Physical Activity as a Determinant of Fecal Bile Acid Levels

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Abstract

Physical activity is protective against colon cancer, whereas colonic bile acid exposure is a suspected risk factor. Although likely related, the association between physical activity and bile acid levels has not been well-studied. Furthermore, the effect of triglycerides, which are known to modify bile acid levels, on this relationship has not been investigated. We conducted a cross-sectional analysis of baseline fecal bile acid levels for 735 colorectal adenoma formers obtained from participants in a phase III ursodeoxycholic acid chemoprevention trial. Compared with the lowest quartile of recreational physical activity duration, the highest quartile was associated with a 17% lower fecal bile acid concentration, adjusted for age, sex, dietary fiber intake, and body mass index (P = 0.042). Furthermore, consistent with a previously established relationship between serum triglyceride levels and bile acid metabolism, we stratified by triglyceride level and observed a 34% lower fecal bile acid concentration (highest versus lowest quartiles of physical activity) in individuals with low triglycerides (<136 mg/dL; P = 0.002). In contrast, no association between physical activity and fecal bile acid concentration was observed for subjects with high triglycerides (≥136 mg/dL). Our results suggest that the biological mechanism responsible for the protective effect of physical activity on the incidence of colon cancer may be partially mediated by decreasing colonic bile acid exposure. However, this effect may be limited to individuals with lower triglyceride levels. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1591–8)

Introduction

Physical activity is one of the strongest protective factors against colon cancer (1). This association is present whether physical activity is based on occupational activity (2), leisure-time activity (3), or both (4). A number of biological mechanisms explaining the protective nature of physical activity have been proposed, including reduced gastrointestinal transit time, enhanced immune function, effects on prostaglandin levels, reduced exposure to insulin, and changes in bile acid metabolism that affect colonic exposure to bile acids (5).

Bile acids, the end products of cholesterol metabolism, have been implicated in colorectal cancer etiology for a number of years (6). Bile acids have been shown to stimulate DNA synthesis (7), and, conversely, the deprivation of bile acids has been shown to inhibit DNA synthesis (8). Individual hydrophobic bile salts, including deoxycholate, lithocholate, cholate, and chenodeoxycholate, have been shown separately to have cancer-promoting properties (6). In cell culture, bile acids have been shown to induce DNA damage (6), but most animal studies have failed to show bile acid induced tumors in the absence of another carcinogen (9). Deoxycholic acid and chenodeoxycholic acid have been shown to induce apoptosis in human colon adenocarcinoma cells (10, 11). Bile acid induced apoptosis has been argued to initiate an adaptive response to the toxic environment that selects for cells with reduced apoptotic ability (10). These apoptosis-resistant cells are thought to propagate as a tumor-prone population of cells.

Multiple ecological studies have shown correlations between high fecal bile acid levels and risks for colorectal cancer (12-14). Case-control studies have shown that patients with colorectal cancer have higher levels of fecal bile acids than controls (15, 16). A recent meta-analysis shows differences in fecal bile acid profiles between colorectal adenoma or carcinoma patients and matched controls (17). Additional evidence favoring a tumorigenic role for bile acids comes from studies of dietary calcium, which has been shown to protect against bile acid-induced mucosal damage and colorectal tumors in animal models (18) and colorectal neoplasias in humans receiving calcium in randomized controlled trials (19), where a 15% reduction in risk of colorectal adenoma was observed. In sum, a wide body of evidence supports the relationship between fecal bile acids and increased colon cancer risk.

