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Effect of Dietary Inclusion of *Lactobacillus acidophilus* ATCC 43121 on Cholesterol Metabolism in Rats

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Abstract This study examined the effects of *Lactobacillus acidophilus* ATCC 43121 (LAB) on cholesterol metabolism in hypercholesterolemia-induced rats. Four treatment groups of rats (n=9) were fed experimental diets: normal diet, normal diet+LAB (2×10^6 CFU/day), hypercholesterol diet (0.5% cholesterol, w/w), and hypercholesterol diet+LAB. Body weight, feed intake, and feed efficiency did not differ among the four groups. Supplementation with LAB reduced total serum cholesterol (25%) and VLDL+IDL+LDL cholesterol (42%) in hypercholesterol diet groups, although hepatic tissue cholesterol and lipid contents were not changed. In the normal diet group, cholesterol synthesis (HMG-CoA reductase expression), absorption (LDL receptor expression), and excretion via bile acids (cholesterol 7α-hydroxylase expression) were increased by supplementation with LAB, and increased cholesterol absorption and decreased excretion were found in the hypercholesterol diet group. Total fecal acid sterols excretion was increased by supplementation with LAB. With proportional changes in both normal and hypercholesterol diet groups, primary bile acids (cholic and chenodeoxycholic acids) were reduced, and secondary bile acids (deoxycholic and lithocholic acids) were increased. Fecal neutral sterol excretion was not changed by LAB. In this experiment, the increase in insoluble bile acid (lithocholic acid) reduced blood cholesterol level in rats fed hypercholesterol diets supplemented with LAB. Thus, in the rat, *L. acidophilus* ATCC 43121 is more likely to affect deconjugation and dehydroxylation during cholesterol metabolism than the assimilation of cholesterol into cell membranes.

Keywords: Hypocholesterolemic effect, *L. acidophilus* ATCC 43121, rat

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Coronary heart disease (CHD) continues to be the major cause of death in developed countries. Several studies [24, 27, 28] have reported high correlations between the elevated concentrations of total cholesterol and LDL cholesterol in blood with the incidence of CHD and atherosclerosis. In addition, dietary fat and cholesterol levels are highly correlated with blood cholesterol concentrations [9, 25, 34].

Many studies in animals and humans have suggested that probiotics or equivalent fermentation products can have health benefits on the host [6, 20, 21]. Hypoglycemic [23, 26] and hypocholesterolemic effects are their health benefits on lipid metabolism. Lactic acid bacteria may influence cholesterol levels in blood by affecting exogenous and endogenous cholesterol assimilation [14, 16] and also by affecting the deconjugation and dehydroxylation of primary bile acids within the intestinal tract under anaerobic conditions [15]. Several studies have indicated that the hypocholesterolemic effects of lactic acid bacteria are caused by the inhibition of dietary cholesterol absorption and the suppression of endogenous cholesterol synthesis [7, 9, 12, 14, 19, 22, 38, 39]. In contrast, the lactic acid bacteria have no effect on endogenous cholesterol synthesis [37].

The deconjugation of bile acids is the primary mode of hypocholesterolemic action. Fukushima and Nakano [12, 13] have proposed that a probiotic mixture containing *Bacillus*, *Lactobacillus*, *Streptococcus*, *Saccharomyces*, and *Candida* may act through this mechanism to indirectly lower HMG-CoA reductase activity. An alternative mechanism is the direct inhibition of HMG-CoA reductase; possible inhibitors include hydroxymethylglutaric acid, orotic acid, and uric acid from lactic acid fermentation [33].

Lactic acid bacteria or cultured products containing the bile salt hydrolase might reduce serum cholesterol levels and increase bile excretion in feces via bile salt hydrolase

[9]. This result would be a decreased quantity of bile acid returning to the liver, resulting in reduced feedback inhibition of bile acid synthesis and increased conversion of cholesterol to bile acids. The hypocholesterolemic effect exerted by microbials, however, remains unclear, and improved understanding of how microbials function may lead to more appropriate probiotic use.

