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RESEARCH ARTICLE

Deciphering the Contribution of the Gastrointestinal Tract on Glucose, Lipid, and Energy Metabolism

Exercise training and diet-induced weight loss increase markers of hepatic bile acid (BA) synthesis and reduce serum total BA concentrations in obese women

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Abstract

Regular exercise has profound metabolic influence on the liver, but effects on bile acid (BA) metabolism are less well known. BAs are synthesized exclusively in the liver from cholesterol via the rate-limiting enzyme cholesterol 7 alpha-hydroxylase (CYP7A1). BAs contribute to the solubilization and absorption of lipids and serve as important signaling molecules, capable of systemic endocrine function. Circulating BAs increase with obesity and insulin resistance, but effects following exercise and diet-induced weight loss are unknown. To test if improvements in fitness and weight loss as a result of exercise training enhance BA metabolism, we measured serum concentrations of total BAs (conjugated and unconjugated primary and secondary BAs) in sedentary, obese, insulin-resistant women (N = 11) before (PRE) and after (POST) a ∼14-wk exercise and diet-induced weight loss intervention. BAs were measured in serum collected after an overnight fast and during an oral glucose tolerance test (OGTT). Serum fibroblast growth factor 19 (FGF19; a regulator of BA synthesis) and 7-alpha-hydroxy-cholesten-3-one (C4, a marker of CYP7A1 enzymatic activity) also were measured. Using linear mixed-model analyses and the change in $\dot{V}O_{2peak}$ (mL/min/kg) as a covariate, we observed that exercise and weight loss intervention decreased total fasting serum BA by ∼30% (P = 0.001) and increased fasting serum C4 concentrations by 55% (P = 0.004). C4 was significantly correlated with serum total BAs only in the POST condition, whereas serum FGF19 was unchanged. These data indicate that a fitness and weight loss intervention modifies BA metabolism in obese women and suggest that improved metabolic health associates with higher postabsorptive (fasting) BA synthesis. Furthermore, pre- vs. postintervention patterns of serum C4 following an OGTT support the hypothesis that responsiveness of BA synthesis to postprandial inhibition is improved after exercise and weight loss.

NEW & NOTEWORTHY Exercise and weight loss in previously sedentary, insulin-resistant women facilitates a significant improvement in insulin sensitivity and fitness that may be linked to changes in bile acid metabolism. Diet-induced weight loss plus exercise-induced increases in fitness promote greater postabsorptive bile acid synthesis while also sensitizing the bile acid metabolic system to feedback inhibition during a glucose challenge when glucose and insulin are elevated.

exercise; female; liver; metabolism; weight loss

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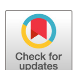

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INTRODUCTION

Regular exercise can increase liver fat oxidation, an adaptation that has been associated with improved metabolic health (1). Aside from fuel catabolism and conversion to chemical energy, the liver also plays an important role in the conversion of energetic substrates into metabolically active bile acids (BAs). BAs are synthesized exclusively in the liver from cholesterol via the rate-limiting enzyme cholesterol 7 alpha-hydroxylase (CYP7A1), stored in the gallbladder, and secreted into the upper small intestine where they facilitate in the solubilization and absorption of lipids. After performing their digestive function, BAs are reabsorbed in the distal small intestine (ileum) where they are recycled back to the liver in a process termed enterohepatic circulation.

Enterohepatic circulation of BAs provides negative feedback on CYP7A1 gene expression through the activation of nuclear receptor farnesoid X receptor (FXR). In hepatocytes, FXR induces small heterodimer partner (SHP) to repress CYP7A1 expression, whereas in human enterocytes, FXR induces fibroblast growth factor 19 (FGF19), which circulates back to the liver via the portal vein to facilitate CYP7A1 repression. Cholic acid (CA; a 12α-hydroxylated BA) and chenodeoxycholic acid (CDCA) are the two main BAs produced by hepatocytes, which can later be transformed by gut bacteria into secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA), respectively. Before secretion into the intestines, most BAs are conjugated to either a glycine or taurine amino acid to form bile salts, which increase their solubility and detergent capability (2). Conjugated BAs are actively absorbed throughout the gastrointestinal (GI) tract, whereas unconjugated BAs are passively absorbed. The reduced recycling efficiency of unconjugated BAs results in them being the primary BAs excreted in feces. About 95% of BAs are reabsorbed in the intestines and transported back to the liver via enterohepatic circulation, whereas 5% are putatively lost in feces and replaced by de novo synthesis in the liver.

Feedback regulation of BAs is essential to keeping a tightly controlled circulating physiological pool, but in certain disease conditions, such as in obesity or insulin resistance, the BA pool can be altered. Obese and insulin-resistant individuals consistently display higher concentrations of circulating BAs (3, 4). A rise in circulating BAs can often be attributed to an increased proportion of conjugated Bas, as they have a greater affinity for transporters involved in enterohepatic circulation (5).

Very little is known about the combined effects of exercise and diet on BA metabolism in humans, especially in the context of a weight loss intervention. A recent study in amateur runners found that a single exercise bout led to a significant decrease in circulating BAs (6). Greater GI motility is often used as an explanation for the beneficial effects of exercise on various digestive and metabolic disorders (7, 8), but it is not clear if this contributes to differences in BAs that stem from weight loss or increased fitness. Another study comparing trained versus sedentary men observed that fecal BA excretion was not different between the two groups (9). However, a recent study comparing low-fit and high-fit women showed that low-fit women have an increase in circulating conjugated BAs, whereas high-fit women demonstrated higher levels of the specific BA, LCA, post oral glucose tolerance test (OGTT) (10). Regardless, no previous studies in humans have specifically acknowledged the impact of exercise training on obesity and weight loss with respect to BA metabolism. Interestingly, rodents exposed to chronic voluntary wheel running increase cholesterol turnover, biliary flow, and fecal BA excretion (11, 12), changes that are associated with a reduction in the circulating BA pool in addition to creating greater BA turnover. Other weight loss tools, such as gastric bypass surgery, tend to have the opposite effect of exercise: higher circulating BA concentrations (13, 14). In the current study, we tested the hypothesis that obese women who experience improvements in fitness and weight loss as a result of an exercise and diet intervention display evidence of modified BA metabolism, as measured by serum BA levels, FGF19, and 7-alpha-hydroxycholesten-3-one (C4; a verified marker of CYP7A1 enzymatic acitivity), both during fasting and during an oral glucose challenge.