Fecal bile acid levels are affected by several dietary factors, including total energy intake, amount and type of dietary fat, and dietary fiber (20). Besides diet, physical activity may indirectly alter bile acid concentrations, possibly explaining how exercise reduces colon cancer risk. This mechanism may involve the lowering of serum cholesterol levels by higher physical activity (21). Few studies have examined the relationship between physical activity and bile acids. Some studies have reported a positive association between exercise and serum bile acid levels in rats (22, 23). One case-control study has shown

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Table 1. Age-adjusted demographic and life style characteristics according to recreational physical activity

Variable	Quartiles of recreational physical activity*			
	1st (n = 178)	2nd (n = 182)	3rd (n = 191)	4th (n = 184)
	<15 min	15 to <45 min	45 to <105 min	≥105 min
Age (y), mean ± SD	65.3 ± 9.0	65.5 ± 8.3	66.9 ± 8.5	69.1 ± 6.8
Male gender, n (%)	90 (50.8)	110 (60.5)	134 (70.3)	147 (80.0)
Dietary fiber (grams/d), mean ± SD	20.1 ± 9.8	21.1 ± 10.9	22.0 ± 11.4	23.0 ± 10.4
Dietary fat (grams/d), mean ± SD	63.0 ± 34.8	63.2 ± 30.8	63.4 ± 31.2	63.6 ± 30.6
Current smoker, n (%)	27 (15.4)	25 (13.6)	23 (11.8)	18 (10.0)
BMI (kg/m^2), mean ± SD	28.1 ± 5.4	27.6 ± 4.5	27.1 ± 4.4	26.6 ± 3.5
Glucose (mg/dL), mean ± SD	106 ± 25.9	106 ± 30.4	106 ± 19.8	105 ± 27.4
Triglycerides (mg/dL), mean ± SD†	173 ± 102	166 ± 105	158 ± 95.2	151 ± 92.0
HDL (mg/dL), mean ± SD†	50.9 ± 15.8	50.9 ± 13.8	50.8 ± 16.5	50.7 ± 14.7
LDL (mg/dL), mean ± SD†	124 ± 33.2	124 ± 29.0	123 ± 32.8	122 ± 32.3
Family history of colorectal cancer, n (%)‡	56 (31.5)	53 (29.1)	51 (26.7)	45 (24.3)

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
*Quartile cut points are rounded to the nearest five-minute interval of recreational physical activity.
†Data missing for triglycerides, HDL, and LDL (n = 25).
‡History of colorectal cancer in a parent, sibling, or child.

lower fecal bile acid concentrations in male distance runners compared with sedentary individuals; however, the sample size was small, and the association with physical activity was not significant after adjusting for dietary fiber intake (24). Given the paucity of data, we assessed the relationship between physical activity and fecal bile acid concentrations to test the hypothesis that physical activity is inversely related to fecal bile acid levels.

Materials and Methods

Study Population. A randomized, double-blind, placebo-controlled phase III clinical trial was conducted to test the efficacy of ursodeoxycholic acid to prevent recurrence of colorectal adenomas (25). Eligible participants had at least one colorectal adenoma with a diameter of at least 3 mm removed during a colonoscopy within the six months before study registration. Participants were between ages 40 and 80 years, had no invasive cancer within the previous five years, and all other colorectal neoplasms, except for sessile rectal polyps smaller than 3 mm, must have been completely removed during colonoscopy for eligibility. A total of 6,570 potential subjects were recruited, of which 1,537 subjects were eligible for random assignment to daily treatment with ursodeoxycholic acid (8-10 mg/kg of body weight) or placebo. After a four-week run-in period, 1,285 participants were eligible to participate in the trial.

For the current work, baseline data from the parent study were analyzed, including information from questionnaires and biological samples collected at clinic study visits. All participants who both completed the Arizona Activity Frequency Questionnaire and supplied stool samples for bile acid analysis were included in the present analysis, for a total of 735 (57%) participants. This study was approved by the Human Subjects Protection Program at the University of Arizona.

