There was a stronger correlation between propionate and light intensity minutes for low fiber consumers (r=0.7909, p=0.006) compared to high fiber consumers (r=0.445, p=0.1976). There were no statistically significant differences between high and low fiber consumers for butyrate (Figure 2).

Table 3 shows multivariate associations between short chain fatty acids and physical activity variables. Daily minutes of light intensity physical activity was significantly and positively associated with butyrate [F(2, 17)=5.684, p=0.014, R^2=0.3992, and propionate [F(2,17)=13.62, p=0.0003, R^2=0.6157] while controlling for dietary fiber intake. More specifically, each additional minute per day of light intensity activity was associated with 1.88 μg/g higher butyrate concentration (β=1.88, 95%CI 0.477 to 3.291) and 1.79 μg/g higher propionate concentration (β=1.79, 95%CI 0.862 to 2.724). There were no associations between MET-hours, MVPA, and sedentary minutes and butyrate or propionate concentrations.

Bile Acids and Physical Activity

Univariate analyses found that moderate to vigorous physical activity was negatively associated with deoxycholic acid (r=0.4468, p=0.042), and ursodeoxycholic acid (r=0.5579, p=0.009). Fiber alone was unrelated to deoxycholic acid (r= 0.0866, p=0.71) and ursodeoxycholic acid (r= 0.077, p=0.75). There was a stronger correlation between between ursodeoxycholic acid and light intensity minutes for high fiber consumers (r=0.6283, p=0.04) compared to low fiber consumers (r=0.5681, p=0.09). There were no statistically significant differences between high and low fiber consumers for deoxycholic acid. (Figure 3).

Table 3 shows multivariate associations between bile acids and physical activity variables. After controlling for dietary fiber, minutes of moderate to vigorous physical activity was no longer significantly associated with deoxycholic acid [F(2,18)=2.377, p=0.1213, R^2=0.209]. However MVPA continued to be significantly, inversely associated with ursodeoxycholic acid [F(2,18)=4.213, p=.032, R^2=.0.3189] after accounting for dietary fiber. Further, each additional minute of MVPA per day was associated with 6.13 μg/g lower deoxycholic acid (β=-6.13, 95%CI -12.14 to -0.11) and 0.45 μg/g

lower ursodeoxycholic acid concentration (β= -0.45, 95%CI -0.80 to -0.12). There were no associations between MET-hours, light intensity physical activity, and sedentary minutes and deoxycholic acid, and ursodeoxycholic acid.

For other primary bile acids (cholic acid, chenodeoxycholic acid, and taurocholic acid), several PA predictors (light intensity, MVPA, and MET-hours) were trending toward significance, but no PA predictors nor dietary fiber were significant predictors of these bile acids. Table 4 shows select bile acid associations for physical activity outcomes measured by activPAL. All remaining bile acid regressions are in Supplemental Table 1.

Table 3: Associations of ActivPAL-Derived Variables and Dietary Fiber with Short Chain Fatty Acids

	Butyrate		**Acetate**		**Propionate**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	1.88 (0.480 − 2.39)	**0.0117**	5.18 (-0.454 − 10.8)	0.0692	1.79 (0.86 −2.72)	**<0.001**
Dietary Fiber Intake	−6.35 (-16.3 − 3.62)	0.1966	−47.59 (-87.5 − -7.70)	**0.0222**	−8.08 (-14.7 − -1.48)	**0.019328**
Whole Model	R^2= 0.3992	**0.01316**	R^2= 0.4132	**0.01077**	R^2= 0.6157	**<0.001**
MVPA Minutes	−0.208 (-5.71 − 5.29)	0.937	6.61 (-13.2 − 26.4)	0.4908	−0.341 (-4.55 − 3.87)	0.866497
Dietary Fiber Intake	−8.50 (-20.4 − 3.43)	0.151	−53.2 (-96.1 − -10.3)	**0.01808**	−10.1 (-19.3 − -0.993)	**0.031791**
Whole Model	R^2= 0.1174	0.3458	R^2= 0.3036	**0.04614**	R^2= 0.244	0.09274
MET-Hours	50.4 (-8.96 − 110)	0.091	164 (-56.8 − 384)	0.1356	33.7 (-12.8 − 80.2)	0.145
Dietary Fiber Intake	−6.86 (-18.0 − 4.25)	0.21	−48.2 (-89.4 − -8.86)	**0.0249**	−9.02 (-17.7 − -0.321)	**0.043**
Whole Model	R^2= 0.2573	0.07977	R^2= 0.3738	**0.01872**	R^2= 0.3342	**0.0315**
Sedentary Minutes	−0.011 (-1.62 − 1.59)	0.988	−0.477 (-6.32 − 5.37)	0.8652	−0.443 (-1.65 − 0.765)	0.4501
Dietary Fiber Intake	−8.46 (-21.0 − 4.08)	0.173	−52.3 (-98.0 − -6.59)	**0.0274**	−9.04 (-18.5 − -0.408)	0.0596
Whole Model	R^2= 0.1171	0.3468	R^2= 0.2846	0.05805	R^2= 0.2684	0.07016