The objectives of this study in rodents was to examine the effects of *L. acidophilus* ATCC 43121 on 1) serum cholesterol levels in induced hypercholesterolemia, 2) on hepatic cholesterol synthesis and bile synthesis as they relate to overall cholesterol homeostasis, and 3) on fecal sterol composition.

Table 1. Composition of experimental diets (g/kg diet).

Composition	Normal diet	Hypercholesterol diet
Milk casein	200	200
Corn starch	240.5	240.5
Sucrose	200	200
Corn oil	200	200
Cellulose	105	100
Mineral mixture[a]	40	40
Vitamin mixture[b]	9	9
DL-Methionine	3	3
Choline chloride	2	2
Cholesterol[c]	–	5
Sodium cholate	0.5	0.5

[a]AIN-76 Mineral mixture: Dyets Inc., Bethlehem, PA, U.S.A.
[b]AIN-76 Vitamin mixture: Dyets Inc., Bethlehem, PA, U.S.A.
[c]Nippon Fine Chemical Co. Ltd., Chuo-ku, Osaka, Japan.

MATERIALS AND METHODS

Source and Maintenance of Bacterial Cultures

The *L. acidophilus* ATCC 43121 used in this animal study was obtained from the ATCC (American Type Culture Collection, Rockville, MD, U.S.A.). The cultures were maintained by subculture in MRS broth (Difco Laboratories, Detroit, MI, U.S.A.) using 1% inocula with incubation at 37°C for 18 h.

Preparation of Freeze-Dried Cells

Five ml of active *L. acidophilus* ATCC 43121 culture was inoculated in 1,000 ml of MRS broth (Difco Laboratories, Detroit, MI, U.S.A.), and incubated at 37°C for 18 h. *L. acidophilus* ATCC 43121 cells were harvested by centrifugation at 2,000 ×*g* for 20 min and frozen at −70°C overnight. The cells were dried under vacuum for 24 h in a chamber-type freeze-drier (TFD5505, Ilshin Lab Co., Kyunggi, Korea). The freeze-dried cells were finely ground with a mortar and pestle; fresh preparations were made each week of the study period.

Animals

A total of 36 male Sprague-Dawley rats (Sam:TacN(SD)BR, Samtako, Kyunggi, Korea), initially weighing 241.6±2.1 g, were housed in individual stainless steel cages and maintained under a constant 12/12 h light/dark cycle. Rats were randomly assigned to four dietary treatment groups of nine rats each. The initial average animal body weight did not differ among the four groups. Feed intake and body weight were measured weekly. The rats had free access to water and to the group-specific diet during experiment. The experiment was terminated after 7 days of hypercholesterolemia conformation (21 days).

Diets and Experimental Design

Animal diets were formulated based on AIN-76 [1, 2] and contained 20.2% (w/w) fat and 18.1% (w/w) protein. Table 1 provides the detailed diet formula. Casein was used as the major source of dietary protein to avoid any possible effects related to soy-based components. The four experimental diets were normal diet (ND), normal diet with *L. acidophilus* ATCC 43121 (NDA), hypercholesterol diet (HD), and hypercholesterol diet with *L. acidophilus* ATCC 43121 (HDA). Both hypercholesterol diets contained 0.5% (w/w) supplemental cholesterol. Freeze-dried *L. acidophilus* ATCC 43121 was supplemented in the NDA and HDA groups based on the feed consumption of the previous week to ensure that its consumption was 2×10^6 CFU/day.

Sampling and Analytical Procedures

Blood samples were collected by cardiac puncture after the administration of ether anesthesia once each week at days 7, 14, and 21. Approximately 4 ml of blood was taken from each rat, the blood transferred to serum-separating tubes (Vacutainer SST, BD Bioscience, Franklin Lakes, NJ, U.S.A.), and kept on ice for 30 min. The tubes were then centrifuged for 20 min at 4°C and 2,000 ×*g*. The serum was collected for the analysis of HDL cholesterol and total cholesterol; both analyte concentrations were determined using commercially available kits (Sigma Diagnostics, St. Louis, MO, U.S.A.).