Bile Acid Concentrations in the Aqueous Phase of the Stool. Bile acid concentrations from fecal water were available for the current study. The aqueous phase of the stool, rather than the solid phase, was selected for these measures as it is thought to have more direct contact with epithelial cells in the colon and to promote carcinogenesis (26). The measurement of fecal bile acids has been described previously (25). Briefly, pooled 72-hour stool samples were collected. During the two day before and throughout the three-day collection period, participants were advised to maintain a constant diet consisting of their typical foods, especially with regard to foods thought to have an effect on bile acids, such as high-fat items (27). Stool samples were stored in metal containers, frozen, transported on dry ice, and stored at −80°C in the laboratory. For analysis, samples were first homogenized for 15 min with an equal weight of deionized water. Two 10-mL aliquots were ultracentrifuged at 4°C in a 70.1 Ti rotor for one hour at 38,500 rpm. The supernatant (aqueous phase) was removed, weighed, and stored at −80°C. Detailed methods for the measurement of bile acid concentrations in fecal water using gas chromatography have been described previously (28, 29). Bile acid concentrations, measured in μg/mL, were determined for lithocholic, deoxycholic, chenodeoxycholic, cholic, ursodeoxycholic, ursocholic, isodeoxycholic, isoursodeoxycholic, 7-ketolithocholic, and 12-ketolithocholic acids. Values of undetectable concentrations were set to zero. Each subject's total fecal bile acid concentration was calculated by summing together the concentrations of the 10 individual acids. The total fecal bile acid concentration was used for the current analysis, as the concentrations of individual bile acids were undetectable in many participants.

Recreational Physical Activity. Study participants self-administered the 59-item scannable Arizona Activity Frequency Questionnaire. This validated questionnaire (30) grouped physical activity by leisure, recreational, household, and ''other'' activity categories. Participants were asked to indicate if, how frequently, and for what duration they had participated in each particular activity during the previous four-week period. Recreational activities included stair-climbing, aerobics, calisthenics, dancing, jogging, walking, racquetball, basketball, volleyball, ski machine or cross-country skiing, floor exercise, lifting free weights, water aerobics, swimming, bowling, tennis, active fishing and/or hunting, yoga/

meditation, bicycling, golfing, and hiking. Each subject's total number of hours per day in recreational physical activities was calculated and adjusted using the following formula: *hrrecrx* = *titype**((24 − *qeax* − *sleep*)/*totacdy*), where *hrrecrx* is the total adjusted hours per day for recreational activities, *titype* is the unadjusted hours per day for recreational activities, *qeax* is the hours per day spent working or volunteering (adjusted for a 28-day period), *sleep* is the hours per day spent sleeping, and *totacdy* is the hours per day reported in all activities (excluding work and sleep). The purpose of adjusting was so that the duration of all reported activities plus working and sleeping totaled 24 hours, thereby reducing measurement error. Results generated using unadjusted measures rather than adjusted values were not substantially different; therefore, only the analyses done using the adjusted measures are presented. Recreational energy expenditure (kJ/d), which incorporates the intensity of the activity besides just duration, was used as an additional measure of physical activity. Recreational physical activity, rather than total activity, was chosen to capture the potential association with intentional exercise and to be consistent with previous studies in the field (3, 31). Occupational activity was not considered due to the high proportion of retirees in this study population.

Statistical Analysis. To compare the characteristics of subjects from the ursodeoxycholic acid study included in the current study with those who were excluded, Student's *t* tests and Fisher's exact tests were used. The relationship between recreational physical activity and fecal bile acid concentration was analyzed using multivariate linear regression. The outcome variable for this cross-sectional analysis was the total concentration of fecal bile acids. The primary explanatory variable, time spent in recreational physical activities, was divided into quartiles (rounded to the nearest five-minute interval) because of extreme right-skewness

Table 2. Relationship of recreational physical activity to total fecal bile acid concentration (*n* = 735)