	Isobutyrate		**Isovalerate**		**Valerate**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	0.302 (-0.219 − 0.8234)	0.239	0.330 (-0.222 − 0.882)	0.226	0.404 (-0.132 − 0.939)	0.131
Dietary Fiber Intake	−2.17 (-5.86 − 1.53)	0.234	−2.17 (-6.08 − 1.75)	0.26	−2.17(-5.96 − 1.62)	0.245
Whole Model	R^2= 0.1658	0.1957	R^2= 0.1622	0.2033	R^2= 0.2067	0.1244
MVPA Minutes	0.379 (-1.36 − 2.12)	0.652	0.425 (-1.42 − 2.27)	0.634	0.395 (-1.44 − 2.22)	0.656
Dietary Fiber Intake	−2.49 (-6.27 − 1.28)	0.182	−2.52 (-6.52 − 1.48)	0.202	−2.62 (-6.58 − 1.36)	0.184
Whole Model	R^2=0.1075	0.1075	R^2= 0.1007	0.3846	R^2= 0.1063	0.3637
MET-Hours	8.81 (-11.3 − 28.9)	0.37	9.59 (-11.8 − 30.9)	0.358	11.9 (-8.96 − 32.8)	0.246
Dietary Fiber Intake	−2.23 (-5.99 − 1.54)	0.23	−2.23(-6.22 − 1.76)	0.256	−2.24 (-6.15 − 1.66)	0.243
Whole Model	R^2= 0.1376	0.2638	R^2= 0.1319	0.2799	R^2= 0.163	0.2016
Sedentary Minutes	−0.0404 (-0.547 − 0.466)	0.869	−0.0701 (-0.607 − 0.467)	0.787	−0.0791 (-0.612 − 0.453)	0.759
Dietary Fiber Intake	−2.41 (- 6.40 − 1.58)	0.22	−2.37(-6.60 − 1.86)	0.254	−2.44 (-6.63 − 1.76)	0.238
Whole Model	R^2= 0.09853	0.3931	R^2= 0.09281	0.4162	R^2= 0.101	0.3837

Table 4: Associations of ActivPAL-Derived Variables and Dietary Fiber with Select Bile Acids