METHODS

Ethical Approval and Human Subjects

All protocols were approved by the University of California at Davis Institutional Review Board, and all subjects provided informed written consent. The study is listed in the ClinicalTrials.gov (NCT01494025). Details regarding diet, recruitment, test week protocols, and other intervention-associated aspects for this cohort are published (15, 16). Briefly, obese modestly hyperinsulinemic 30–50-yr-old females were recruited from the greater Davis and Sacramento, California communities. Body mass index (BMI) ranged from 30–37.5 kg/m^2, and participants reported their weight was stable as defined by <5% change in body mass over the past 6 mo. Participants were insulin resistant at the time of screening as determined by an abbreviated oral glucose tolerance test (OGTT) consisting of an initial blood draw 2 h after consuming a standard 75-g glucose drink. Insulin resistance was defined as one or more of the following: *1*) as per the American Diabetes Association guidelines for prediabetes, fasting glucose $\geq$ 100 and $\leq$ 125 mg/dL or 2-h OGTT glucose $\geq$ 140 and $\leq$ 199 mg; and/or *2*) a target Quantitative Insulin Sensitivity Check Index (QUICKI) score < 0.315, Homeostasis Model assessment (HOMA) > 3.67 (15). Exclusion criteria included any clinical signs of infection, chronic disease, personal history of cardiovascular disease, regular medications other than oral contraceptives, and pregnancy or lactation. The current analyses were performed on a subset of individuals for which matched blood samples were available before (PRE) and after (POST) a ~14-wk exercise and diet-induced weight loss intervention.

Exercise Intervention Protocol

Participants (n = 11 participants) completed testing before (*test wk 1*, PRE) and after (*test wk 2*, POST) an exercise and weight-loss intervention (14–17 wk) designed to improve fitness and insulin sensitivity (15, 16). During each test week, participants were provided with lot-matched foods, and all ate identical diets. Participants also refrained from exercise and were weight stable at the time of testing. Body mass was measured weekly on an electronic scale (Scale-Tronic model

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6002; Wheaton, IL) to the nearest 0.1 kg; height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (Ayrton Stadiometer model S100; Prior Lake, MN), and BMI (kg/cm^2) was calculated. On *day 3* or *4* of each test week period, fasting participants were administered a standard 75-g glucose OGTT. EDTA-treated blood samples were taken before the glucose drink and at 30, 60, 90, and 120 min after ingestion. Plasma glucose was determined using a Beckman Coulter clinical analyzer DXC800; serum insulin was analyzed using standard methods as per manufacturer's instructions (ADVIA Centaur, Siemans). The insulin sensitivity index ("Matsuda index") was calculated as described (17). On *day 4* or *5* of each test week, a graded cycle ergometer test (SRM ergometer, Colorado Springs, CO) was performed to determine $\dot{V}O_{2peak}$. Participants completed a 5-min warm-up, followed by a graded exercise test to exhaustion (initial workout load of 50 W, increased by 20 W every 2 min until volitional fatigue). Relative $\dot{V}O_{2peak}$ was determined as the highest O_2 uptake (ml/kg/min). Following "test wk 1," participants were prescribed a self-selected calorie-restricted diet and using the dietary reference intake (DRI) equation to target a 10% body mass loss over 14 wk (ca. 500–600 kcal/day reduction); participants recorded daily food intake in diaries and received weekly counseling from a registered dietitian. Participants also engaged in a prescribed exercise regimen as directed by the Western Human Nutrition Research Center (WHNRC) exercise physiologists (15, 16). During the first 4 intervention weeks, participants exercised aerobically 4 days/wk for 30 min each (treadmill or cycle ergometer) at an intensity of 60%–70% of their maximal HR as determined in the $\dot{V}O_{2peak}$ test. During intervention *wk 5–8*, exercise sessions were increased to 40 min, 4 days/wk; during intervention *wk 9* onward intensity increased to a HR of 75% of maximal. Participants wore HR monitors during all exercise sessions to ensure that they were exercising at the appropriate intensity for the prescribed amount of time, with digital information downloaded by staff weekly to ensure compliance. "Testing wk 2" was completed following the exercise and weight loss intervention.

LC-MS Analysis of 7α-Hydroxy-4-Cholesten-3-One

The presence and quantification of 7α-hydroxy-4-cholesten-3-one (C4) was determined in fasting serum samples (30 µL) with the use of LC-MS as previously described (10). Serum lipids were extracted using a modified Bligh and Dyer method (18). Chromatographic separation was performed on an UltiMate 3000 UHPLC system (Thermo Scientific; Waltham, MA) using a 100 × 2.1 mm, 1.7 µm Acquity BEH C18 column (Waters Corp, Milford, MA) with detection on a Q Exactive Orbitrap high-resolution accurate mass (HRAM) spectrometer (Thermo Scientific) with positive heated electrospray ionization (HESI +). Data were acquired using targeted-selective ion monitoring scanning and analyzed using Xcalibur 4.0 and TraceFinder 3.3 software (19). Calibration curves, 0–65 nM, showed linearity $>r^2 = 0.997$. The limit of detection for C4 and C4-d7 was 0.97 nM and 0.58 nM, respectively. The limit of quantitation for both compounds was 1.9 nM. C4 concentrations were corrected using percentage recovery of the internal standard.

Quantifying Serum FGF19 Concentrations

FGF19 concentrations in fasting serum were determined by ELISA (Quantikine Human FGF-19 ELISA kit, R&D Systems Inc, Minneapolis, MN) as per manufacturer's instructions.