On day 7, three rats per group were euthanized for hepatic tissue cholesterol and lipid determinations, and at the end of the study (day 21), the remaining six rats per group were euthanized for hepatic mRNA quantification and hepatic tissue cholesterol and lipid determinations. Liver samples were taken from each rat after cervical separation. The middle lobe of each rat liver was standardized as the sampling region, based on a previous report regarding the gradient distribution of cholesterol 7α-hydroxylase expression (CYP7A1 gene) in rat liver [29]. Dissected liver samples were rinsed with DEPC treated PBS (0.1% diethylpyrocarbonate in 0.01 M PBS, pH 7.4).

Table 2. Primer sequences for RT-PCR and the expected product size (bp).

	Nucleotide	Product size (bp)
LDL receptor		
Upstream	5'-ATTTTGGAGGATGAGAAGCAG-3'	931
Downstream	5'-CAGGGCGGGGAGGTGTGAGAA-3'	
HMG-CoA reductase		
Upstream	5'-GCGTGCAAAGACAATCCTGGAG-3'	245
Downstream	5'-GTTAGACCTTGAGAACCCAATG-3'	
Cholesterol 7α-hydroxylase		
Upstream	5'-GCCGTCCAAGAAATCAAGCAGT-3'	306
Downstream	5'-TGTGGGCAGCGAGAACAAAGT-3'	
GAPDH		
Upstream	5'-GCCATCAACGACCCCTTCATT-3'	702
Downstream	5'-CGCCTGCTTCACCACCTTCTT-3'	

Total lipid was extracted from the whole liver (except the middle lobe) according to Folch *et al.* [11]. Total liver cholesterol was determined from the total extracted lipid using a modified enzymatic method.

The acid guanidium-phenol-chloroform method described by Chomczynski and Sacchi [5] and Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.) were used to isolate total RNA from the liver samples. The RNA samples were subjected to cDNA synthesis using Accupower RT PreMix (Bioneer, Daejeon, Korea) and an oligo(dT) 12–18 primer (Invitrogen, Carlsbad, CA, U.S.A.).

The PCR analysis was conducted using 2.0 μl of cDNA, 1.25 U of EX Taq polymerase (Takara, Tokyo, Japan), 2.0 μl of 10× PCR buffer (Takara, Tokyo, Japan), 1.6 μl of dNTP mix (2.5 mM each; Takara, Tokyo, Japan), and 1.0 μl of 25 mM upstream and downstream primers (Bioneer, Daejeon, Korea). Table 2 shows the primer sequences and expected product sizes. The initial PCR cycle consisted of denaturation at 94°C for 3 min, annealing at 60°C for 1 min, and extension at 72°C for 2 min, followed by 20 (GAPDH), 25 (LDL receptor), or 30 (HMG-CoA reductase and cholesterol 7α-hydroxylase) cycles of 94°C for 1 min, 60°C for 1 min, and extension at 72°C for 2 min, with a terminal extension at 72°C for 10 min.

The PCR products were electrophoresed in 1.5% agarose gels (SeaKem LE, BioWhittaker Molecular Applications, Rockland, ME, U.S.A.) and stained with ethidium bromide. LabWorks software 4.0 (UVP, Inc., Upland, CA, U.S.A.) was used for gel image acquisition, band identification, and band optical density determination. Band optical density of GAPDH was used as baseline, and the relative density of the LDL receptor, HMG-CoA reductase, and cholesterol 7α-hydroxylase to GAPDH was compared.

Whole fecal droppings were collected over a 2-day period (days 20 and 21). The feces were carefully collected using disposable wooden chopsticks to avoid contamination by feed or rodent hair. The samples were dried in a drying oven (55°C) for 2 days, after which any remaining hair or dust contaminants were blown away using an air blower; the remaining material was finely ground using an analytic mill (A-10, Ika Works, Wilmington, NC, U.S.A.) for further analysis.

Fecal acid and neutral sterols were extracted following a slightly modified version of the method described by Grundy *et al.* [18]. Separated neutral steroids and acidic steroids were silyated, and the derivatized steroids were analyzed by gas chromatography on a CP-SIL 19CB-1 capillary column (25 m×0.32 mm ID×0.2 μm; Varian BV, Middelburg, The Netherlands) using helium as the carrier gas at a flow rate of 1.5 ml per min, with an injector and detector final temperature of 295°C. An initial oven temperature of 265°C was retained for 20 min, and the final temperature of 295°C was attained by incremental increases of 5°C/min.

