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□ ORIGINAL ARTICLE □

The Associations between Circulating Bile Acids and the Muscle Volume in Patients with Non-alcoholic Fatty Liver Disease (NAFLD)

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Abstract

Objective Non-alcoholic fatty liver disease (NAFLD) is frequently associated with obesity, dyslipidemia and type-2 diabetes mellitus. Bile acids (BAs) bind to the farnesoid X receptor (FXR) and G protein-coupled receptor 5 (TGR5), which are involved in lipid and glucose metabolism and energy expenditure. The present study aimed to determine associations between the circulating BAs and the skeletal muscle volume (SMV), and lipid and glucose metabolism in patients with NAFLD.

Methods Serum BAs and metabolic parameters were measured in 55 patients with NAFLD (median age, 55 years). The changes (Δ) in serum BA (ΔBA) and metabolic parameters were determined in 17 patients (male, n=10; female, n=7) who received nutritional counseling for 12 months.

Results Spearman's test revealed that the levels of 12α-hydroxysterol (12α-OH) BAs, including deoxycholic acid (DCA), were inversely correlated with the SMV of the upper and lower limbs and the total SMV. A multivariate analysis revealed that the level of DCA was correlated with a reduced total SMV, whereas non-12α-OH BAs, including chenodeoxycholic acid (CDCA), were correlated with an increased SMV of the lower limbs. Changes in CDCA were positively correlated with the ΔSMV of the lower limbs, and inversely correlated with the Δwaist-hip ratio and Δtotal cholesterol. Changes in the total non-12α-OH BA level were positively correlated with the ΔSMV of the lower limbs.

Conclusion Circulating BAs were associated with SMV. The 12α-OH BAs, including DCA were associated with reduced SMV levels, whereas non-12α-OH BAs including CDCA were associated with increased SMV levels. The molecular mechanisms underlying the association between the BA levels and the SMV remain to be explored.

Key words: farnesoid X receptor, G-protein-coupled bile acid receptor 5, waist-hip ratio, cholesterol metabolism, insulin resistance

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is frequently associated with metabolic diseases such as obesity, dyslipidemia, cardiovascular disease, chronic kidney disease, and type 2 diabetes mellitus (T2DM) (1).

Bile acids (BAs) are essential for dietary lipid absorption and the cholesterol metabolism. Recent studies have revealed that BAs bind to farnesoid X receptors (FXRs) and G protein-coupled receptor 5 (TGR5) (2-5). Various nuclear receptors regulate many biological processes, including lipid

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and glucose metabolism in the liver of NAFLD patients (6). The decreased expression of hepatic FXR protein has been associated with the progression of NAFLD (7). Bile acids activate nuclear receptor FXR-α, which binds to the farnesoid X response element of DNA together with the retinoid X receptor (RXR) to regulate the transcription of genes involved in lipid and glucose metabolism and inflammation (8, 9). In addition, BAs bind to the membrane-type receptor TGR5, which is involved in the secretion of incretin hormone glucagon-like peptide and the regulation of energy homeostasis (5, 8, 10). The plasma BA levels are increased in NAFLD patients (11).

Bile acids are synthesized by cholesterol oxidation in the liver via classical (neural) and alternative (acidic) major pathways. Cholesterol is initially hydroxylated to 7α-hydroxycholesterol by 7α-hydroxylase in the classical pathway and to 27-hydroxycholesterol by 27-hydroxylase in the alternative pathway. Both are then metabolized to chenodeoxycholic acid (CDCA) and cholic acid (CA). The catabolic conversion of CDCA to CA proceeds via 12α-hydroxylase, which is encoded by CYP8B1 (9). Both CDCA and CA are conjugated with glycine or taurine in the liver and are excreted into the duodenum. These primary BAs can be dehydroxylated by the gut microbiota to produce secondary BAs, which predominantly consist of deoxycholic (DCA) and lithocolic acids that are efficiently absorbed in the terminal ileum and returned via the portal vein to the liver, where they are absorbed by the hepatocytes and secreted into the bile (8). Thus, various BAs circulate in the blood via the entero-hepatic circulation. Several BA transporters, including sodium/taurocholate co-transporting polypeptide, the bile salt export pump and an apical sodium-dependent BA transporter are involved in the BA recycling system. Sodium/taurocholate co-transporting polypeptide and the bile salt export pump are associated with the progression of NAFLD (7). The 12α-OH BAs include CA, DCA, glycocholic, taurocholic, glycodeoxycholic, and taurodeoxycholic acids, and non-12α-OH BAs include CDCA, glucochenodeoxycholic, taurochenodeoxycholic, ursodeoxycholic, glycoursodeoxycholic, tauroursodeoxycholic, lithocolic, glycolithocholic, and taurolithocholic acids. Low ratios of 12α-OH BAs to non-12α-OH BAs correlate with insulin resistance in humans (12).