LC-MS Analysis of Serum Bile Acids

Glycine-conjugated, taurine-conjugated and nonconjugated BA concentrations were quantified in fasting serum using LC-MS methodology. Serum samples (50 µL) were prepared as previously described (19). Chromatic separation was performed on an Ultimate 3000 UHPLC system fitted with an Acquity BEH C18 column, 100 × 2.1 mm, 1.7 µm (Waters, Milford, MA) as described (10). Identification was carried out on a Q-Exactive HRAM, and data were acquired by an ESI-Full-MS scan and analyzed by using Xcalibur 4.0 and TraceFinder 3.3 software. Individual BAs were identified by exact mass and retention time as shown in Table 1. Peak area measurements normalized to the internal standard(s) were used to quantitate; calibration curves, 0–5,000 nM, showed linearity >0.99.

Statistics

Body weight, fitness ($\dot{V}O_{2peak}$), and glucose homeostasis indices measured PRE versus POST intervention were analyzed using Student's paired *t* test or Wilcoxon Rank test. Serum C4, BAs, and FGF19 concentrations were analyzed using a mixed-effects linear model including collection period, time of blood samples within collection periods, the time by collection interaction and relative $\dot{V}O_{2peak}$ (mL/min/kg) as a covariate. VO_{2peak} was used as a covariate to control

Table 1. *The precision and accuracy of each bile acid quantified by LC/MS*

Bile Acid	Exact Mass (M-H)	RT (min)	QC (1.8 µM)		ISTD
			%RSD	%RME	
CA	407.28030	15.80	8.30	6.48	CA-D4
CDCA	391.28538	17.66	6.02	0.41	DCA-D4
DCA	391.28538	17.92	4.90	0.28	DCA-D4
GCA	464.30176	13.79	7.52	5.69	GCA-D4
GCDCA	448.30685	15.42	8.44	2.25	GCDCA-D9
GDCA	448.30685	15.83	9.64	1.52	GCDCA-D9
GLCA	432.31193	17.18	11.36	0.53	LCA-D4
GUDCA	448.30685	12.60	7.10	4.39	GCDCA-D9
LCA	375.29047	19.44	2.62	−10.80	LCA-D4
TCA	514.28440	13.64	5.31	4.40	GCA-D4
TCDCA	498.28948	15.24	9.72	1.21	GCDCA-D9
TDCA	498.28948	15.64	4.83	1.22	GCDCA-D9
TLCA	482.29457	16.97	9.08	−1.45	LCA-D4
TUDCA	498.28948	12.50	7.99	4.48	GCDCA-D9
UDCA	391.28538	14.88	0.33	2.63	DCA-D4

Conjugated and nonconjugated bile acids identified by LC-MS. QC samples were used throughout the runs to verify calibration; precision (%RSD) is determined from the average standard deviation of QC values from multiple runs; accuracy (%RME) is determined form the average calculated QC values of multiple runs. BA, bile acid; CA, cholic acid, CDCA, chenodeoxycholic acid; C4, 7-alpha-hydroxy-4-cholesten-3-one; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GUDCA, glycoursodexoycholic acid; ITSD, internal standard; QC, qualilty control; TBAs, total bile acids; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

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for the impact of exercise to improve aerobic fitness. To obtain estimates of variance components for the mixed model, we used restricted maximum likelihood estimation (REML). Repeated measures were used to account for multiple blood samples (i.e., time) within the same subject, and subject ID was deemed a random effect. Data are presented for each BA by collection period as least-square means and associated standard errors. Values of $P \leq 0.05$ were considered statistically significant. Unadjusted medians with interquartile range for each serum BAs, C4, and FGF19 PRE versus POST intervention are presented in Table 2. Changes in serum BAs, C4, and FGF19 concentrations during an OGTT PRE versus POST intervention were determine by repeated measures ANOVA followed by Student–Newman–Keuls post hoc analysis. Correlations between serum C4 concentrations and serum BAs (unconjugated, conjugated, and total) PRE versus POST intervention were determined by Spearman's Rank correlation coefficient. Statistical analyses were performed in SAS software (Version 9.4, SAS Institute, Cary, NC) or in Prism 6 (GraphPad Software, San Diego, CA).

RESULTS

Anthropometrics, Metabolic Health, and Fitness

We reported previously that the fitness and weight loss intervention led to significant reductions in body weight and adiposity in the larger cohort, with expected marked improvements of fitness ($\dot{V}O_{2peak}$) and substantially improved insulin sensitivity (15). As planned, weight and a strict diet were maintained during each of the test weeks, PRE and POST. Several phenotype parameters related to body weight, fitness, and insulin sensitivity are presented for context in Table 3.

Table 2. *Serum concentrations of BAs and BA synthesis indices in obese insulin-resistant women PRE vs. POST intervention*

Variable	PRE, $n = 11$	POST, $n = 11$
CA (μM)	0.02 (0.01–0.05)	0.03 (0.01–0.05)
CDCA (μM)	0.07 (0.02–0.11)	0.06 (0.02–0.13)
DCA (μM)	0.14 (0.10–0.20)	0.11 (0.06–0.15)
UDCA (μM)	0.01 (0.005–0.02)	0.01 (0.004–0.03)
GCA (μM)	0.12 (0.04–0.17)	0.08 (0.05–0.11)
GCDCA (μM)	0.35 (0.11–0.65)	0.32 (0.13–0.42)
GDCA (μM)	0.12 (0.04–0.24)	0.06 (0.05–0.09)
GUDCA (μM)	0.04 (0.01–0.11)	0.03 (0.01–0.05)
TCA (μM)	0.01 (0.01–0.02)	0.01 (0.01–0.02)
TCDCA (μM)	0.03 (0.02–0.11)	0.03 (0.02–0.07)
TDCA (μM)	0.02 (0.01–0.08)	0.01 (0.01–0.02)
TUDCA (μM)	0.002 (0.001–0.002)	0.001 (0.001–0.005)
TBAs (μM)	0.99 (0.44–1.8)	0.76 (0.46–1.3)
C4 (nM)	11.0 (3.4–15)	19.0 (5.3–21)
FGF19 (pg/ml)	57 (36–99)	63 (41–97)