Statistical Analysis

Data were analyzed using the SAS general linear model procedure [35]. Differences among means were tested using Duncan's new multiple range test. Means and standard error of the means were calculated from replicate determinations.

RESULTS AND DISCUSSION

All rats appeared healthy throughout the study; the body weight gain, feed intake, and feed efficiency were unchanged for diet supplementation with 0.5% cholesterol or *L. acidophilus* ATCC 43121 (Table 3), whereas rats fed the hypercholesterol diets exhibited slightly higher body weights; however, no significant differences appeared among the four treatment groups.

Table 4 presents the effects of dietary cholesterol and *L. acidophilus* ATCC 43121 on serum levels of total cholesterol, VLDL+IDL+LDL cholesterol, and HDL cholesterol levels. Hypercholesterolemia was confirmed

Table 3. Weight gain, feed intake, and feed efficiency ratio of rats (n=6 per group) fed experimental diets for 21 days.

Diet treatment	Weight gain (g)	Feed intake (g)	FER (g/kg)
ND[a]	115.66	432.66	270.27
NDA	99.87	388.75	256.85
HD	121.01	451.24	271.06
HDA	115.67	456.03	256.03
SEM[b]	4.38	14.84	11.80

[a]ND, Normal diet; NDA, Normal diet+*L. acidophilus* ATCC 43121; HD, Hypercholesterol diet; HDA, Hypercholesterol diet+*L. acidophilus* ATCC 43121.
[b]SEM: Standard error of the means.

by serum total cholesterol levels. Serum total cholesterol levels were increased in rats fed the hypercholesterol diets, and the levels reached a plateau after at least 14 days ($P<0.05$).

After dietary cholesterol is ingested, it is absorbed in the gastrointestinal tract and transported to the liver by chylomicrons. Cholesterol is secreted from hepatocytes within very low density lipoprotein (VLDL) particles to extrahepatic tissues. VLDL loses apolipoprotein C to become IDL or LDL. Liver and extrahepatic tissues take up IDL and LDL via specific LDL receptors [30]. In rats fed the hypercholesterol diets, the increase in VLDL+IDL+LDL cholesterol caused the increase in total serum cholesterol, whereas the serum HDL cholesterol level was decreased compared with that in rats fed the normal diets ($P<0.05$). The hypercholesterolemic effect of dietary cholesterol and the increase in VLDL+IDL+LDL cholesterol was consistent with the findings of Fukushima and Nakano [12, 13] and Usman and Hosono [40]. When compared with the serum levels in rats fed the hypercholesterol diets, the serum VLDL+IDL+LDL cholesterol was lower in rats fed the hypercholesterol diets supplemented with *L. acidophilus* ATCC 43121, resulting in a lower total serum cholesterol ($P<0.05$); however, the serum HDL cholesterol level was not altered. Supplementation with *L. acidophilus* ATCC 43121 had no effect on total, HDL, or VLDL+IDL+LDL serum cholesterol levels in rats fed the normal diets. The hypocholesterolemic effects of *L. acidophilus* ATCC 43121 were restricted to VLDL+IDL+LDL cholesterol levels and supported the experimental results of Danielson *et al.* [7] and de Rodas *et al.* [8], who reported that *L. acidophilus* ATCC 43121 supplementation was beneficial in lowering total serum cholesterol and LDL cholesterol levels in pigs.

Table 4. Serum total cholesterol, HDL cholesterol, and VLDL+IDL+LDL cholesterol concentrations (mg/dl) in rats fed experimental diets.