	Cholic Acid		**Chenodeoxycholic Acid**		**Taurocholic Acid**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	−0.601 (-1.29 −0.0880)	0.0836	−0.3191 (-0.68 - 0.044)	0.0814	−0.00251 (-0.00817 − 0.003158428)	0.3651
Dietary Fiber Intake	−1.74 (-6.62 − 3.15)	0.4647	−1.63 (-4.21- 0.938)	0.1986	−0.00138 (-0.0415 − 0.0387)	0.9432
Whole Model	R^2= 0.1653	0.1968	R^2= 0.2004	0.1336	R^2=0.04609	0.654
MVPA Minutes	−1.86 (-4.10 − 0.379)	0.0981	−0.9334 (-2.12- 0.256)	0.1164	−-0.0150 (-0.032 − 0.00217)	0.0832
Dietary Fiber Intake	−1.14 (-5.99 − 3.72)	0.6287	−1.3138 (-3.90 - 1.27)	0.2991	0.000777 (-0.0364 − 0.0380)	0.9655
Whole Model	R^2= 0.1529	0.2246	R^2= 0.1739	0.1792	R^2= 0.1578	0.2132
MET-Hours	−22.5 (-48.7 −3.78)	0.0889	−12.121 (-25.91- 1.67)	0.0813	−-0.180 (-0.382 − 0.0209)	0.076
Dietary Fiber Intake	−1.78 (-6.69 − 3.13)	0.4562	−1.663 (-4.24 - 0.915)	0.192	−0.00437 (-0.0420 − 0.0333)	0.81
Whole Model	R^2= 0.1605	0.207	R^2= 0.2005	0.1334	R^2= 0.1648	0.1977
Sedentary Minutes	0.341 (-0.340 −1.02)	0.307	0.1985 (-0.158 - 0.555)	0.258	0.000669 (-0.00474 − 0.00608)	0.798
Dietary Fiber Intake	−1.89 (-7.25 −3.48)	0.469	−1.7571 (-4.57 - 1.05)	0.205	−0.000159 (-0.0427 − 0.0424)	0.994
Whole Model	R^2 =0.067	0.5357	R^2= 0.1161	0.32925	R^2=0.004067	0.964

	Deoxycholic Acid		**Ursodeoxycholic Acid**	
	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	−0.387 (-2.47 − 1.69)	0.7005	−0.0688 (-0.190 − 0.0526)	0.2492
Dietary Fiber Intake	−-3.02 (-17.7 − 11.7)	0.6723	−0.214 (-1.07 − 0.647)	0.6081
Whole Model	R^2= 0.01591	0.8656	R^2= 0.07831	0.48
MVPA Minutes	−6.125 (-12.1 − -0.114)	**0.04623**	−0.461 (-0.797− -0.124)	**0.01**
Dietary Fiber Intake	−2.864 (-15.9 − 10.2)	0.65024	− 0.157 (-0.887 − 0.573)	0.6572
Whole Model	R^2=0.209	0.1213	R^2= 0.3189	**0.03155**
MET-Hours	−61.85 (-135 − 11.4)	0.0929	−-4.45(-8.71 − -0.203)	**0.041**
Dietary Fiber Intake	−-4.582 (-18.3 − 9.11)	0.4909	−0.280 (-1.07 − 0.515)	0.4694
Whole Model	R^2=0.15543	0.2188	R^2= 0.2166	0.111
Sedentary Minutes	0.0807 (--1.87 − 2.03)	0.932	0.0627 (-0.0510 − 0.176)	0.262
Dietary Fiber Intake	−2.78 (-18.1 − 12.6)	0.709	−-0.289 (-1.18 − 0.606)	0.506
Whole Model	R^2= 0.00797	0.9305	R^2= 0.07471	0.4972