Data are median with interquartile range. BA, bile acid; CA, cholic acid, CDCA, chenodeoxycholic acid; C4, 7-alpha-hydroxy-4-cholesten-3-one; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GUDCA, glycoursodexoycholic acid; POST, post-fitness and weight loss intervention; PRE, pre-fitness and weight loss intervention; TBAs, total bile acids; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

Table 3. *Body weight, fitness, and glucose homeostasis indices in sedentary obese women PRE- and POST-fitness and weight-loss intervention*

Variable	PRE, $n = 11$	POST, $n = 11$	P Value
BMI, kg/m^2	33.9 ± 0.72	32.0 ± 0.77	**0.0006†**
Absolute $\dot{V}O_{2peak}$, L/min	1.7 ± 0.22	2.1 ± 0.19	**0.0002†**
Relative $\dot{V}O_{2peak}$, ml/kg/min	19.9 ± 0.90	24.2 ± 0.78	**<0.0001†**
Fasting glucose, mg/dL	86.9 ± 1.56	83.7 ± 1.44	**0.048‡**
Fasting insulin, IU/ml	19.3 ± 2.73	12.3 ± 1.93	**0.024‡**
Matsuda Index	1.9 ± 0.20	2.87 ± 0.37	**0.010†**

Data are means ± SE. The bold P values indicate $P < 0.05$ using †Student's paired t tests or ‡Wilcoxon rank test. BMI, body mass index, $\dot{V}O_{2peak}$, peak oxygen consumption.

Fasting Serum BA Concentrations and Species Profile

Glycine-conjugated, taurine-conjugated, and unconjugated BAs were measured in fasting serum. BA species profiles were similar at PRE and POST intervention, with glycine-conjugated BAs representing ≥ 60% of the serum BA pool, followed by unconjugated BAs (30%) and taurine-conjugated BAs (≤10%; Fig. 1). Differences in serum concentrations of individual BA species between PRE and POST intervention are presented in Table 4. With respect to glycine- and taurine-conjugated BAs, we observed a 13% increase in GCA and 15% increase in GCDCA concentrations POST versus PRE intervention. In contrast, GDCA and TDCA concentrations decreased significantly by 37% and 44% POST versus PRE intervention. We also observed similar changes in the unconjugated BA pool. CDCA concentrations increased by 20%, while CA, DCA, and UDCA concentrations decreased by 25%, 23%, and 44%, respectively, in POST versus PRE intervention (Table 4). GUDCA, TCA, TCDCA, and TUDCA concentrations remained unchanged between PRE and POST intervention.

BA Synthesis in Relation to BA Species

7-Alpha-hydroxy-4-cholesten-3-one (C4) is a validated surrogate serum marker of hepatic CYP7A1 activity and BA synthesis (20). As shown in Fig. 2*A*, fasting serum C4 concentrations were 24% higher POST versus PRE intervention. Fibroblast growth factor 19 (FGF19) is an intestinal hormone released into circulation to repress hepatic BA synthesis via receptor signaling mechanisms (21). Fasting serum FGF19 concentrations did not differ between PRE versus POST intervention (Fig. 2*B*). We also observed a positive significant Spearman's coefficient correlation between C4 and total BA (TBA) concentrations in POST samples (Fig. 2*D*), but no significant correlation between C4 and TBA concentrations PRE intervention (Fig. 2*C*). Table 5 lists the Spearman's coefficient correlations between C4 and individual BA concentrations PRE versus POST intervention. We observed positive correlations between C4 and GCDCA, CA, CDCA, and DCA concentrations at POST intervention. Additional positive correlations between C4 and GDCA, GUDCA, TUDCA, and UDCA were observed with P values trending toward significance, $P > 0.05 < 0.10$.

BA Concentrations during an OGTT

Fasting concentrations of BAs and BA synthesis markers may not provide the complete picture of BA metabolism,

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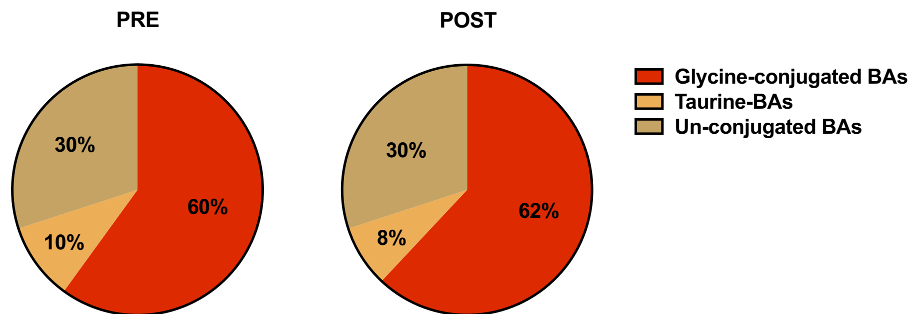

Figure 1. Effect of exercise and weight loss intervention on fasting serum BA profiles in obese insulin-resistant women. The proportion of glycine-, taurine-, and unconjugated BAs did not change PRE vs. POST intervention. Data represent % of total serum BAs for conjugated and unconjugated BAs. BA, bile acid.

since the latter is known to be responsive to meals (22). To address this, we examined BAs, C4, and FGF19 during an OGTT in the PRE and POST conditions both as a relative percent change (Fig. 3) and absolute value (Table 6). During the OGTT, total conjugated BA concentrations increased at 30 min, and concentrations remained elevated through the remainder of the time course in both PRE and POST (Fig. 3*A*). In contrast, changes in total unconjugated BA concentrations differed between PRE and POST intervention (Fig. 3*B*). Unconjugated BA concentrations decreased after 30 min in the OGTT time course at PRE intervention, while unconjugated BA concentrations increased significantly at 30 min POST intervention and then remained at levels near the baseline concentration through 120 min (Fig. 3*B*). BA synthesis, as measured by serum C4 concentration, tracked upward in PRE intervention at 60 min and continued to remain near baseline levels until 90 min, then rose at 120 min (Fig. 3*C*). In contrast, POST intervention, C4 concentrations trended downward during the OGTT. Concentrations of serum FGF19, a repressive hormone of BA synthesis, were not different PRE and POST intervention throughout the OGTT and displayed a pattern suggestive of an acute modest reduction at 30 and 60 min, followed by increases at 90 min onward (Fig. 3*D*).