	Day 7	Day 14	Day 21
Total cholesterol			
ND[1]	99.42	107.18[b]	107.60[b]
NDA	127.63	108.90[b]	107.20[b]
HD	118.27	152.24[a]	148.16[a]
HDA	101.54	125.57[b]	112.01[b]
SEM[2]	5.41	5.27	5.73
HDL cholesterol			
ND	57.95	61.52	51.21[a]
NDA	38.86	62.00	51.45[a]
HD	40.37	51.64	36.11[b]
HDA	47.15	54.32	38.86[b]
SEM	3.85	1.71	2.12
VLDL+IDL+LDL cholesterol			
ND	41.46[c]	41.92[c]	50.27[b]
NDA	68.76[a]	38.09[c]	55.74[b]
HD	77.88[ab]	103.17[a]	112.05[a]
HDA	54.39[bc]	71.25[b]	66.38[b]
SEM	6.48	6.52	7.14

[1]ND, Normal diet; NDA, Normal diet+*L. acidophilus* ATCC 43121; HD, Hypercholesterol diet; HDA, Hypercholesterol diet+*L. acidophilus* ATCC 43121.
[2]SEM: Standard error of the means.
[abc]Mean values in the column with different superscripts are significantly different as determined by GLM and Duncan's multiple range tests ($P<0.05$).

High dietary cholesterol increased the cholesterol content in liver ($P<0.05$) after day 7; however, the lipid content was significantly increased after 21 days of cholesterol supplementation. *L. acidophilus* ATCC 43121 supplementation lowered both of these levels numerically in rats fed either normal or hypercholesterol diets (Table 5). Fukushima and Nakano [12] reported that the hepatic cholesterol level was affected by hypercholesterol diets and by the dietary inclusion of *L. acidophilus* and *S. faecalis.*

The body's cholesterol pool is regulated by three distinct pathways: absorption, synthesis, and excretion. LDL receptor-mediated endocytosis controls plasma cholesterol levels by hepatic absorption, and the LDL receptor is regulated by a transcriptional control mechanism [4]. HMG-CoA reductase, a regulatory enzyme in the cholesterol

Table 5. Hepatic cholesterol (mg/g) and total lipids (mg/g) in rats fed experimental diets.

Treatment	ND[1]	NDA	HD	HDA	SEM[2]
Cholesterol					
Day 7 (n=3)	2.98[c]	3.35[bc]	4.62[a]	3.98[ab]	0.22
Day 21 (n=6)	2.94[bc]	2.47[c]	4.78[a]	3.98[ab]	0.31
Lipid					
Day 7 (n=3)	8.17	11.36	15.12	14.35	1.53
Day 21 (n=6)	10.83[bc]	7.00[c]	18.81[a]	15.86[ab]	1.20

[1]ND, Normal diet; NDA, Normal diet+*L. acidophilus* ATCC 43121; HD, Hypercholesterol diet; HDA, Hypercholesterol diet+*L. acidophilus* ATCC 43121.
[2]SEM: Standard error of the means.
[abc]Mean values in the row with different superscripts are significantly different as determined by GLM and Duncan's multiple range tests ($P<0.05$).

synthesis pathway, catalyzes the synthesis of mevalonate from HMG-CoA [17] and is regulated at the post-transcriptional level. Thus, hepatic HMG-CoA reductase expression is correlated with the rate of cholesterol synthesis [17, 36]. The excretion of bile salts formed from cholesterol in the liver is the only direct path for reducing the total pool of cholesterol, and hepatic cholesterol 7α-hydroxylase regulates bile acid synthesis from cholesterol.

Cholesterol synthesis (HMG-CoA reductase) and absorption (LDL receptor) represent the cholesterol inputs to the liver, and bile acid synthesis (cholesterol 7α-hydroxylase) is a measure of hepatic cholesterol reduction. The hypercholesterol diets decreased the input of cholesterol to the liver, especially that from cholesterol synthesis (HMG-CoA reductase) (Fig. 1, $P<0.05$). Bile acids, cholesterol, and mevalonate are known to inhibit HMG-CoA reductase expression [31], and the present study demonstrated that supplementation of the normal diets with cholesterol had an inhibitory effect on the HMG-CoA reductase expression. Supplementation with *L. acidophilus* ATCC 43121 increased hepatic HMG-CoA reductase mRNA expression in the normal diets groups ($P<0.05$), but there were no significant differences when *L. acidophilus* ATCC 43121 was supplemented in hypercholesterol diets. Cholesterol absorption via the LDL receptor (LDL receptor mRNA expression) was not affected