Skeletal muscle plays a key role in the development of insulin resistance and subsequent T2DM (13), and it releases muscle-derived cytokines (myokines) and metabolites (myometabolites), which regulate physiological homeostasis, aging and the progression of disease in the brain, liver, pancreas, adipose tissue, cardiovascular system, gut and other organs (14). The plasma BA levels are associated with glycemic control, body weight, and insulin sensitivity (15, 16). However, the association between BAs and the skeletal muscle volume (SMV) has not been investigated in detail. The present study aimed to determine the associations between circulating BAs and metabolic parameters, including the SMV in patients with NAFLD.

Materials and Methods

Fifty-five patients (male, n=34; female, n=21; median age, 55 years) who had been diagnosed with NAFLD [as described (17)], who attended our outpatient department during 2014 were enrolled in this study (Table 1). Briefly, the features of NAFLD were: ultrasonographic findings of a diffuse bright echo of liver parenchyma and impaired visualization of the peripheral vein, undetectable levels of antinuclear antibody, anti-mitochondrial antibody, anti-smooth muscle antibody, hepatitis B surface antigen and antibody to hepatitis C virus, an alcohol intake of <210 g/week for men and <140 g/week for women, the absence of hepatocellular carcinoma, and not being under treatment with steatosis-inducing drugs (such as corticosteroids). Thirty-nine (67.2%), 31 (56.3%), 27 (49.0%), and 9 (16.3%) patients were under medication at the time of enrollment for dyslipidemia, T2DM, hypertension, and gout respectively.

The physical parameters were assessed and serum levels of alanine aminotransferase (ALT), aspartate amino transferase (AST), γ-glutamyl transpeptidase (γ-GTP), total cholesterol (T-Chol), TG, creatinine (Cr), uric acid (UA), FBG, glycated hemoglobin A1c (HbA1c), and fasting immunoreactive insulin (IRI) were measured after an overnight fast of at least 12 hours. The homeostasis model assessment of insulin resistance (HOMA-R) was calculated according to the following formula (18): [fasting IRI (μU/mL) × FBG (mg/dL)]/405. The homeostasis model assessment beta (HOMA-β) value was calculated according to the following formula (19): [fasting IRI (μU/mL) ×360]/[FBG (mg/dL)-63]. The fasting serum BA levels were measured by HPLC-MS/MS using a 6410 Triple Quad LC/MS device (Agilent Technologies, Santa Clara, CA, USA) (20).

The body composition of all 55 patients was measured at entry in the diet counseling room at Mie University Hospital, and then registered dieticians (RD) started nutritional counseling as described (17). The physical parameters, which included body weight, body mass index (BMI), SMV, ratio of body fat (%FAT), waist-hip ratio (WHR), and visceral fat, were determined by multiple-frequency bioimpedance using an InBody 720 body composition analyzer (Biospace Co. Ltd., Seoul, Korea) (17). Dual energy X-ray absorptiometry (DXA) remains the standard modality for the analysis of body composition. However, under normal conditions the bioimpedance and magnetic resonance imaging findings are closely correlated (21). The European Working Group on Sarcopenia in Older People recommended that bioelectronic impedance might serve as a portable alternative to DXA (22). The InBody is an accurate substitute for DXA when measuring body composition (23). A recent study has shown that the SMV measured by computed tomography and by a bioimpedance analysis are significantly and positively correlated (24).

Seventeen of the enrolled patients (males, n=10; females, n=7; median age, 63 years) agreed to undergo regular nutri-

Table 1. Basic and Physical Characteristics of All Patients (n=55).