Table 4. *Fitness and weight-loss intervention alters serum BA concentrations in obese insulin-resistant women*

Serum BA (μM)	PRE, *n* =11	POST, *n* = 11	*P* Value
Conjugated BAs			
GCA	0.26 ± 0.073	0.30 ± 0.073	**0.013**
GCDCA	0.82 ± 0.19	0.93 ± 0.19	**0.0009**
GDCA	0.27 ± 0.042	0.17 ± 0.042	**0.002**
GUDCA	0.15 ± 0.060	0.13 ± 0.06	0.150
TCA	0.034 ± 0.010	0.031 ± 0.010	0.8213
TCDCA	0.11 ± 0.019	0.10 ± 0.019	0.232
TDCA	0.076 ± 0.013	0.042 ± 0.013	**0.007**
TUDCA	0.007 ± 0.0034	0.006 ± 0.003	0.860
Unconjugated BAs			
CA	0.048 ± 0.007	0.0386 ± 0.0078	**<0.0001**
CDCA	0.10 ± 0.023	0.12 ± 0.023	**<0.0001**
DCA	0.17 ± 0.018	0.13 ± 0.18	**<0.0001**
UDCA	0.037 ± 0.012	0.021 ± 0.012	**0.002**
Sum total BAs			
Conjugated BAs	1.75 ± 0.37	1.72 ± 0.37	**0.009**
Unconjugated BAs	0.36 ± 0.029	0.31 ± 0.029	0.428
BAs	2.11 ± 0.39	2.04 ± 0.39	**0.001**

Data are least squared means ± SE. The bold *P* values indicate $P < 0.05$ using mixed-effects linear modeling using relative VO_{2peak} and insulin as covariates. BA, bile acid; CA, cholic acid, CDCA, chenodeoxycholic acid; C4, 7-alpha-hydroxy-4-cholesten-3-one; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GUDCA, glycoursodexoycholic acid; POST, post-fitness and weight loss intervention; PRE, pre-fitness and weight loss intervention; TBAs, total bile acids; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid; VO_{2peak}, peak oxygen consumption.

DISCUSSION

In the current study, we demonstrated that a ∼14-wk exercise and diet intervention aimed at improving fitness and weight loss in sedentary, obese, insulin-resistant women induced significant changes in BA metabolism both during fasting and following an oral glucose challenge. Exercise has long been known to have positive metabolic effects and is often used as a therapeutic tool in obese and insulin-resistant populations to improve glucose homeostasis or insulin sensitivity, lower blood lipids, and improve body composition (23, 24). BAs are active metabolic signals that act as a bridge between hepatic mitochondria oxidation of substrate and lipid synthesis. To date, very little research has tried to specifically understand the relationships between exercise and diet-induced weight loss to changes in BA metabolism. In the present study, we show that a regimented diet and exercise weight-loss program in women increases a marker of BA synthesis and modifies responses of BA metabolism to an oral glucose challenge. The latter suggests a change in nutrient-associated feedback signals related to BA synthesis or flux in hepatic or intestinal tissues. Importantly, these effects are specific to women and it is unknown if these same effects would be found in men.

The circulating BA pool relies upon feedback signaling that is partially mediated through the nuclear receptor FXR in the intestine, resulting in the release of FGF19, which travels to the liver and inhibits BA synthesis. A secondary redundant level of feedback inhibition is the return of BAs to the liver (enterohepatic circulation) and the direct downregulation BA synthesis via hepatic FXR activation. Immediately following synthesis, the majority of BAs are conjugated to an amino acid (primarily glycine in humans) (2). Conjugated BAs form the greatest proportion of recycled BAs as their reabsorption relies on active transport, whereas unconjugated BAs are passively absorbed and make up most of the BAs found in feces (5). Unconjugated BA levels are relatively unregulated and more accurately reflect gut microbiome activity as the resident bacteria perform deconjugation in competition with

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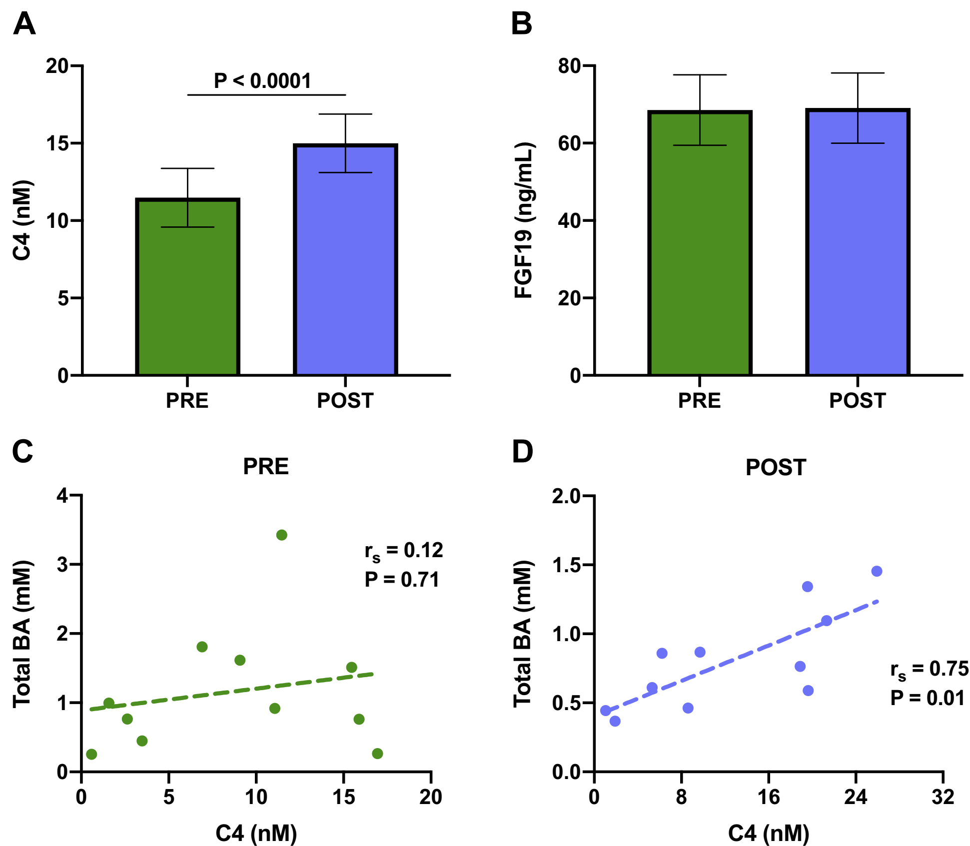