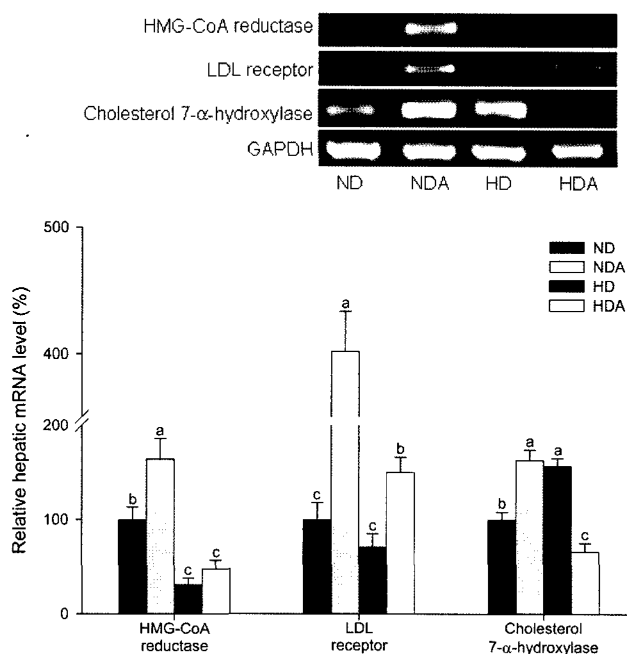

Fig. 1. Hepatic HMG-CoA reductase, LDL receptor, and cholesterol 7-α-hydroxylase of mRNA expression levels.

Levels of mRNA were calculated as a percentage of the values of the normal diet group. [abc]Mean values in the row with different superscripts are significantly different as determined by GLM and Duncan's multiple range test ($P<0.05$). Each value is mean±standard error. ND, Normal diet; NDA, Normal diet+*L. acidophilus* ATCC 43121; HD, Hypercholesterol diet; HAD, Hypercholesterol diet+*L. acidophilus* ATCC 43121.

Table 6. Fecal acid sterol concentration (mg/day) for rats fed experimental diets.

	CA[1]	CDCA	DCA	LCA	MCA	UBA	Total	P/B
ND[2]	2.28^{b}	1.30^{b}	6.23^{c}	9.88^{c}	3.18^{b}	1.82	25.41^{d}	0.65^{b}
NDA	1.70^{c}	0.85^{c}	6.88^{b}	11.60^{b}	3.22^{b}	1.74	26.58^{c}	0.71^{a}
HD	2.73^{a}	1.55^{a}	7.20^{b}	10.35^{c}	4.65^{a}	1.93	29.70^{b}	0.62^{c}
HDA	1.43^{d}	1.38^{b}	7.90^{a}	14.93^{a}	4.58^{a}	1.82	33.20^{a}	0.71^{a}
SEM[3]	0.13	0.05	0.13	0.52	0.38	0.15	0.62	0.01

[1]CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; MCA, α-, β-, and ω-muricholic acid; UBA, unidentified bile; P/B, Secondary bile concentration per total bile acid.
[2]ND, Normal diet; NDA, Normal diet+*L. acidophilus* ATCC 43121; HD, Hypercholesterol diet; HDA, Hypercholesterol diet+*L. acidophilus* ATCC 43121.
[3]SEM: Standard error of the means.
[abc]Mean values in the column with different superscripts are significantly different as determined by GLM and Duncan's multiple range tests ($P<0.05$).

by supplementation with cholesterol in the diet; however, LDL receptor mRNA expression increased with *L. acidophilus* ATCC 43121 supplementation in both the normal and hypercholesterol diets fed groups ($P<0.05$). The reaction by cholesterol 7α-hydroxylase is the rate-limiting step in bile acid synthesis from cholesterol, and their transcription and activity are increased by cholesterol of endogenous and dietary origins [31]. Diets supplemented with cholesterol resulted in increased bile acid synthesis (~40% increase in cholesterol 7α-hydroxylase mRNA expression; $P<0.05$), and supplementation with *L. acidophilus* ATCC 43121 in hypercholesterol diets reduced hepatic cholesterol 7α-hydroxylase mRNA expression.