Parameter		Total (n = 55)	Male (n=34)	Female (n=21)	p
Age	(y)	67 (21-81)	63 (43-78)		
BMI	(kg/m^2)	24.6 (20.1-39.6)	24.6 (20.1-30.0)	24.9 (20.6-39.6)	0.3759
%FAT	(%)	29.5 (14.3-50.7)	26.8 (14.3-33.1)	37.6 (28.2-50.7)	0.0000†
WHR		0.88 (0.81-0.99)	0.89 (0.84-0.96)	0.87 (0.81-0.99)	0.2701
SMV of upper limb	(kg/m^2)	1.90 (1.21-2.78)	2.03 (1.52-2.78)	1.56 (1.21-2.34)	0.0007†
SMV of lower limb	(kg/m^2)	5.56 (4.45-8.28)	5.70 (4.84-8.28)	4.86 (4.45-6.30)	0.0004†
Total SMV	(kg/m^2)	8.33 (6.54-12.59)	9.15 (7.65-12.59)	7.07 (6.54-11.64)	0.0001†
Visceral fat	(cm^3)	105.6 (31.9-168.1)	105.5 (31.9-154.1)	101.5 (65.3-168.1)	0.7319
AST	(IU/L)	31 (15-141)	36 (19-141)	28 (15-80)	0.1697
ALT	(IU/L)	34 (12-133)	38 (16-133)	31 (12-76)	0.0700
γ-GPT	(IU/L)	42 (17-353)	57 (17-203)	27 (17-353)	0.0028†
T-Chol	(mg/dL)	188 (126-252)	184 (126-240)	205 (130-251)	0.0070†
TG	(mg/dL)	151 (41-591)	141 (41-591)	162 (57-320)	0.2066
Cr	(mg/dL)	0.69 (0.41-1.06)	0.75 (0.55-1.06)	0.64 (0.41-0.78)	0.0002†
UA	(mg/dL)	5.7 (3.5-9.2)	6.2 (4.4-9.2)	5.1 (3.5-6.7)	0.0004†
HbA1c	(%)	6.0 (5.2-7.1)	6.3 (5.6-7.1)	6.0 (5.2-6.9)	0.3861
FBG	(mg/dL)	107 (64-166)	109 (64-149)	106 (79-166)	0.8217
Fasting IRI	(μU/mL)	12.5 (2.1-50.0)	10.9 (2.1-50.0)	16.2 (4.4-22.6)	0.2965
HOMA -IR		3.37 (0.49-15.06)	3.00 (0.49-15.06)	4.52 (1.49-7.62)	0.3869
HOMA -β		102.4 (19.0-825-9)	87.3 (24.3-825.9)	118.8 (19.0-396.0)	0.3252
PLT	($10^4/\mu L$)	21.8 (7.9-32.2)	21.3 (9.3-25.9)	24.5 (7.9-32.2.)	0.2211

† $p < 0.01$, male vs. female

Table 2. Basic and Physical Characteristics of the Patients who Received Regular Nutritional Counseling for 12 Months (n=17).

Parameter		Nutritional counseling (n = 17)		
		at baseline	After 12 months	p
Male (%)		10 (58.8%)		
Age	(y)	63 (43-78)		
BMI	(kg/m^2)	24.8 (21.2-39.6)	24.4 (21.3-38.4)	0.0703
%FAT	(%)	29.2 (17.5-50.7)	29.9 (14.3-54.6)	0.554
WHR		0.89 (0.81-0.99)	0.88 (0.82-0.97)	0.816
SMV of upper limb	(kg/m^2)	1.84 (1.27-2.78)	1.98 (1.39-2.71)	0.4691
SMV of lower limb	(kg/m^2)	5.55 (4.57-8.28)	5.66 (4.45-8.66)	0.9246
Total SMV	(kg/m^2)	8.21 (7.21-12.59)	8.39 (7.23-13.66)	0.5383
Visceral fat	(cm^3)	114.4 (57.5-168.1)	81.4 (31.9-151.5)	0.0007†
AST	(IU/L)	35 (19-92)	33 (21-80)	0.438
ALT	(IU/L)	36 (16-83)	32 (18-76)	0.8871
γ-GPT	(IU/L)	31 (17-154)	36 (18-253)	0.9246
T-Chol	(mg/dL)	190 (156-245)	184 (126-229)	0.0684
TG	(mg/dL)	129 (51-591)	140 (41-315)	0.421
Cr	(mg/dL)	0.74 (0.48-1.02)	0.65 (0.41-1.02)	0.0179*
UA	(mg/dL)	5.4 (4.2-9.2)	5.7 (4.2-7.6)	0.2343
HbA1c	(%)	6.3 (5.8-7.1)	6.1 (5.1-6.9)	0.7461
FBG	(mg/dL)	104 (87-151)	99 (44-166)	1
Fasting IRI	(μU/mL)	16.0 (2.9-33.2)	12.1 (2.1-32.0)	0.7564
HOMA -IR		3.85 (0.7-8.35)	2.89 (0.4-10.6)	0.7764
HOMA -β		118.1 (19.0-825.9)	85 (24.4-295.4)	0.554
PLT	($10^4/\mu L$)	22.5 (16.0-130)	24.0 (11.1-25.9)	0.6417