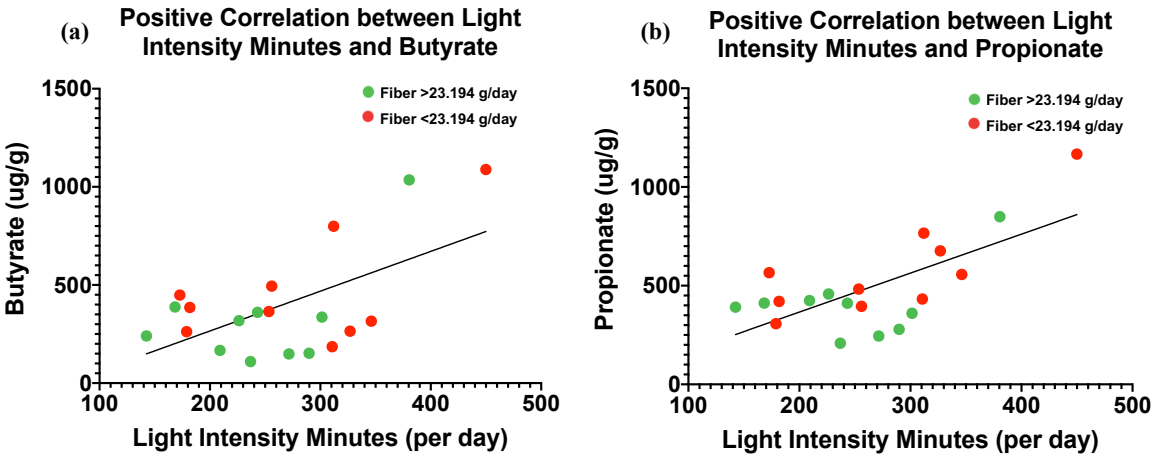

Figure 2. Correlations between light intensity minutes per day with fecal butyrate and propionate. (a) Positive correlation between light intensity minutes and fecal butyrate (r=0.5791, p=0.007) with dietary fiber separated by median value. (b) Positive correlation between light intensity minutes and fecal propionate (r= 0.6818, p=0.0009) with dietary fiber separated by median value.

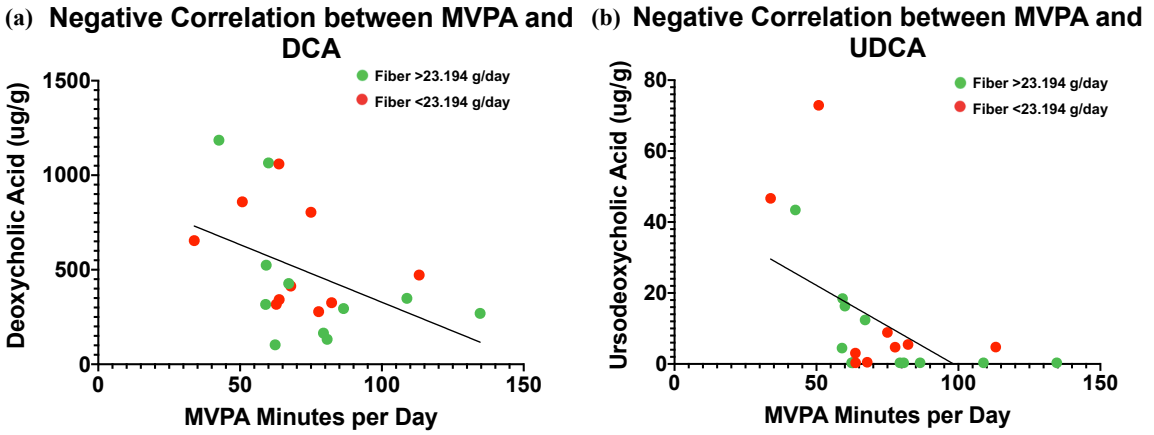

Figure 3. Correlations between MVPA minutes per day with fecal deoxycholic acid and ursodeoxycholic acid. (a) Negative correlation between MVPA minutes and fecal deoxycholic acid (R^2=0.4468, p=0.04) with dietary fiber separated by median value. (b) Negative correlation between MVPA minutes and fecal ursodeoxycholic acid (R^2=0.5579, p=0.009) with dietary fiber separated by median value.

4. DISCUSSION AND CONCLUSIONS

The aim of this study was to examine the relationships between physical activity and the stool metabolome in individuals at high risk for colorectal cancer. This is the first study to test associations of both endogenous and microbial derived stool metabolites and physical activity in individuals at high risk for colorectal cancer. Both butyrate and propionate were positively correlated with light intensity physical activity when accounting for dietary fiber intake. Conversely, both deoxycholic and ursodeoxycholic acid were negatively correlated with MVPA when accounting for dietary fiber intake. These findings suggest that both moderate to vigorous intensity physical activity and light intensity physical activity may be important for colorectal cancer primary and secondary prevention through different mechanisms.

Relationships between Physical Activity and Fecal Short Chain Fatty Acids

This study found that there was a positive correlation between butyrate and propionate and light intensity physical activity when accounting for dietary fiber intake. Similarly, rodent studies have suggested that voluntary exercise increases butyrate concentration in the cecum,[42] and in humans, Allen et al[21] (2018) found that a 6-week exercise intervention in previously sedentary but healthy individuals led to increases in short chain fatty acid producing species as well as increased absolute abundance of short chain fatty acids in lean individuals.