Figure 2. Changes in serum analytes relevant to BA synthesis in obese insulin-resistant women after a fitness and weight loss intervention. *A*: fasting serum C4 concentrations were higher POST vs. PRE intervention. *B*: fasting serum FGF19 concentrations did not differ between PRE vs. POST intervention. Data represent least squares means ± SE ($n = 11$ for PRE; $n = 11$ for POST). P value < 0.05 for PRE vs. POST. *C*: serum C4 and total bile acids (TBAs) did not correlate PRE intervention. *D*: serum C4 and TBAs were positively correlated POST intervention. Unadjusted data were used in Spearman's coefficient correlations analyses (r_s). BA, bile acid; C4, 7-alpha-hydroxy-cholesten-3-one; FGF19, fibroblast growth factor 19.

active transport uptake (19). Conjugated BAs generally play the biggest role in feedback inhibition, tracking closely with circulating FGF19 (22). This close association is implied from our findings: both conjugated BAs and FGF19 blood patterns were not different between PRE and POST intervention (e.g., overnight fasted or OGTT patterns). Conjugated BAs released after meal consumption stimulate FGF19 release which actively inhibits BA synthesis, and therefore, should reduce C4 concentrations. This level of control appeared evident in the POST condition, where C4 dropped following an OGTT. In contrast, there was a maintenance and then delayed induction of BA synthesis (C4) in the PRE intervention group during the OGTT. This change in OGTT C4 response PRE versus POST intervention might be attributed to differences in circulating glucose and insulin.

Indeed, both glucose and insulin are strong inducers of BA synthesis and CYP7a1 activity, albeit through different mechanisms (25, 26). Acute glucose excursions can increase histone acetylation and decrease histone methylation on the CYP7a1 gene promoter, inducing its expression (25, 27). However, in chronic hyperglycemic conditions, such as that seen in type 2 diabetes, basal expression of CYP7a1 becomes elevated and is unresponsive to nutrient signals or FXR signaling (25). Similarly, an acute insulin response from the consumption of a meal also induces CYP7a1 expression through binding to the transcription factor, forkhead box protein O1 (FOXO1), which prevents its inhibition of the CYP7a1 gene (26). Constant high insulin levels reduces the ability of insulin to increase CYP7a1 expression due to impaired FOXO1 signaling paired with constant upregulation of SREBP-1c (26, 28). In the current study, the intervention improved insulin sensitivity and lowered circulating glucose and insulin [Table 3; also see (15)]. In the PRE intervention time point, evidence suggests that BA synthesis (measured by C4) is not being turned off appropriately, i.e., there is a lack of feedback response to the released conjugated BAs after the OGTT. This is likely due to participants in the PRE-condition being more insulin resistant and having elevated circulating glucose and insulin levels postprandially (data not shown). In insulin-resistant or diabetes states, prolonged high insulin exposure may reduce CYP7a1 expression, but the epigenetic changes induced by glucose may possibly override, and still induce CYP7a1. We hypothesize that as insulin sensitivity improves (as seen POST-intervention herein), the regulation of BA synthesis becomes more sensitive to postprandial signals. Under this working model, the system becomes more attuned to its feedback signals, like FGF19, causing C4 concentrations drop in response to circulating conjugated BAs returning from the intestine.

In the postabsorptive (fasting) state, because there is no meal or nutrient stimulus, BAs are not being pulled through the GI tract and this greatly reduces the need to synthesize new BAs. In this case, BAs are being recycled back to the liver and induce feedback inhibition on CYP7a1 and reducing synthesis. Hence, in the PRE (fasting) condition, new BA synthesis contributes very little to the circulating BA pool. The lack of correlation between serum C4 and total BA concentrations supports this model. In contrast, in the POST condition,

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Table 5. *Correlations between serum C4 concentrations and individual BA species PRE vs. POST intervention in women*

Serum BA	PRE, $n = 11$		POST, $n = 11$	
	r_s	*P*	r_s	*P*
Conjugated BAs				
GCA	0.010	0.49	−0.11	0.37
GCDCA	0.12	0.35	0.65	**0.01**
GDCA	0.12	0.35	0.50	0.06
GUDCA	−0.22	0.25	0.46	0.07
TCA	−0.018	0.48	−0.11	0.36
TCDCA	0.054	0.44	0.23	0.23
TDCA	0.24	0.22	−0.10	0.37
TUDCA	−0.37	0.13	0.46	0.06
Unconjugated BAs				
CA	0.31	0.17	0.64	**0.01**
CDCA	0.49	0.06	0.83	**0.001**
DCA	0.25	0.22	0.79	**0.002**
UDCA	−0.036	0.46	0.51	0.05

r_s: Spearman's correlation coefficient. The bold *P* values indicate $P < 0.05$. BA, bile acid; CA, cholic acid, CDCA, chenodeoxycholic acid; C4, 7-alpha-hydroxy-4-cholesten-3-one; DCA, deoxycholic acid; FGF19, fibroblast growth factor 19; GCA, glycocholic acid; GCDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GUDCA, glycoursodexoycholic acid; POST, post-fitness and weight loss intervention; PRE, pre-fitness and weight loss intervention; TBAs, total bile acids; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid.