* $p < 0.05$, † $p < 0.01$, at baseline vs. after 12 months

tional counseling. Briefly, counselors recommended 30 kcal/ideal body weight (kg) and a fat energy fraction of 20% as the daily intake and three bouts of aerobic exercise for >20 minutes every week. Their body composition was assessed during every meeting with the RD. The changes in the patients' anthropometric and laboratory parameters after 12 months of regular nutritional counseling were calculated and are expressed as delta (Δ). Table 2 shows anthropometric and laboratory values at baseline and after nutritional consultation. The physical parameters of patients without regu-

Table 3. Correlations among Serum Levels of 12α-hydroxysterol Bile Acids, and Physical and Laboratory Parameters Determined Using Spearman's Test.

Parameter	DCA		DCA+GDCA+TDCA		Total 12α -OH	
	ρ	p	ρ	p	ρ	p
BMI	0.1071	0.6885	0.0848	0.7510	0.1107	0.6787
%FAT	0.4786	0.0733	0.4643	0.0824	0.8143	0.0023†
WHR	-0.4509	0.0916	-0.4848	0.0697	-0.3107	0.2450
SMV of upper limb	-0.5929	0.0145*	-0.6125	0.0219*	-0.8536	0.0014†
SMV of lower limb	-0.5911	0.0309*	-0.5768	0.0309*	-0.8054	0.0026†
Total SMV	-0.7179	0.0072†	-0.7089	0.0080†	-0.8304	0.0019†
Visceral fat	0.3393	0.2043	0.3286	0.2189	0.3589	0.1793
AST	-0.1750	0.5126	-0.1911	0.4747	-0.0902	0.7358
ALT	-0.0688	0.7970	-0.0571	0.8307	-0.1071	0.6885
γ-GPT	-0.5732	0.0320*	-0.5741	0.0317*	-0.4714	0.0777
T-Chol	0.1732	0.5169	0.1875	0.4830	0.4938	0.0647
TG	-0.2027	0.4482	-0.1938	0.4685	0.3098	0.2464
Cr	-0.2268	0.3961	-0.2411	0.3671	-0.4339	0.1045
UA	-0.5357	0.0450*	-0.5313	0.0468*	-0.5563	0.0374*
FBS	0.3518	0.1881	0.3768	0.1586	0.4045	0.1302
Fasting IRI	0.4536	0.0897	0.4321	0.1059	0.5089	0.0569
HOMA -IR	0.4107	0.1244	0.3964	0.1380	0.5179	0.0527
HOM A-β	0.3518	0.1881	0.3143	0.2396	0.4571	0.0872
PLT	0.4304	0.1073	0.4321	0.1059	0.0938	0.7258

*p < 0.05, †p < 0.01. 12α-OH: 12α-hydroxysterol bile acids, DCA: deoxycholic acid, GDCA: glycodeoxycholic acid, TDCA: taurodeoxycholic acid

lar nutritional counseling were only measured at entry.

The correlation coefficients between the serum BA levels and the metabolic parameters were calculated by univariate analyses using Spearman's rho (ρ) test. The correlations between ΔBAs and the Δmetabolic parameters were analyzed using Pearson's test. Statistical differences between males and females and between baseline and the end of nutritional counseling were calculated using the Wilcoxon rank-sum test. A multivariate analysis was performed using multivariate logistic regression models. All of the patients provided their written informed consent to participate in the present study, which was approved by the Ethics Committee at Mie University.

Results

The correlations among the serum bile acid levels, and the physical and laboratory parameters

Spearman's test revealed that the serum DCA level was inversely correlated with the SMV of the upper or lower limbs, the total SMV, γ-GTP, and UA. The total 12α-OH BA level (including DCA), were positively correlated with %FAT, and inversely correlated with the SMV of the upper or lower limbs, the total SMV and UA (Table 3). The serum non-12α-OH BA levels were only correlated with UA. The ratio of 12α-OH BAs to non-12α-OH BAs was inversely correlated with the SMV of the lower limbs and the total SMV. The total serum BA level was inversely correlated with the SMV of the upper limbs (Table 4).