Several mechanisms could explain why physical activity may alter fecal short chain fatty acids. One mechanism would simply be mixing of intestinal contents during exercise and thus more surface area for bacterial fermentation of fiber.[43] A second mechanism could be increased anaerobic fermentation due to reduced colonic oxygen saturation during exercise.[43] Additionally, exercise is associated with colonic pH changes which may select for certain bacterial genera, and this can decrease transformation of primary bile acids to secondary bile acids.[44] Physical activity also increases gut motility which could result in increased excretion, rather than absorption, of short chain fatty acids.[44] Finally, exercise induces increased

expression of bacterial butyrate-acetoacetate CoA-transferase (BCoAT). BCoAT transfers co-A from butyrlcoA to acetate which forms butyrate and acetyl coA.[21] No previous studies have showed a relationship between light, but not moderate to vigorous, physical activity and stool short chain fatty acids. This could be due to gastrointestinal upset due to vigorous activity[45] that may cause a pro-inflammatory immune response and thus blunt the production of short chain fatty acids. Furthermore, this could be unique to those at high risk for colorectal cancer survivors and thus be an important consideration for exercise prescription for these individuals.

Weir et al (2013) found that colorectal cancer survivors had significantly lower percentage of butyrate in their stool compared to healthy controls as well as higher percentages of acetic, valeric, isobutyric, isovaleric acids[46]. Furthermore, there were fewer butyrate producing bacterial species in the colorectal cancer survivors compared to healthy controls. However, it is unknown if this is due to cancer or cancer treatment versus being causative of cancer. Butyrate and propionate, but not acetate, inhibit histone deacetylases in immune cells and colonocytes. This results in downregulation of pro inflammatory cytokines. Butyrate and propionate also are involved in anti-inflammatory effects that regulate colonic regulatory T cells.[47] We showed a relationship between light intensity activity and butyrate and propionate, but not acetate. This is a potential important mechanistic link between physical activity and colorectal cancer prevention.

Relationships between Physical Activity and Fecal Bile Acids

The current study found that there was a negative correlation between ursodeoxycholic acid and deoxycholic acid and moderate to vigorous physical activity, although this was not significant for deoxycholic acid when accounting for dietary fiber intake. This finding is different from a previous study by Wertheim et al (2012), who found that colorectal cancer survivors who were in the lowest quartile of recreational physical activity duration had a 17% *lower* fecal bile acid concentration compared to the highest quartile. Thus, based on the preliminary data from this study, it is uncertain whether the pattern exists in those who are at high risk for colorectal cancer (but do not have cancer). Imray et al (1992)

found that individuals who had adenomatous polyps removed had higher concentrations of secondary fecal bile acids than healthy controls.[48] Furthermore, those with colorectal cancer had higher total bile acids and primary bile acids in their stool compared to both healthy controls and those who underwent adenomatous polyp removal. It is unknown whether or not physical activity can mediate this relationship. Secondary bile acids, in particular deoxycholic acid and lithocholic acid, may be cancer-promoting through several different mechanisms. They both cause cell membrane damage resulting in destruction of intestinal epithelium. Additionally, these secondary bile acids cause cell proliferation through epidermal growth factor receptors (EGFRs) and post-EGFR/ERK (extracellular signal-related kinase) signaling. Finally, they lead to increased reactive oxygen species and reactive nitrogen species through activation of NADH and NADPH oxidases and phospholipase A2 and activation of NF-kB which is important in cellular processes such as apoptosis and inflammation.[49] The present study found a negative correlation between ursodeoxcholic acid and moderate to vigorous physical activity. Consequently, ursodeoxycholic acid may be a chemoprotective agent against colorectal cancer. In a study of 21,000 adults, those who supplemented ursodeoxycholic acid had significantly reduced risk of cancer (HR 0.60, 95% CI 0.39-0.92).[25] Mechanistically, it may suppress deoxycholic-acid induced apoptosis and deoxycholic acid-induced epidermal growth factor activation.

Taurocholic acid, a primary bile acid, is also known to be genotoxic and pro-inflammatory. It can lead to an increase in *Bilophilia wadsworthia* which can lead to an accumulation of H_2S which may be cancer promoting.[50] In the current study we saw a trending negative relationship between taurocholic acid and moderate to vigorous physical activity ($p=0.07$).