there was significantly higher fasting C4 concentration compared to PRE, and serum total BAs were strongly correlated with C4. This suggests that improved fitness and metabolic health somehow promotes sustained BA synthesis even in the postabsorptive state, and that this new synthesis contributes to the circulating pool of total BAs. We postulate that the lower serum total BAs in the POST state may be due to higher net fecal excretion of BAs. Unfortunately, this question will need to await future studies to be addressed, as no fecal samples were available from the current cohort. However, a previous study in mice has shown that daily exercise can increase fecal BA loss (12). The idea of increased BA synthesis in response to higher BA fecal excretion is demonstrated in individuals treated with BA sequestrants (29). BA sequestrants act by binding BAs in the intestine, disrupting enterohepatic circulation, and promoting their excretion. Interestingly, colesevelam, a well-known BA sequestrant, has been extensively studied for its ability to help lower lipid and glucose levels in individuals with type 2 diabetes (30–32). It is unclear in the current study whether improved insulin sensitivity driven by exercise and diet-induced weight loss promoted BA synthesis, or if increasd BA synthesis contributed to improved insulin sensitivity.

Previous studies examining the impact of acute exercise on BA metabolism are helpful in understanding how chronic exercise induces changes (6, 33). A recent study explored both acute resistance and aerobic exercise effects on markers of BA metabolism in healthy young males (33). Both types of exercise acutely influenced BA composition by increasing lithocholic acid (LCA), a secondary BA and potent activator of TGR5. Acute resistance exercise lowered circulating total BA, whereas acute aerobic exercise had no effect on total BA levels (33). However, the current study only included females and utilized a complete lifestyle intervention that combined both chronic aerobic exercise and weight loss, which modified outcomes known to influence BA metabolism (fitness and insulin sensitivity). Thus, it is difficult to tease out if the acute effects of each bout of exericse contributed to the findings here beyond other factors (e.g., diet, exercise, improved insulin sensitivity, or overall weightloss) that impacted BA metabolism. Moreover, it is also unknown if our findings would translate to men. We are unaware of research in human subjects showing differences between men and women in BA metabolism let alone changes in response to exercise or weight loss. Haeusler et al. (34) examined BA metabolism in relation to insulin sensitivity in a large cohort of males and females and did not note sex-based differences despite using sex as a covariate in their analysis. One study has shown that an age related reduction in CYP7a1 activity may be greater in women than men (35). However, rodent research has clearly indicated that females have chronically elevated circulating BA levels compared with males during aging (36), an effect likely driven by estrogen. It is apparent that more extensive research is required to understand the exact mechanisms linking BA metabolism, glucose/insulin regulation, and exercise training/fitness, and if these effects are regulated differently between males and females.

CYP8B1, another enzyme involved in the BA synthesis pathway, is responsible for determining the ratio of CA to CDCA. Like CYP7a1, glucose induces CYP8B1 activity; however, the nuclear exclusion of FOXO1 by insulin has the opposite effect on CYP8B1, resulting in its inhibition. A previous study has confirmed this by showing that greater insulin resistance is associated with a greater ratio of CA to CDCA (34). In fact, accumulating research is starting to identify negative effects associated with higher ratios of CA to CDCA. CA has a lower critical micelle concentration, making it more efficient at facilitating intestinal cholesterol absorption (25, 37). The secondary form of CA, DCA, has been associated to undesirable changes in gut microbiome (38), an effect that is replicated in an obese rodent model and after exposure to a high-fat diet (39). It is interesting to note in the current study that POST intervention, the most significant increase in absolute BA species and greatest correlation coefficient to C4, was CDCA, both in the glycine conjugated form and unconjugated form (Tables 4 and 5). POST intervention, we also noted a positive correlation between C4 and unconjugated CA and to a lesser extent with DCA. The correlation with CA and DCA seemed to be mostly driven by the increase in BA synthesis. Regardless, both conjugated and unconjugated concentrations of CA and DCA generally decreased POST intervention. Taken together, these data suggest that improved insulin sensitivity evoked by exercise and weight loss not only triggers improved control over BA synthesis but also alters BA composition in a manner that is most likely facilitated by the activation or rather, deactivation, of CYP8B1.

In summary, the current study provides evidence supporting the idea that exercise and weight loss in previously sedentary, insulin-resistant women facilitates a significant improvement in insulin sensitivity and fitness that may be linked to changes in BA metabolism. Our data suggest that diet-induced weight loss plus exercise-induced increases in fitness promote greater postabsorptive BA synthesis while

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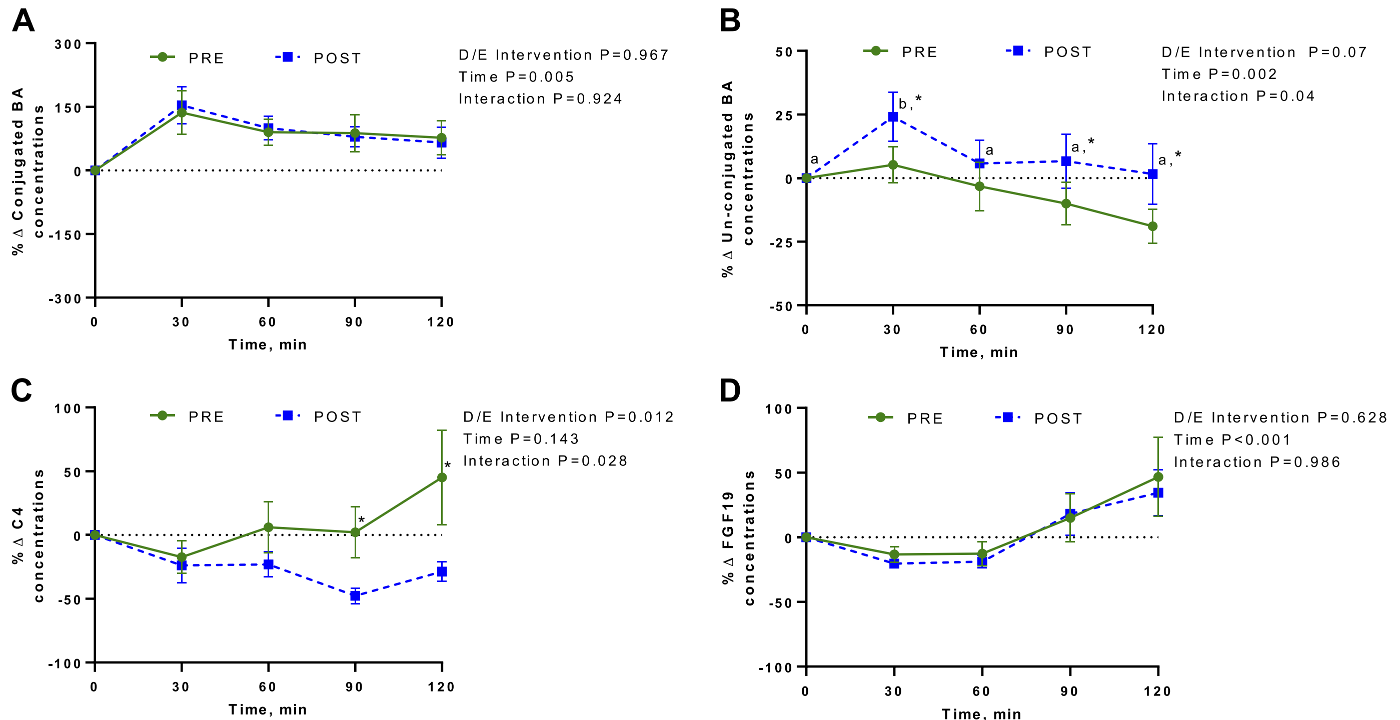