Spearman's test revealed that the total 12α-OH BA level was inversely correlated with the SMV of the lower limbs (ρ=-0.5528, p=0.0322) and the total SMV (ρ=-0.6029, p=0.0195) in men, but that there was no significant correlation between the serum BA levels and the SMV in women.

A multivariate analysis adjusted for %FAT, the SMV of the upper limbs, the SMV of the lower limbs, the total SMV, γ-GTP, and UA revealed that DCA was inversely correlated with γ-GTP, %FAT, and the total SMV. The total 12α-OH BA level was inversely correlated with the level of UA. The levels of chenodeoxycholic acid and total non-12α-OH BA were positively correlated with the SMV of the lower limbs, whereas the total BA level was positively correlated with the level of γ-GTP (Table 5).

The correlations between the changes in the serum bile acid levels and metabolic parameters after regular nutritional counseling

The visceral fat and serum Cr values were significantly reduced among the 17 patients who underwent regular nutritional counseling for 12 months (Table 2). The BMI and the serum level of T-Chol tended to be reduced, but the difference did not reach statistical significance. Eleven (70.5%) patients continued to walk quickly for >20 minutes at least three times each week during nutritional consultation. The SMV of the upper limbs, lower limbs, and the total SMV were increased in 9, 8, and 9 out of the 11 the patients who walked, respectively. However, the SMV was not observed to change to a statistically significant extent in any of the 17 patients who received nutritional counseling (Table 2).

The changes in the total serum 12α-OH BA (Δ12α-OH BA) level were positively correlated with the ΔFBG,

Table 4. Correlations among Serum Levels of Non-12α-hydroxysterol Bile Acids, and Physical and Laboratory Parameters Determined Using Spearman's Test.

Parameter	CDCA		CDCA+GCDCA+TCDCA		Total non-12α-OH		12α-OH /non-12α-OH		Total BAs	
	ρ	p	ρ	p	ρ	p	ρ	p	ρ	p
BMI	0.3250	0.2240	-0.1589	0.5521	0.0036	0.6787	0.0750	0.9893	0.0670	0.8022
%FAT	0.3036	0.2560	0.0429	0.8726	0.3821	0.1528	0.4321	0.1528	0.4804	0.0723
WHR	0.2304	0.3887	0.0607	0.8203	-0.0625	0.8151	-0.1750	0.5126	-0.0491	0.8542
SMV of upper limb	-0.0714	0.7893	-0.1786	0.5040	-0.4982	0.0623	-0.3643	0.1279	-0.5411	0.0429*
SMV of lower limb	-0.0750	0.7790	-0.0518	0.8464	-0.2536	0.3437	-0.5964	0.0256*	-0.3179	0.2343
Total SMV	0.0768	0.7739	0.0804	0.7637	-0.1732	0.5169	-0.6321	0.0180*	-0.2679	0.3162
Visceral fat	0.1518	0.5701	-0.1821	0.4955	0.0964	0.1793	0.3429	0.7182	0.1321	0.6210
AST	-0.0232	0.9308	0.4848	0.0697	0.1991	0.7358	-0.1107	0.4563	0.1464	0.5838
ALT	-0.3313	0.2152	0.1946	0.4664	0.0714	0.6885	-0.075	0.7893	0.0455	0.8647
γ-GPT	0.2563	0.3377	0.5357	0.0450*	0.2259	0.0777	-0.7196	0.3980	0.1661	0.5343
T-Chol	0.1214	0.6496	0.2143	0.4227	0.3946	0.0647	0.1375	0.1398	0.3982	0.1362
TG	0.3366	0.2079	0.5188	0.0523	0.5036	0.2464	-0.1143	0.0595	0.5	0.0614
Cr	-0.4938	0.0647	-0.4679	0.0800	-0.3982	0.1045	-0.0804	0.1362	-0.3634	0.1739
UA	-0.2134	0.4246	0.2402	0.3688	-0.0491	0.0374*	-0.3696	0.8542	-0.1304	0.6257
FBS	-0.2964	0.2674	-0.3875	0.1471	-0.1214	0.1302	0.5179	0.6496	-0.0661	0.8047
Fasting IRI	-0.0375	0.8884	-0.2446	0.3600	-0.0125	0.0569	0.4786	0.9627	0.0768	0.7739
HOMA-IR	0.0018	0.9947	-0.2518	0.3461	-0.0089	0.0527	0.4786	0.9733	0.0875	0.7434
HOMA-β	0.1125	0.6738	-0.1196	0.6544	-0.0107	0.0872	0.4357	0.9680	0.0607	0.8203
PLT	-0.0598	0.8229	0.0429	0.8726	0.0286	0.7258	-0.0232	0.9149	0.0188	0.9441