Model	β coefficient	95% CI	Predicted total fecal bile acids (μg/mL)*
Minutes of recreational physical activity per day			
Crude			
<15 min	Reference	—	275
15 to <45 min	−0.265	−0.444 to −0.086	211
45 to <105 min	−0.272	−0.449 to −0.095	210
≥105 min	−0.358	−0.536 to −0.179	192
P_{trend} < 0.001	—	—	—
Adjusted†			
<15 min	Reference	—	301
15 to <45 min	−0.237	−0.414 to −0.059	238
45 to <105 min	−0.216	−0.393 to −0.039	243
≥105 min	−0.248	−0.432 to −0.065	235
P_{trend} = 0.015	—	—	—
Adjusted‡			
<15 min	Reference	—	284
15 to <45 min	−0.200	−0.376 to −0.024	233
45 to <105 min	−0.150	−0.327 to 0.026	245
≥105 min	−0.190	−0.372 to −0.007	235
P_{trend} = 0.082	—	—	—
kJ of recreational energy expenditure per day			
Crude			
0 to <289	Reference	—	282
289 to <868	−0.270	−0.446 to −0.093	215
868 to <1,981	−0.338	−0.515 to −0.162	201
1,981 to <14,775	−0.399	−0.576 to −0.223	189
P_{trend} < 0.001	—	—	—
Adjusted†			
0 to <289	Reference	—	303
289 to <868	−0.243	−0.418 to −0.067	237
868 to <1,981	−0.273	−0.452 to −0.094	230
1,981 to <14,775	−0.286	−0.472 to −0.100	227
P_{trend} = 0.003	—	—	—
Adjusted‡			
0 to <289	Reference	—	290
289 to <868	−0.223	−0.396 to −0.050	232
868 to <1,981	−0.218	−0.396 to −0.041	233
1,981 to <14,775	−0.235	−0.420 to −0.051	229
P_{trend} = 0.019	—	—	—

NOTE: Total fecal bile acid concentration, dietary fiber, and BMI are natural log-transformed.
Abbreviation: CI, confidence interval.
*Calculated for females at median age (68.3 y), dietary fiber (19.4 grams), and BMI (26.6 kg/m^2).
†Adjusted for age and sex.
‡Adjusted for all confounders (change β coefficient > 10%): age, sex, dietary fiber, and BMI.

Table 3. Relationship of recreational physical activity to total fecal bile acid concentration by triglyceride status (*n* = 710)

Level of triglycerides*	Daily recreational physical activity (min)	β coefficient†	95% CI	Predicted total fecal bile acids (μg/mL)‡
<136 mg/dL	<15	Reference	—	283
	15 to <45	−0.310	−0.574 to −0.046	208
	45 to <105	−0.275	−0.530 to −0.019	215
	≥105	−0.419	−0.683 to −0.155	186
≥136 mg/dL	<15	−0.007	−0.262 to 0.249	281
	15 to <45	−0.083	−0.346 to 0.180	261
	45 to <105	−0.026	−0.296 to 0.243	276
	≥105	−0.040	−0.231 to 0.310	295

NOTE: Model is adjusted for age, sex, dietary fiber, and BMI. Total fecal bile acid concentration, dietary fiber, and BMI are natural log-transformed.
*Likelihood–ratio test for interaction between physical activity and triglycerides $P = 0.079$.
†All β coefficients are relative to the reference group: triglycerides of <136 mg/dL and physical activity of <15 min/d.
‡Calculated for females at median age (68.3 y), dietary fiber (19.3 grams), and BMI (26.6 kg/m^2).

and a large spike in the distribution near zero. Thus, daily recreational physical activity was divided into the following rounded quartiles: <15 min per day (reference category), 15 to <45 min, 45 to <105 min, and ≥105 min. Daily recreational energy expenditure (kJ/d) was also divided into quartiles.

Several variables were assessed as potential confounders, including demographics, dietary intake, and medical history. Factors that changed at least one of the β coefficients for the three physical activity dummy variables by >10% were considered confounders (32). Thus, the multivariate model is adjusted for age, sex, dietary fiber intake, and body mass index (BMI). Concentration of fecal bile acids (the outcome variable), dietary fiber intake, and BMI were extremely right-skewed, so the natural log transformation was used to normalize their distributions. Potential interactions of physical activity by age, sex, dietary fiber, BMI, and triglyceride level were investigated using likelihood-ratio tests. None of these interactions was significant ($P_{interaction} > 0.2$ in all cases) except for that with triglycerides. A stratified analysis was pursued using the median triglyceride value as the cutpoint (≥136 mg/dL). This value was selected to maximize power by having equal sample sizes in each group. Further analyses using the cutpoint used in the clinical definition for metabolic syndrome (≥150 mg/dL) generated results that were consistent with the median cutpoint but lacked statistical significance ($P_{interaction} = 0.30$). Tests for trend were based on the significance of a single trend variable coded as the category of exposure. All statistical analyses were done using Stata 10.1 (StataCorp), and all reported P values are two sided.