Reverse cholesterol transport may be the mechanism responsible for exercise-induced reductions in stool bile acids.[26,51] Lecithin -cholesterol acyltransferase may be upregulated by physical activity and thus increasing reverse cholesterol transport. Bile acids interact with gut epithelial cell receptors faenesoid X receptor (FXR) and TGR5. This can alter glucose, lipid, and energy homeostasis. This could be a link between physical activity and bile acid metabolism, mediated by the microbiome.[52]

Most of the inflammatory and/or carcinogenic bile acids are secondary bile acids. It is unknown how physical activity modulates microbes with genes for bile salt hydrolase and/or 7-α hydroxylase that convert primary bile acids to secondary bile acids. More work must be done to elucidate the effects of physical activity on the microbiome, in particular deoxycholic acid producing microbes.

Strengths and Limitations

Two limitations of this study are the cross sectional design as well as the highly active population whom all met physical activity guidelines. This population lacked variability and it is unknown the threshold at which physical activity may modulate bile acids and short chain fatty acids. Furthermore, BMI, cholesterol, and dietary fat intake could be meaningful predictors of these outcomes but we lacked the sample size to add these to the models. Other studies have stratified by BMI and plasma triglycerides but we lacked the sample size to do so. Due to its self-report nature, dietary fiber intake could have been inaccurate due to inaccurate food logs. Finally, these results from microbial-derived metabolites could be augmented with microbiome analysis which is not reported in this paper.

There are several strengths of this study. This is the first study to test associations of both endogenous and microbial derived stool metabolites and physical activity in individuals at high risk for colorectal cancer. Additionally, many studies use self-reported physical activity and we used objectively measured physical activity. Finally, all intensities of physical activity were measured which provides a more accurate spectrum of movement throughout the day.

Conclusions

In conclusion, these data provide evidence that both MVPA and light intensity physical activity may reduce bile acids, and increase short chain fatty acids, respectively after controlling for dietary fiber intake. This study is an important step in understanding the mechanistic relationship between physical activity and colorectal cancer risk, with an emphasis on microbial derived metabolites. Additional insight from large physical activity intervention trials in sedentary individuals at high risk for colorectal cancer

are necessary to clarify and/or confirm these findings. An increase in physical activity could induce similar modulations to these metabolites. If confirmed, a spectrum of physical activity intensity may be important for primary and secondary prevention of colorectal cancer.

5. FUTURE DIRECTIONS

This study was underpowered to utilize all predictors that may be necessary to predict fecal bile acids. For deoxycholic acid and moderate to vigorous physical activity with 4 regressors (moderate to vigorous physical activity, dietary fiber intake, BMI, and plasma triglycerides) based on an R^2 of 0.209 (f^2=0.2642), with p=0.05 and 80% power, a sample size of 51 would be required.

Furthermore, a more practical measure of these outcomes would be an exercise intervention in previously sedentary individuals at high risk for colorectal cancer. Based on the findings in the current study, the intervention could use a spectrum of physical activity intensity due to the differential effects on fecal short chain fatty acid and bile acids.

Finally, fecal measures, while practical to collect, are not an accurate representation of colon metabolism. A more accurate representation could be colon tissue collected during a colonoscopy. These same measures could be repeated on colon tissue to examine the effects of physical activity on short chain fatty acids and bile acids.

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APPENDIX I

Supplementary Table 1: Associations of ActivPAL-Derived Variables and Dietary Fiber with Bile Acids