*p < 0.05. 12α-OH: 12 α-hydroxysterol , BA: bile acid, CDCA: chenodeoxycholic acid, GCDCA: glycochenodeoxycholic acid, TCDCA: taurochenodeoxycholic acid

Table 5. Multivariate Analysis of Physical Parameters and Laboratory Data Associated with Serum Bile Acids Levels.

	Logistic regression coefficient	F	95% CI (lower, upper)		p
DCA vs.					
γ-GPT	-1.3999	15.0244	-2.1386	-0.6613	0.0006
%FAT	-0.0535	10.3935	-0.0875	-0.0196	0.0031
Total SMV	-8.4497	9.3899	-14.0893	-2.8100	0.0047
DCA+GDCA+TDCA vs.					
γ-GPT	-0.9002	4.2244	-1.7960	-0.0044	0.0490
%FAT	-0.0755	14.0704	-0.1167	-0.0344	0.0001
Total SMV	-11.2010	11.2202	-18.0402	-4.3619	0.0023
12α-OH vs.					
UA	-5.7543	6.3005	-10.4429	-1.0657	0.0179
CDCA vs.					
SMV of lower limbs	0.4026	7.8695	0.1091	0.6961	0.0089
CDCA+GCDCA+TCDCA vs.					
γ-GPT	1.2126	7.6142	0.3138	2.1114	0.0099
SMV of lower limbs	0.3276	7.4701	0.0824	0.5727	0.0106
Non-12α-OH vs.					
γ-GPT	1.0640	5.2462	0.1139	2.0140	0.0295
SMV of lower limbs	0.3243	6.5541	0.1247	0.5834	0.0159
12α-OH /non -12α-OH vs.					
UA	-5.0978	4.6963	-9.9089	-0.2867	0.0386
%FAT	-0.0591	4.6562	-0.1151	-0.0031	0.0394
SMV of lower limbs	-0.3659	5.0730	-0.6982	-0.0336	0.0320
Total BAs vs.					
γ-GPT	1.0525	13.4492	0.4655	1.6395	0.0010

Table 6. Correlations between Changes in Serum Bile Acids and Metabolic Parameters after Regular Nutritional Counseling for 12 Months.

BA	Parameter	Correlation coefficient	95% CI (lower, upper)		p
Δ Total 12α-OH vs.					
	Δ FBG	0.5597	0.8262	0.0886	0.0242
Δ CDCA vs.					
	Δ T-Chol	-0.5664	-0.0763	-0.8361	0.0277
	Δ WHR	-0.5018	-0.0081	-0.7988	0.0477
	Δ SMV of lower limbs	0.5942	0.8419	0.1395	0.0152
Δ Total non-12α-OH vs.					
	Δ SMV of lower limbs	0.5731	0.8323	0.108	0.0203

Changes in serum bile acids and metabolic parameters after 12 months of regular nutritional counseling were expressed as Δ. 12α-OH: 12α-hydroxysterol bile acids, CDCA: chenodeoxycholic acid, FBG: fasting blood glucose, SMV: skeletal muscle volume, T-Chol: total cholesterol, WHR: waist-hip ratio

