Hypocholesterolemic Effect of Dietary Apple Polyphenol Is Associated with Alterations in Hepatic Gene Expression Related to Cholesterol Metabolism in Rats

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***Abstract*-Apple polyphenol (AP) mainly consists of procyanidins and has been reported to improve blood cholesterol levels and promote excretion of cholesterol in rats fed high-cholesterol diets. To understand the molecular mechanisms underlying the effects of AP, we investigated whether dietary AP changed the hepatic expression of genes related to cholesterol metabolism and steroid transport. Four-week-old male Sprague-Dawley rats were pair-fed with diets containing 0.5% cholesterol together with 0% (control), 0.2%, and 0.5% AP, respectively, for 30 days. Administration of 0.5% AP was found to improve serum total cholesterol levels (0.69-fold vs. control, $p < 0.05$), and increase hepatic LDL receptor (LDLR) mRNA (1.59-fold vs. control, $p < 0.0001$). There was a negative correlation between serum non-HDL cholesterol and LDLR mRNA ($p < 0.001$). Administration of 0.5% AP increased excretion of primary bile acids (2.96-fold vs. control, $p < 0.0001$) and up-regulated the expression of steroid catabolism genes such as sterol 12α-hydroxylase (CYP8B1). However, improvements in cholesterol levels were not associated with the hepatic expression of cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid biosynthesis. Expression of farnesoid X receptor (FXR), which is involved in the regulation of bile acid biosynthesis, was up-regulated by 0.5% AP (1.56-fold vs. control, $p < 0.01$), and FXR mRNA levels correlated positively with bile acid excretion ($p < 0.01$). These results show that dietary AP improved blood cholesterol levels with adequate intake of LDL from blood by increasing hepatic LDLR expression and promoting production of bile acid with an up-regulation of genes related to steroid catabolism.**

Keywords- Apple Polyphenol; Procyanidin; Cholesterol Metabolism; LDL Receptor

I. INTRODUCTION

Hypercholesterolemia is the presence of an excess of cholesterol in the bloodstream, which is caused by continuous intake of high levels of cholesterol from food or derangement of cholesterol metabolism, and may contribute to many diseases linked to hyperlipemia such as cardiovascular disease [1]. The liver plays a major role in metabolizing cholesterol, in which cholesterol biosynthesis and catabolism, steroid metabolites transport, and internal cholesterol homeostasis are regulated. Statin is used as a typical pharmaceutical for hypercholesterolemia to lower cholesterol levels by inhibiting HMG-CoA reductase, a rate-limiting enzyme involved in cholesterol biosynthesis [2]. However, its effect of lowering cholesterol is not enough for patients with low levels of cholesterol biosynthesis in the liver or high absorption of steroid compounds in the small intestine. Furthermore, adverse reactions to statin when it is used as a drug, such as impaired liver function, remain problematic. Hypercholesterolemia is mainly caused by a lifestyle of excessive eating or lack of exercise. Therefore, preventive treatment against hypercholesterolemia with daily safely ingestible food products without side effects is desirable.

Polyphenol extracted from unripe apples contains a significantly higher proportion of procyanidins (PC), which are polymerized with epicatechin or catechin. Apple polyphenol (AP) shows potent anti-oxidant activity [3]. Many physiological functions of AP have been reported recently, including anti-allergy [4], anti-tumor [5], and anti-dental caries [6] functions and the promotion of hair growth [7]. The bioavailability of AP has been reported previously [8]. Shoji et al. revealed that apple PC, which range in length from dimers to pentamers, were detectable in rat plasma after their oral administration. We recently demonstrated that PC from apples had Sir2-dependent anti-ageing effects in worms (*Caenorhabditis elegans*) [9], and that PC suppressed amyloid-β protein aggregation [10]. Moreover, we reported that dietary AP regulated mitochondrial ROS production and ameliorated the phenotypes of heart/muscle-specific manganese-superoxide dismutase-deficient mice [11]. Osada et al. also reported that intake of AP modulated blood triglycerides and reduced body fat in rats [12], supported by a previous study where dietary AP decreased expression of lipogenic genes and increased expression of lipolytic genes in the liver of rats fed high-fat or normal diets [13]. In addition, it has been reported that dietary AP improves cholesterol levels in blood and livers of rats fed high cholesterol [14], and also improves blood cholesterol levels in humans [15]. However, the mechanism underlying this effect

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is unclear. To address the molecular mechanisms of the anti-hypercholesterolemia effect of AP, we examined hepatic expression profiles of genes related to cholesterol metabolism, such as cholesterol biosynthesis, steroid catabolism, and steroid transport, in hypercholesterolemia rats treated with AP.