Results

The majority of participants were white (94.7%), male (65.6%), overweight or obese (BMI, ≥25 kg/m^2; 68.7%), or nonsmokers (87.3%). The mean age in this study population was 66.7 years, and the mean amount of time spent in recreational physical activities was 75 minutes per day (median, 45 minutes per day). The distribution of total fecal bile acid concentrations was extremely right skewed, with a mean of 326.4 μg/mL and median of 213.4 μg/mL. On average, fecal bile acids were composed primarily of deoxycholic acid (54%), followed by lithocholic (9%), cholic (8%), ursocholic (5%), ursodeoxycholic acid (3%), and chenodeoxycholic acid (2%).

Trends in the demographic and life style characteristics of subjects by quartiles of time spent in recreational physical activities were examined (Table 1). Compared with the lowest quartile, participants in the highest quartile of physical activity were older with more males and more nonsmokers, consumed more dietary fiber, had a lower BMI, and had lower triglycerides. The most active group also included a lower proportion of subjects with a family history of colorectal cancer. In addition, characteristics of the 735 participants in the current study were compared with the 550 excluded subjects from the parent study to determine if there were any substantial differences between the two samples. Overall, there were no important differences detected between the current study population and the parent study (Supplementary Table S1).

The relationship between recreational physical activity and fecal bile acid concentration is presented in Table 2. The crude model reveals that higher levels of physical activity were significantly associated with reduced concentrations of fecal bile acids, and this relationship was strengthened as the duration of physical activity increased ($P_{trend} < 0.001$); this is supported further in the multivariate models. At the median values of age (68.3 years), dietary fiber (19.4 grams/day), and BMI (26.6 kg/m^2), participants in the highest quartile of physical activity had a predicted 17% lower concentration of fecal bile acids compared with the reference group (a drop from 284 to 235 μg/mL for females and from 227 to 188 μg/mL for males) in the fully adjusted model ($P = 0.082$). The inverse association between physical activity and fecal bile acid concentration was further supported when quartiles of recreational activity energy expenditure (kJ/day) were used instead of time. Additionally, the relationship between physical activity and the total concentration of only hydrophobic bile acids (deoxycholic acid, chenodeoxycholic acid, cholic, ursocholic, and lithocholic) was virtually identical to that with total bile acids (data not shown).

A relationship between serum triglyceride levels and bile acid metabolism has been established previously (33, 34). Therefore, triglyceride level was tested as a potential effect modifier by means of stratified analysis (Table 3). For subjects with low triglycerides (<136 mg/dL),

there was a significant inverse relationship between physical activity and fecal bile acid concentration with a clear dose-response effect ($P_{trend} = 0.001$). Participants in the highest quartile of physical activity had a predicted 34% lower concentration of fecal bile acids compared with the reference group ($P = 0.002$). In contrast, there was no association between physical activity and fecal bile acid concentration for subjects with high triglycerides (≥136 mg/dL). Thus, triglycerides may modify the inverse association between recreational physical activity and total fecal bile acid concentration. When quartiles of recreational activity energy expenditure (kJ/day) were included in these stratified models instead of time, the results were similar (data not shown).

Discussion

The aim of this study was to assess the relation between physical activity and fecal bile acid concentration in a population of colorectal adenoma formers. The results show that time spent in recreational physical activity is significantly inversely associated with total fecal bile acid concentrations. Participants in the highest quartile of physical activity had a 17% lower fecal bile acid concentration compared with the lowest quartile. A stratified analysis revealed that this association was limited to individuals with low levels of triglycerides (<136 mg/dL). Within this group, participants in the highest quartile of physical activity had a 38% lower fecal bile acid concentration compared with the lowest quartile. In contrast, there was no relationship for those with high triglycerides (≥136 mg/dL). These results were observed irrespective of whether daily recreational physical activity duration (minutes) or intensity (kJ) was measured. These findings suggest that disturbances in metabolic homeostasis related to triglycerides may modify the relationship between physical activity and fecal bile acid levels. Thus, the protective effect of physical activity observed for colon cancer, which may be mediated by influences on colonic bile acid exposure, may be less important in individuals with high triglycerides.