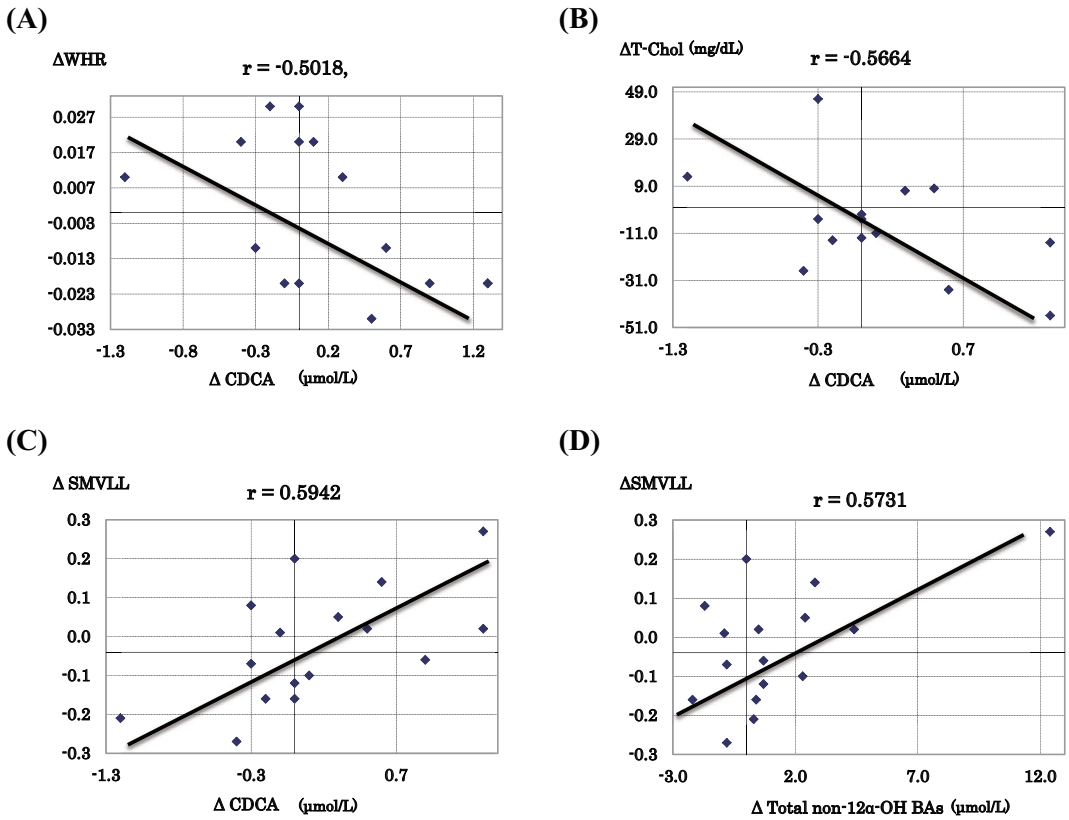

Figure. The correlations between the changes in the serum bile acid levels and the metabolic parameters after regular nutritional counseling for 12 months. (A) Changes (Δ) in the waist hip ratio (WHR) and serum levels of chenodeoxycholic acid (CDCA). (B) The serum levels of total cholesterol (T-Chol) and CDCA. (C) The skeletal muscle volume of lower limbs (SMVLL) and CDCA. (D) The SMVLL and the total 12α-hydroxysterol bile acid (non-12α-OH BA) level.

whereas the ΔCDCA was inversely correlated with the ΔT-Chol and ΔWHR, and positively correlated with the ΔSMV of the lower limbs. The changes in the total non-12α-OH BA (Δnon-12α-OH BA) level were positively correlated with the ΔSMV of the lower limbs (Table 6, Figure). The degrees of change in the physical and laboratory parameters of males and females did not differ to a statistically significant extent.

Discussion

The present findings associated fasting levels of serum BA with the SMV. Spearman's test revealed that the circulating levels of 12α-OH BAs were associated with a reduced

SMV. The increase in the %FAT value that was found in this investigation might be due to the reduction of the SMV. The multivariate analysis revealed that the circulating levels of DCA were associated with a reduced SMV, %FAT, and γ-GTP values. These findings indicated that 12α-OH BAs, including DCA, are involved in protein catabolism and energy consumption. Few studies have investigated the effects of serum BAs on the SMV, but incubating skeletal muscle with a synthetic TGR5 agonist increases the activity of type 2 deiodinase (D2) and energy expenditure (25). Activated D2 increases the expression of the active form of the thyroid hormone triiodothyronine and heat production in brown fat tissue (5, 8). In comparison to CDCA, deoxycholic acid binds to TRG5 with higher affinity (8, 26). Thus, 12α-OH BAs can enhance the general energy expenditure through the TGR5 signaling pathway, which appears consistent with the finding that the %FAT and γ-GTP values were reduced. The activation of TGR5 has been shown to increase O_2 consumption in primary human myocytes (5), but evidence supporting the TGR5-mediated autocatalysis of skeletal muscle has not come to light. The underlying mechanisms that might explain the correlation between the 12α-OH BA levels and the reduction in the SMV remain to be elucidated. Deoxycholic acid can potentially serve as a biomarker of sarcopenia, which frequently arises in patients with advanced NAFLD (27). Increased energy expenditure would help to improve metabolic syndrome, whereas increased muscle catabolism would exacerbate metabolic syndrome. Aerobic exercise might be necessary to maintain the SMV and to benefit from the 12α-OH BA-mediated metabolic effects.