II. MATERIALS AND METHODS

A. Animals and Diets

Three-week-old male Sprague-Dawley strain rats (CLEA Japan) were housed individually in cages at a controlled temperature (20-23 ℃) with light (08:00-20:00). After one week of acclimatization with AIN-93G diets (Oriental Yeast, Japan), rats were divided into 3 groups consisting of 8 or 9 animals on the basis of body weights, and were fed AIN-93G diets containing 0.5% cholesterol. Table 1 shows the compositions of diets determined according to the AIN-93G formulation: AP-free diet (control), 0.2%, and 0.5% AP-supplemented diet (AP-0.2%, and AP-0.5%, respectively). AP was purified by Asahi Breweries, Ltd., and components of AP were analyzed according to Shoji et al [16]. Detailed experimental procedures involved in the preparation and analysis of AP have been previously reported [9]. PC accounted for 63.8% of AP. The volume of the diet fed to the control group was adjusted and normalized according to that fed to the AP-0.5% group under pair-feeding conditions because dietary AP may cause a slight reduction in food intake. After 30 days, rats were fasted overnight, anesthetized lightly using diethyl ether, bled from the abdominal aorta, and various tissues were immediately excised. Ten days before rats were dissected, feces were collected for 2 days from each rat and lyophilized. All experiments were performed under the guidelines of the Ethical Committee for Experimental Animal Care at Hirosaki University.

TABLE I EXPERIMENTAL DIET COMPOSITIONS

Ingredient	**Control**	**AP-0.2%**	**AP-0.5%**
		g/kg	
Cornstarch	412.5	410.5	407.5
Casein	200.0	200.0	200.0
α-Cornstarch	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0
Soybean oil	50.0	50.0	50.0
Cellulose	50.0	50.0	50.0
Mineral mix (AIN-93)	35.0	35.0	35.0
Vitamin mix (AIN-93)	10.0	10.0	10.0
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
Cholesterol	5.0	5.0	5.0
Apple polyphenol	0.0	2.0	5.0

B. Lipid and Fecal Steroid Analysis

Liver and serum lipids and fecal steroids were measured as previously described [14]. The amount of primary bile acids was defined as the sum of chenodeoxycholic acid (CDCA), cholic acid (CA), α-muricholic acid (α-MCA), and β-muricholic acid (β-MCA).

C. RNA Preparation

Livers cut into little pieces were fixed in RNAlater (Ambion, U.S.A.) overnight at 4 ℃ and frozen at -80 ℃ after removal of fluids. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. After confirming no degradation using the Agilent 2100 Bioanalyzer and RNA 6000 Nano-LabChip kit (Agilent technologies, U.S.A.), total RNA was used for real-time RT-PCR and DNA microarray analyses.

D. Real-Time RT-PCR

Single-stranded cDNA from 1 μg of total RNA was obtained using the High-Capacity cDNA Archive Kit (Applied Biosystems, U.S.A.) according to the manufacturer's protocol. Real-time RT-PCR was performed with cDNA as the template using a SYBR Green PCR Universal Master Mix and ABI PRISM 7700 Sequence Detector (Applied Biosystems). Specific primers were designed using Primer Express v1.5 software (Applied Biosystems) (Table 2). The following PCR cycle was performed: Step 1: 50 ℃ for 2 min, Step 2: 95 ℃ for 10 min, and Step 3: (95 ℃ for 15 s and 60 ℃ for 1 min) x 40 cycles. Gene expressions were normalized to glyceraldehyde-3-phosphate dehydrogenase gene signals.