Fecal excretions of acid and neutral sterol were presented in Tables 6 and 7. Diets supplemented with cholesterol increased the fecal excretion of acid and neutral sterols ($P<0.05$). Supplementation with *L. acidophilus* ATCC 43121 in hypercholesterol diets increased fecal acid sterol excretion ($P<0.05$); however, there was no effect on neutral sterol excretion. In fecal acid sterol, supplementation with *L. acidophilus* ATCC 43121 decreased excretion of primary bile salts, cholic acid (CA) and chenodeoxycholic acid (CDCA), and increased excretion of secondary bile salts, deoxycholic acid (DCA) and lithocholic acid (LCA) ($P<0.05$), and thus the secondary bile acid concentration per total acid sterol concentration (P/B) was increased ($P<0.05$). The cholic acid, chenodeoxycholic acid, and muricholic acid are primary bile acids, which are secreted as conjugates with taurin and glycin. Secreted primary bile acids are deconjugated and 7α-dehydroxylated by microbial enzymes in the intestine, and form secondary bile acids (deoxycholic acid and lithocholic acid) [31]. The decrease in primary bile salt excretion was attributable mainly to a decrease of CA, whereas the increase in secondary bile salt excretion was due mainly to an increase of LCA. Fecal excretion of muricholic acid was not

Table 7. Fecal neutral sterol concentrations (mg/day) for rats fed experimental diets.

	Cholesterol	Coprostanol	Coprostanone	Total neutral sterols
ND[1]	1.15[b]	6.40	0.23[b]	7.78[b]
NDA	1.39[b]	5.84	0.26[b]	7.51[b]
HD	7.32[a]	5.95	0.48[a]	13.76[a]
HDA	6.68[a]	5.56	0.55[a]	12.81[a]
SEM[1]	0.64	0.26	0.05	0.78

[1]ND, Normal diet; NDA, Normal diet+*L. acidophilus* ATCC 43121; HD, Hypercholesterol diet; HDA, Hypercholesterol diet+*L. acidophilus* ATCC 43121.
[2]SEM: Standard error of the means.
[abc]Mean values in the column with different superscripts are significantly different as determined by GLM and Duncan's multiple range tests ($P<0.05$).

affected by supplementation with *L. acidophilus* ATCC 43121, but it was by supplementation with cholesterol ($P<0.05$). Cholesterol and coprostanone excretion was increased with cholesterol supplementation, whereas *L. acidophilus* ATCC 43121 had no effect on cholesterol excretion ($P<0.05$). Coprostanol excretion was not changed by both cholesterol and *L. acidophilus* ATCC 43121 ($P>0.05$).

Supplementation with *L. acidophilus* ATCC 43121 in both normal and hypercholesterol diets increased fecal excretion of acid sterols. However, the serum total cholesterol level was only lowered in hypercholesterol diets supplemented with *L. acidophilus* ATCC 43121. This difference might be due to the different hepatic cholesterol metabolism between normal and hypercholesterol diets fed rats. Rudling [34] found that serum cholesterol levels coordinated the regulation of HMG-CoA reductase and LDL receptor mRNA levels in the liver, when mice were fed normal and hypercholesterol diets. In normal diets, increased fecal acid sterols excretion reduced the hepatic cholesterol level, and cholesterol absorption might be increased to maintain a normal hepatic cholesterol level; however, this might not be enough to maintain a normal hepatic cholesterol level, and cholesterol synthesis might be increased. In hypercholesterol diets, increased cholesterol absorption might be enough to hepatic cholesterol level, so cholesterol synthesis would not be changed when *L. acidophilus* ATCC 43121 was supplemented. This increased cholesterol absorption lowered serum VLDL+IDL+LDL cholesterol levels. Interestingly, in normal diets, bile acid synthesis (cholesterol 7α-hydroxylase) was increased when *L. acidophilus* ATCC 43121 was supplemented. Cholesterol 7α-hydroxylase activity is influenced by hepatic cholesterol levels and bile acids concentration in enterohepatic circulation [31]. Liver cholesterol 7α-hydroxylase mRNA expression is downregulated by bile acids through the activation of the farnesoid X receptor (FXR) and other orphan nuclear receptors [33]. The primary bile acid chenodeoxycholic acid (CDCA) is a more potent activator of FXR than are the secondary bile acids, lithocholic acid (LCA) and deoxycholic acid (DCA). Thus, the reduced CDCA level in the bile acids of rats fed the normal diets supplemented with *L. acidophilus* ATCC 43121 decreased FXR activity, and then cholesterol 7α-hydroxylase mRNA expression increased in this group. Supplementation with *L. acidophilus* ATCC 43121 in hypercholesterol diets decreased liver cholesterol 7α-hydroxylase mRNA expression ($P<0.05$). Chenodeoxycholic acid (CDCA) might not influence liver cholesterol 7α-hydroxylase mRNA expression, because of their similar concentration in normal diets and hypercholesterol diets supplemented with *L. acidophilus* ATCC 43121 (Table 6). Endogenous cholesterol from serum VLDL+IDL+LDL cholesterol might increase liver cholesterol 7α-hydroxylase mRNA expression.