Figure 3. OGTT-associated changes in serum BA concentrations and analytes relevant to BA synthesis in obese insulin-resistant women PRE vs. POST an exercise and weight loss intervention. *A*: the % increase of conjugated BAs from 0 to 120 min was not different between PRE vs. POST intervention. *B*: the % change of unconjugated BAs was higher at 30-, 90-, and 120-min POST vs. PRE intervention. *C*: the % change of serum C4 from 0 to 120 min was higher at 90- and 120-min PRE vs. POST intervention. *D*: the % change of serum FGF19 from 0 to 120 min was not different between PRE vs. POST intervention. Data are presented as % change ± SE over the OGTT time course: 30, 60, 90, 120 min. Significance was determined by two-way RMANOVA (D/E intervention × time) followed by Student–Newman–Keuls post hoc analysis; for changes over time within groups, $P < 0.05$, a < b (values with different superscript letters are significantly different following post hoc analysis); *P value <0.05 PRE vs. POST at each time point, $n = 11$ participants. BA, bile acid; C4, 7-alpha-hydroxy-cholesten-3-one; D/E intervention, diet and exercise intervention; FGF19, fibroblast growth factor 19; OGTT, oral glucose tolerance test.

also sensitizing the BA metabolic system to feedback inhibition during a glucose challenge when glucose and insulin are elevated. Whether these changes in BA metabolism are a direct result of exercise and weight loss or are driven by improvements in insulin sensitivity is yet to be determined and warrants further research.

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Table 6. *Absolute values of BAs and BA synthesis indices during the OGTT in obese insulin-resistant women pre- and post-weight loss and fitness intervention*

Serum Variable	Time, Min 0	30	60	90	120	P Value D/E Intervention	Time	Interaction
Conjugated BAs, μM								
PRE	1.00 (0.2)	1.67 (0.3)	1.48 (0.3)	1.29 (0.2)	1.26 (0.2)	0.04	0.014	0.994
POST	0.58 (0.08)	1.27 (0.1)	1.11 (0.2)	0.97 (0.2)	0.84 (0.1)			
Unconjugated BAs, μM								
PRE	0.28 (0.05)	0.29 (0.5)	0.26 (0.05)	0.23 (0.04)	0.21 (0.03)	0.967	0.002	0.522
POST	0.27 (0.05)	0.30 (0.05)	0.24 (0.03)	0.24 (0.04)	0.22 (0.03)			
C4, nM								
PRE	9.1 (1.9)[a]	8.8 (1.6)[a]	10.8 (2.5)[a]	8.8 (1.7)[a]	14.5 (4.1)[b]	0.934	0.063	0.001
POST	14.2 (2.7)[b]	10.4 (2.2)[a]	9.4 (1.2)[a]	7.5 (1.8)[a]	9.5 (1.6)[a]			
FGF19, pg/mL								
PRE	81.6 (19.7)	63.4 (12.1)	60.1 (9.9)	70.3 (9.6)	70.3 (9.6)	0.76	0.134	0.932
POST	70.3(10.7)	55.8 (8.3)	54.6 (6.8)	77.3 (13.5)	87.1 (13.5)			

Values are means ± SE. C4, 7-alpha-hydroxy-4-chlosten-3-one; D/E intervention, diet and exercise intervention; FGF19, fibroblast growth factor 19; OGTT, oral glucose tolerance test. Significance was determined by two-way RMANOVA (D/E intervention × time) followed by Student–Newman–Keuls post hoc analysis; for changes over time within groups, $P < 0.05$ a < b; $P < 0.05$ between groups (PRE vs. POST) at each time point, $n = 11$ participants.

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DISCLOSURES

S. H. Adams is founder and principal of XenoMed, LLC, and has previously served as a consultant to Abitec, LLC.

AUTHOR CONTRIBUTIONS

K.E.M. conceived and designed research; K.E.M., G.C., N.K., J.N., G.H., J.F., W.T.G., M-E.H., C.H., S.H.A., J.T., L.M.P., K.O-M., C.C., C.J.C., D.B., and E.S. performed experiments; K.E.M., A.M., L.M.P., and B.J.S. analyzed data; K.E.M. and A.M. interpreted results of experiments; K.E.M., A.M., and B.J.S. prepared figures; K.E.M. and A.M. drafted manuscript; K.E.M., G.C., N.K., J.N., G.H., J.F., W.T.G., M-E.H., C.H., S.H.A., J.T., A.M., K.O-M., C.C., C.J.C., D.B., and E.S. edited and revised manuscript; A.M. approved final version of manuscript.

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