Bile acid production and absorption are key steps in the tightly regulated cholesterol biosynthesis pathway (enterohepatic circulation of bile acids; Fig. 1). Cholesterol is converted into bile acids, which are necessary for intestinal absorption of dietary fat that the body converts to free fatty acids for energy. As key components of fuel use, bile acids are absorbed for recycling via enterohepatic circulation. More than 95% of bile acids are absorbed, leaving only a small portion to be excreted in the feces. Unabsorbed bile acids pass through the colon where they are thought to play a role in the initiation of carcinogenesis by inducing DNA damage and/or apoptosis resistance in colonic epithelial cells.

There is an established relationship between bile acid metabolism and serum triglyceride levels (33, 34). Interruption of bile acid enterohepatic circulation using bile acid sequestrants or biliary drainage has been shown to increase production of very low–density lipoprotein (VLDL), the major carrier of triglycerides (35, 36). Likewise, bile acid synthesis suppression using chenodeoxycholic acid can decrease VLDL production (35, 37). Furthermore, it has been shown that bile acids lower serum triglyceride levels by stimulating gene expression of SREBP-1c (38), which produces a sterol regulatory element binding protein isoform that controls fatty acid synthesis (39). The inverse relationship between bile acids and triglycerides provides a negative feedback mechanism in the bile acid cycle. The final step in this pathway is the synthesis of LDL from VLDL. Because bile acids are synthesized from cholesterol, lower triglyceride levels should ultimately result in decreased bile acid production. Indeed, case-control studies have reported that patients with elevated VLDL triglycerides have increased rates of bile acid synthesis compared with controls (40, 41). Overall, bile acid metabolism is highly regulated and involves a negative feedback mechanism controlled by bile acid regulation of triglycerides.

In this study, we postulate that a low level of serum triglycerides may be a marker for metabolic health. Under such conditions, physical activity is significantly inversely associated with fecal bile acid concentrations, possibly owing to the effect of exercise on decreasing levels of serum cholesterol and/or triglycerides (42). Such lipid reductions would theoretically decrease bile acid production while increasing the efficiency of bile acid absorption as fuel, resulting in reduced bile acid excretion into the feces (43). Physical activity, under these conditions, is likely contributing to the tight regulation of bile acid metabolism by maintaining energy balance through efficient fuel use. An alternative hypothesis to explain the relationship between physical activity and reduced fecal bile acid concentrations involves the effect of exercise on accelerated colon transit (5). In support of this alternative hypothesis, studies have shown that physical activity decreases colonic transit time (44), whereas physical inactivity has the opposite effect (45). However, this mechanism does not explain the observed effect modification by triglyceride level.

In contrast, we observed no relation between physical activity and fecal bile acid levels in people with high levels of triglycerides. Although we are unable to address causality with cross-sectional analyses, our results suggest that individuals with elevated triglyceride levels may have a defect in enterohepatic circulation. A link between hypertriglyceridemia and impaired enterohepatic recycling of bile acids resulting in high bile acid levels has been established previously (33, 40) and is consistent with this suggestion. This relationship may result from lower levels of the ileal apical sodium bile acid transporter in patients with high triglycerides (46) or a disruption in leptin signaling (47). Impaired enterohepatic circulation blocks bile acid absorption, thereby propagating elevated triglycerides while increasing bile acid transfer to the colon for fecal excretion (Fig. 1B). In this study population, we suspect that a high triglyceride level may be a marker for an underlying disease state involving disturbed metabolism, such as metabolic syndrome. The loss of the association between physical activity and lower fecal bile acid concentrations in individuals with high triglycerides might be explained by a failure of physical activity to overcome disruptions in bile acid signaling (i.e., blunted response).