Two possible mechanisms through which *L. acidophilus* ATCC 43121 could act to lower cholesterol levels are cholesterol assimilation and bile acid deconjugation. Bottazzi *et al.* [3] and Gilliland and Walker [14] suggested that cholesterol excretion could be enhanced by including lactic acid bacteria or an equivalent microbial environment in the diet. Their *in vitro* studies indicated that the appearance of cholesterol in microbial cells was associated with the disappearance of cholesterol from the medium; the assimilation of cholesterol by *L. acidophilus* ATCC 43121 occurred only when the culture was maintained in an anaerobic condition with bile acids [14]. De Smet *et al.* [10] hypothesized that bile acids accumulate in feces through bile acid deconjugation and dehydroxylation, and *Lactobacillus plantarum* with high activity of bile salts hydrolase decreased blood cholesterol level in hypercholesterolemia-induced rats [20]. Lactic acid bacteria contain active bile salt dehydrolases that may influence the body's bile salt metabolism to reduce the amount of bile salts available for resorption. Consequently, the feedback inhibition mechanism of bile salt synthesis is decreased, and the synthesis of bile salts from cholesterol is increased, yielding reduced serum cholesterol levels.

The composition of fecal acid and neutral sterols indicated that adding *L. acidophilus* ATCC 43121 to the diet did not increase cholesterol excretion via feces, suggesting that the cholesterol assimilated by *L. acidophilus* ATCC 43121 did not participate in the cholesterol-lowering effect. Previous studies [14, 32], however, suggested that *L. acidophilus* ATCC 43121 might produce a cholesterol-lowering effect by assimilating cholesterol. *In vitro* trials [32] have demonstrated that 4×10^9 cells/ml of *L. acidophilus* ATCC 43121 could incorporate up to 48 μg/ml cholesterol in cell membranes. Our experiment allowed a daily consumption of only 2×10^6 CFU of *L. acidophilus* ATCC 43121, which is much lower than the quantities used by Noh *et al.* [32].

Diets supplemented with *L. acidophilus* ATCC 43121 resulted in increased deconjugation and 7α-dehydroxylation

of primary bile acids in rat gastrointestinal tracts. The transformation of primary bile acids (CA and CDCA) increased the production of secondary bile acids (DCA and LCA). DCA is absorbed by the colon and enters the enterohepatic circulation [31]; in contrast, LCA is not absorbed by the colon because of its insolubility. As shown in Table 6, the fecal excretion of LCA exceeded that of DCA. Therefore, increased bile synthesis from cholesterol might be required to compensate for reduced enterohepatic bile acid circulation. In this experiment, hepatic cholesterol 7α-hydroxylase expression was reduced by supplementation with *L. acidophilus* ATCC 43121 in hypercholesterol diets. Other factors, dietary cholesterol, and endogenous cholesterol might increase hepatic cholesterol 7α-hydroxylase expression, and more intensive research must be conducted.

In the present study, supplementing rat diets with *L. acidophilus* ATCC 43121 increased bile acid deconjugation and dehydroxylation, resulting in increased cholesterol excretion. *L. acidophilus* ATCC 43121 had a negligible effect on cholesterol assimilation. These results indicate that the hypocholesterolemic effects of *L. acidophilus* ATCC 43121 are primarily the result of bile acid deconjugation and dehydroxylation.

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