In contrast to the 12α-OH BA levels, a multivariate analysis revealed that the serum non-12α-OH BA levels were correlated with an increased SMV of the lower limbs. Changes in the serum levels of non-12α-OH BAs, including CDCA, were positively correlated with the changes in the SMV of the lower limbs, which is consistent with the results of the multivariate analyses. The SMV of the lower limbs was far larger than that of the upper limbs. In addition, aerobic exercises such as walking can exert a greater influence on the changes in the SMV of the lower limbs. This might have contributed to the positive association between the serum non-12α-OH BA levels and the SMV of the lower limbs.

The present study found that the ΔCDCA value was inversely correlated with the ΔT-Chol and ΔWHR values. The degrees of BA affinity for FXR are in the order of CDCA > DCA > LCA > CA (4, 28). Activated FXR reduces the hepatic expression of fatty acid synthetase and of sterol regulatory element binding protein-1c, which is a key regulator of lipogenetic genes. In addition, the activation of FXR promotes lipid oxidation in the liver mitochondria by inducing the expression of peroxisome proliferator-activated receptor and pyruvate dehydrogenase kinase isoenzyme 4 (8). The correlation between CDCA and reduced WHR might be in line with the metabolic effects of BA via the FXR signaling pathway in the liver and fat tissues, since FXR is not expressed in skeletal muscle (29). The molecular mechanism(s) underlying the association between the serum non-12α-OH BA levels and the SMV have yet to be clarified. However, the effects of CDCA on the proliferation of myocytes and/or the influence of physical activities on CDCA metabolism, including the entero-hepatic circulation might be involved.

The multivariate logistic regression models revealed that the total BA level was correlated with elevated serum levels of γ-GTP, suggesting that fatty liver and/or an impaired hepatic biliary system can influence the total level of BA after fasting.

There were significant differences in the baseline physical and laboratory data of men and women. Men had a significantly higher SMV, γ-GTP, Cr, and UA levels, but lower levels of %FAT and T-Chol in comparison to women. The sex-related hormone estrogen regulates the activity and expression of the key enzymes that are involved in glucose metabolism, fatty acid oxidation, energy balance, and body composition (30). Gender bias should be taken into consideration when analyzing metabolic parameters. However, the separate analyses of men and women in the multivariate analysis did not uncover a significant correlation between the serum levels of BA and the SMV, which might have been due to the small number of samples.

Multiple-frequency bioimpedance technology (InBody) has proven useful for rapidly evaluating anthropometric data, including the skeletal muscle volume without exposing patients with chronic liver disease to radiation (14, 23, 24). Thus, the information collected from each patient at every visit served as the basis for the tailoring of nutritional advice. Nutritional counseling resulted in a significant reduction of visceral fat and the performance of >20 minutes of aerobic exercise at least three times per week might have partly helped to maintain the SMV of the patients in the present study.

The Third National Health and Nutrition Examination Survey in the USA found that the SMV was inversely associated with insulin resistance and the prevalence of prediabetes (31). Regular exercise promotes improved the glucose uptake in patients with diabetes by attenuating the epigenetic modification of glucose transporter 4, peroxisome proliferator activated receptor gamma coactivator 1α, and its downstream regulators in skeletal muscle (32). Thus, a reduction in the SMV mediated by 12α-OH BAs can influence the elevation of the level of FBS, whereas a reduction mediated by non-12α-OH BAs can favorably affect glucose metabolism. However, we did not find a significant association between non-12α-OH BAs and glucose metabolism in either univariate or multivariate analyses. This might be due to the fact that, at the time of entry into the study, 56.3% of patients had already received medication for T2DM, which might have masked associations between the BA levels and glucose metabolism.

One limitation of the present study is that a search of the literature did not uncover any mechanisms that could explain the association between the serum BA levels and the SMV. In addition, the high proportion of patients with

NAFLD who were received medication for the treatment of metabolic diseases might have interfered with associations between the serum BAs and metabolic parameters.

In summary, circulating BAs were associated with the SMV in patients with NAFLD. The 12α-OH BAs, including DCA, were associated with a reduced SMV. In contrast, non-12α-OH BAs, including CDCA were associated with an increased SMV. The underlying molecular mechanisms of the association between the BAs and the SMV remain to be explored.

The authors state that they have no Conflict of Interest (COI).

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