	Lithocholic Acid		**Nutriacholic Acid**		**7alpha-Hydroxy-3-oxo-5beta-cholan-24-oic acid**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	−1.028 (-7.90 - 5.85)	0.757	−1.223 (-3.36 - 0.915)	0.245	−0.17582 (-0.394 - 0.0428)	0.1084
Dietary Fiber Intake	−4.182 (-52.90 - 44.5)	0.859	4.223 (-10.9 - 19.4)	0.565	0.02486 (-1.52 - 1.57)	0.9735
Whole Model	R2= 0.006396	0.9439	R2= 0.1031	0.3757	R2= 0.1408	0.2551
MVPA Minutes	-12.631 (-7.90 - 5.85)	0.2291	−5.530 (-12.1 - 1.08)	0.096	−0.1043 (-0.861 - 0.652)	0.775
Dietary Fiber Intake	−3.603 (-52.9 - 44.5)	0.8719	5.36 (-8.99 - 19.7)	0.4431	0.2209 (-1.42 - 1.86)	0.781
Whole Model	R2=0.08016	0.4714	R2= 0.1729	0.1812	R2= 0.009222	0.92
MET-Hours	−100.065 (-357 - 157)	0.424	−57.096 (-137 - 22.4)	0.149	−1.9551(-10.8 - 6.92)	0.649
Dietary Fiber Intake	−6.254 (-54.3 - 41.8)	0.788	3.769 (-11.1 - 18.6)	0.601	0.1624 (-1.50 - 1.82)	0.84
Whole Model	R2=0.03673	0.714	R2= 0.1399	0.2577	R2= 0.01628	0.8627
Sedentary Minutes	0.8406 (-5.59 - 7.27)	0.787	0.7743 (-1.27 - 2.82)	0.436	0.17426(-0.0280 - 0.377)	0.0871
Dietary Fiber Intake	−-5.0703 (-55.7 - 45.6)	0.836	3.7206 (-12.4 - 19.8)	0.633	−0.20196 (-1.79 - 1.39)	0.793
Whole Model	R2= 0.005121	0.9548	R2= 0.06413	0.5507	R2= 0.1578	0.2131

	Hyodeoxycholic Acid		**3_Oxocholic Acid**		**3alpha-6beta-7beta-trihydroxycholenoic acid**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	−0.17343 (-0.402 - 0.0554)	0.129	−0.006844 (-0.0205 - 0.00687)	0.308	−0.02310 (-0.0554 - 0.00920)	0.1503
Dietary Fiber Intake	−0.09014 (-1.71 - 1.53)	0.908	−0.018560 (-0.116 - 0.0785)	0.693	−0.08703 (-0.316 - 0.142)	0.4347
Whole Model	R2= 0.1243	0.3028	R2= 0.06031	0.5713	R2= 0.1252	0.3002
MVPA Minutes	−0.275 (-1.05 - 0.500)	0.465	−0.03014 (-0.0731 - 0.0128)	0.158	−0.04720 (-0.155 - 0.0607)	0.37
Dietary Fiber Intake	0.09515 (-1.59 - 1.78)	0.907	−0.01216 (-0.105 - 0.0811)	0.787	−0.06285(-0.297 - 0.171)	0.58
Whole Model	R2= 0.03092	0.7538	R2= 0.1102	0.3496	R2= 0.05959	0.5752
MET-Hours	−0.03219 (-9.31 - 9.25)	0.994	−0.06675 (-0.602 - 0.468)	0.796	−0.08383 (-1.38 - 1.22)	0.894
Dietary Fiber Intake	0.1071 (-1.63 - 1.84)	0.898	−0.01290 (-0.113 - 0.0871)	0.79	−0.06334 (-0.306 - 0.180)	0.591
Whole Model	R2= 0.0009841	0.9912	R2= 0.006679	0.9415	R2= 0.01646	0.8613
Sedentary Minutes	0.20209 (-3.24e-03 - 0.407)	0.0534	0.013064 (0.00156 - 0.0246)	**0.0282**	0.03149 (0.00351 - 0.0595)	**0.0295**
Dietary Fiber Intake	−0.38800 (-2.00 - 1.23)	0.6203	−0.042809 (-0.133 - 0.0478)	0.3339	−0.13792(-0.358 - 0.0823)	0.2048
Whole Model	R2= 0.1927	0.1456	R2= 0.2425	0.08216	R2= 0.2488	0.07614

Supplementary Table 1 continued: Associations of ActivPAL-Derived Variables and Dietary Fiber with Bile Acids