The consistent association between obesity, sedentary behavior, and colorectal cancer has been attributed to a number of factors, including presumed higher bile acid

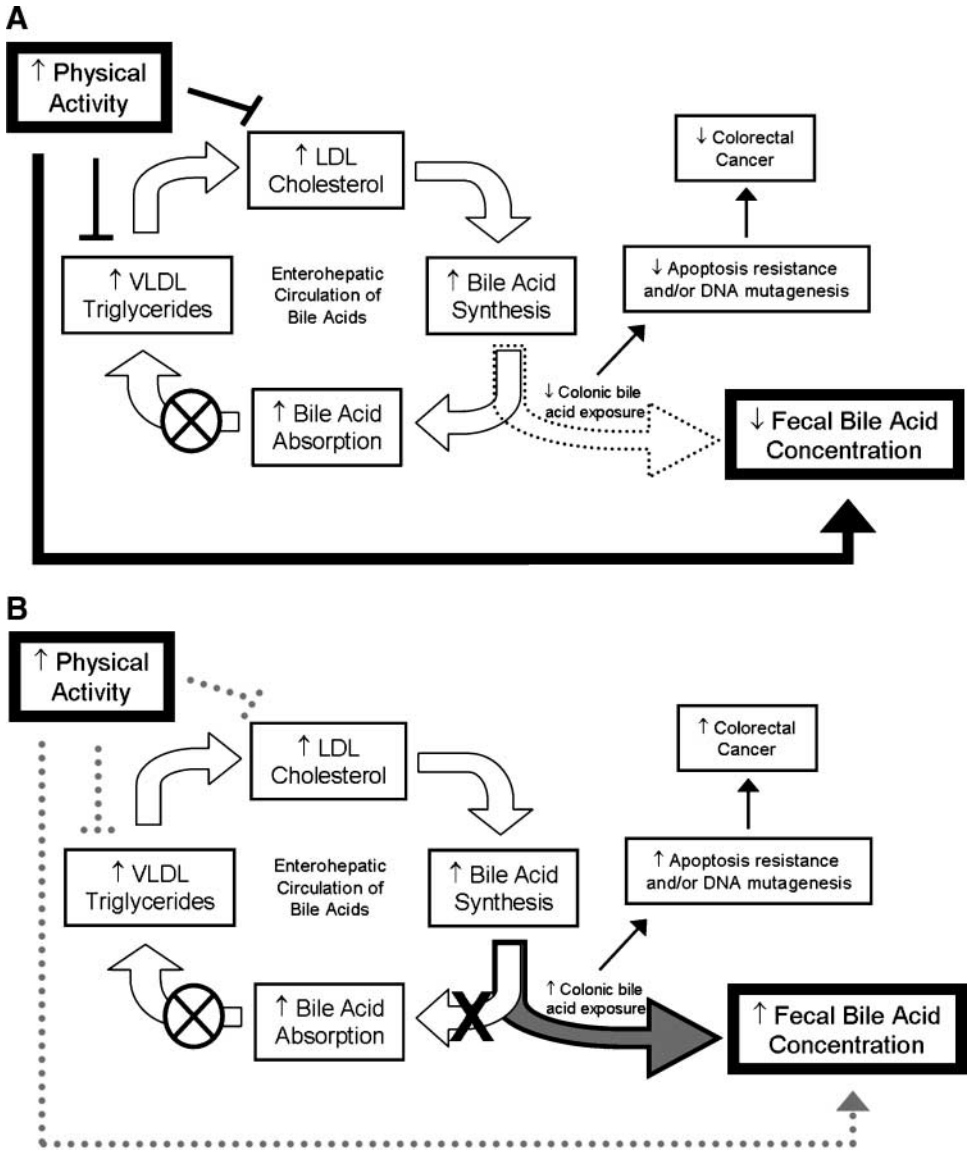

Figure 1. Illustration of bile acid metabolism. The enterohepatic circulation of bile acids (*curved block arrows*) is regulated by a negative feedback mechanism, wherein high bile acid levels reduce triglyceride levels (*circled X*). **A.** Bile acid metabolism under homeostatic regulation. Homeostatic regulation of the cycle results in few bile acids being lost (*dashed block arrow*) and low fecal bile acids. Physical activity is *inversely* related to triglyceride and/or cholesterol levels (*blunted arrows*), further regulating bile acid metabolism. Our results show that physical activity is inversely related to fecal bile acid levels, consistent with its effects on triglyceride and/or cholesterol levels (*bold arrow*). We hypothesize that physical activity in normal enterohepatic circulation may decrease colorectal cancer risk by reducing exposure of colonic epithelial cells to carcinogenic secondary bile acids (*thin arrows*). VLDL, very low–density lipoprotein; LDL, low-density lipoprotein. **B.** Bile acid metabolism under impaired homeostatic regulation. The loss of homeostatic regulation of triglyceride levels impairs bile acid absorption (*bold X*) and causes more bile acids to be lost from the cycle (*gray-shaded block arrow*). The resulting high bile acid levels in the feces is hypothesized to increase risk for colorectal cancer via tumorigeneic effects of secondary bile acids that include induction of apoptosis resistance and/or DNA mutagenesis of colonic epithelial cells (*thin arrows*). Some associations with physical activity that were present under homeostasis are no longer true under metabolic instability (*dotted arrows*). The results of this study show no relationship between physical activity and fecal bile acid concentrations for people with high triglycerides, a possible marker of metabolic imbalance.

levels (48) and metabolic disturbances that manifest in the obese as a consequence of insulin resistance and lipid abnormalities (49). Elevated triglyceride levels are a component of metabolic syndrome and are commonly elevated with obesity and in older individuals. Although information on the role of triglyceride levels and colorectal cancer risk is limited, Bird et al., found higher risk for colorectal adenoma in subjects with