	Glycodeoxycholic Acid		**3beta-hydroxy-5- cholenoic acid**		**Glycochenodeoxycholic Acid**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	0.0004682 (-0.0272 - 0.0281)	0.972	−0.00621 (-0.0291 - 0.0167)	0.5758	−0.02581(-0.0633 - 0.0112)	0.1658
Dietary Fiber Intake	−0.0946338 (-0.290 - 0.101)	0.324	0.169352 (0.00699 - 0.332)	**0.0418**	−0.11767 (-0.384 - 0.148)	0.3649
Whole Model	R2= 0.05617	0.5944	R2= 0.2398	0.08478	R2= 0.1255	0.299
MVPA Minutes	−0.004707 (-0.0937 - 0.0843)	0.913	0.01183 (0.0624 - 0.0861)	0.7417	−0.0720 (-0.195 - 0.0507)	0.2334
Dietary Fiber Intake	−0.095391(-0.289 - 0.0979)	0.314	0.17702 (0.0158 - 0.338)	**0.0332**	−0.09156 (-0.359 - 0.175)	0.4793
Whole Model	R2= 0.05675	0.5911	R2= 0.2309	0.09418	R2= 0.1002	0.3866
MET-Hours	−0.38887 (-1.42 - 0.643)	0.439	0.0812 (-0.796 - 0.958)	0.848	−1.7030 (-2.95 - -0.4556)	**0.01022**
Dietary Fiber Intake	−0.10779(-0.301 - 0.0852)	0.256	0.1791(0.0151 - 0.343)	**0.034**	−0.1434 (-0.377 - 0.0898)	0.21266
Whole Model	R2= 0.08786	0.4371	R2= 0.2277	0.09773	R2= 0.3303	**0.02711**
Sedentary Minutes	−0.01178 (-0.0369 - 0.0134)	0.339	0.011459 (-0.00939 - 0.0323)	0.2634	−0.07200 (-0.0484 - 0.0248)	0.2334
Dietary Fiber Intake	−0.06625(-0.264 - 0.132)	0.491	0.148327 (-0.0159 - 0.313)	0.0738	−0.09156 (-0.347 - 0.229)	0.4793
Whole Model	R2= 0.1042	0.3715	R2= 0.2795	0.05236	R2= 0.1002	0.6391

	Sulfolithocholic Acid		**Glycocholic Acid**		**Taurodeoxycholic Acid**	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Light Intensity Minutes	−0.2799 (-0.826 - 0.266)	0.296	−0.02429 (-0.0564 - 0.00781)	0.1293	0.0001108 (-0.00489 - 0.00511)	0.9634
Dietary Fiber Intake	−0.7047 (-4.57 - 3.16)	0.706	−0.10384 (-0.331 - 0.124)	0.3501	−0.0226531(-0.0581 - 0.0128)	0.1961
Whole Model	R2= 0.06276	0.558	R2= 0.1442	0.2462	R2= 0.09429	0.4101
MVPA Minutes	−1.4133 (-3.09 - 0.260)	0.093	−0.05140 (-0.159 - 0.0561)	0.3283	−0.010100 (-0.0254 - 0.00522)	0.18304
Dietary Fiber Intake	−0.4514 (-4.08 - 3.18)	0.797	−0.07849 (-0.312 - 0.155)	0.4887	−0.023257 (-0.0565 - 0.0100)	0.15912
Whole Model	R2= 0.1508	0.2297	R2= 0.07595	0.4912	R2= 0.1814	0.1651
MET-Hours	−26.540 (-43.4 - -9.67)	**0.00393**	−0.61326 (-1.88 - 0.652)	0.322	−0.12299 (-0.303 - 0.0570)	0.1682
Dietary Fiber Intake	−1.246 (-4.40 - 1.91)	0.41738	−0.09597 (-0.333 - 0.141)	0.405	−0.02677 (-0.0604 - 0.00688)	0.1119
Whole Model	R2= 0.3792	**0.0137**	R2= 0.07722	0.4852	R2= 0.1873	0.1547
Sedentary Minutes	−0.16577 (-0.686 - 0.354)	0.511	0.02055 (-0.00983 - 0.0509)	0.172	−0.0004546 (-0.00513 - 0.00422)	0.84
Dietary Fiber Intake	0.02228 (-4.07 - 4.12)	0.991	−0.12652 (-0.366 - 0.113)	0.281	−0.0216637 (-0.0584 - 0.0151)	0.232
Whole Model	R2= 0.02661	0.7845	R2= 0.1226	0.3083	R2= 0.09628	0.4021