higher serum triglyceride levels (50). Physical activity, in contrast, is one of the stronger modifiable protective factors for colon cancer (51). One possible antitumor effect of physical activity is its potential to improve regulation of bile acid metabolism and prevent malabsorption of bile acids and colonic exposure. Alternatively, the relationship between fecal bile acids and colon cancer observed in the past may reflect more global disturbances in insulin, triglyceride levels, and glucose, perhaps serving as an indirect surrogate of these metabolic disturbances. We would argue that the protective nature of physical activity against colon cancer, which is much more consistent than that for breast or prostate cancer, may reflect the joint benefits of physical activity for metabolic health and reduced fecal bile acids, which is an exposure that likely is exclusive to the gastrointestinal tract.

Our data are the first to suggest that physical activity could be less effective in lowering the exposure of the colorectum to bile acids in individuals with elevated triglyceride levels. Although additional work is needed to better understand the apparent complex relationship between metabolic state and physical activity on fecal bile acid levels and their relevance to colorectal cancer risk, we speculate that behavior (more intense exercise or weight loss) and/or pharmacologic strategies that improve triglyceride control may prove useful in restoring the effects of physical activity on enterohepatic circulation of bile acids and risk reduction.

To our knowledge, this is the first large study to assess the relationship between physical activity and fecal bile acids. The strengths of this study include the large sample size and the ability to assess and adjust for confounding from a large number of demographic and life style characteristics. There are also several limitations, beginning with the cross-sectional study design, which fails to show causality. In addition, physical activity measures were based on self-report of activity during the previous four-week period. Because the mean amount of time spent doing recreational physical activities was 75 minutes per day, there may have been overreporting. Another limitation concerns the measure of bile acids in stool, which is highly dependent on recent dietary intake. Although participants were directed to consume their regular diet before and during the period of stool collection, it cannot be confirmed whether these instructions were followed. Finally, our study population included only individuals who have had a colorectal adenoma, so our findings cannot necessarily be applied to the general population. Given these limitations, further studies are needed to determine if physical activity has a causal relationship with fecal bile acid levels.

Overall, our results provide evidence that physical activity may reduce the risk of colon cancer via decreased colonic exposure to bile acids. Although additional research is required to further investigate this relationship, the findings presented here provide a critical step toward understanding the relationships between physical activity, bile acids, and colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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