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TABLE II PRIMERS USED FOR REAL-TIME RT-PCR

Genes	Forward primer sequence	Reverse primer sequence
Gapd	CCGAGGGCCCACTAAAGG	TGCTGTTGAAGTCACAGGAGACA
Hmgcr	CCTGCAGATGCTAGGTGTTCAA	CATCACAGTGCCACACACAATT
Cyp7a1	CAAGTCAAGTGTCCCCCTCTAGA	ACTCAATATCATGTAGTGGTGGCAAA
Cyp8b1	TCTGCCATGCTCCCTGTAAGA	ACCACAGGTTTTCCTCCTGAGA
Ldlr	AGCATAAACTTTGACAACCCAGTCT	TCCTGGCTGCGGCAAAT
Fxr	CCTCTGCTCGATGTCCTACAAA	GGAGGCAGGCGAAATGCT

E. DNA Microarray Analysis

DNA microarray analysis was conducted using the GeneChip system (Affymetrix, U.S.A.). Total RNA from rats in each group were pooled together in equal amounts. Two μg of total RNA was used for each array experiment. RNA labeling, hybridization, washing, and staining were performed according to the manufacturer's protocol. Labelled cRNA was hybridized on a Rat Expression Array 230A (with 15923 gene transcripts). Fluorescence signals were scanned using a Gene Chip Scanner GC3000. Two arrays were used for each group. Fluorescence signals were converted into a numeric value using an Affymetrix Gene Chip Operation System v1.0. Appropriate probes for analysis were selected by quality control procedures on the basis of flag and raw values [13]. We selected a probe that had a "present" or "marginal" flag in at least 5 arrays, and selected a probe that had a raw value of 20 or over in at least 2 groups or that had a raw value of 50 or over in at least 1 group. Data from selected probes were analyzed using GeneSpring v7.3 (Silicon Genetics, Agilent Technologies), gene expression analysis software.

F. Statistical Analysis

Statistical comparisons were performed by a 1-way ANOVA using SPSS v11.5 software (SPSS Inc., U.S.A.). A multiple comparison between groups was performed with Dunnett's test as post-hoc analysis. Differences in comparison were considered significant with $p < 0.05$. Results were expressed as mean ± SEM. Pearson's linear regression analysis was used to identify significant correlations.

III. RESULTS

In our preliminary experiment, serum total cholesterol levels in rats fed diets containing 0.5% cholesterol for 30 days were significantly higher than noncholesterol-containing diets (1.68 ± 0.10 mM, and 1.28 ± 0.11 mM, n = 8, p = 0.0021). Moreover, serum non-HDL cholesterol levels in rats fed 0.5% cholesterol-containing diets were also significantly higher than noncholesterol-containing diets (1.00 ± 0.09 mM, and 0.31 ± 0.06 mM, n = 8, p < 0.0001). Rats fed diets containing 0.5% cholesterol were considered to be appropriate hypercholesterolemic models.

In this study, there was no significant change in total food intake among the groups (Table 3). AP-0.2% increased body weight whereas AP-0.5% did not (Table 3). Hepatic weight and mesenteric fat mass were significantly decreased by AP-0.5% (Table 3). Intake of AP-0.5% tended to lower hepatic cholesterol (Fig. 1A, p = 0.0933), and serum cholesterol and non-HDL cholesterol was significantly lower than that of the control group (Figs. 1B, 1C, p = 0.0171 and p = 0.0066). Fecal cholesterol was higher with intake of AP-0.5% than that of the control group (Fig. 2A, 1.63-fold, p < 0.0001). AP intake in a dose-dependent manner also increased fecal primary bile acids (Fig. 2B, AP-0.2%, 1.89-fold vs. control, p = 0.0124; AP-0.5%, 2.96-fold vs. control, p < 0.0001). As shown in Fig. 2C, results demonstrated a significant negative correlation between serum non-HDL cholesterol and fecal primary bile acids (r = -0.6251, p = 0.0011).

TABLE III GROWTH PARAMETERS AND ORGAN WEIGHTS OF RATS FED THE CHOLESTEROL- LOADED DIET CONTAINING AP FOR 30 DAYS

	Control	AP-0.2%	AP-0.5%
Initial body weight (g)	95.6 ± 2.1	95.6 ± 2.0	95.9 ± 2.2
Weight gain (g)	147 ± 3	158 ± 3 *	148 ± 3
Body weight (g)	243 ± 2	253 ± 3 *	244 ± 4
Total food intake (g)	496 ± 3	497 ± 3	497 ± 5
Liver weight / BW (%)	3.51 ± 0.06	3.34 ± 0.06	3.23 ± 0.11 *
Mesenteric WAT weight / BW (%)	1.35 ± 0.06	1.22 ± 0.08	1.11 ± 0.08 *
n	9	8	8

Values are mean ± SEM *Different from the control group, p < 0.05. BW, body weight; WAT, white adipose tissue

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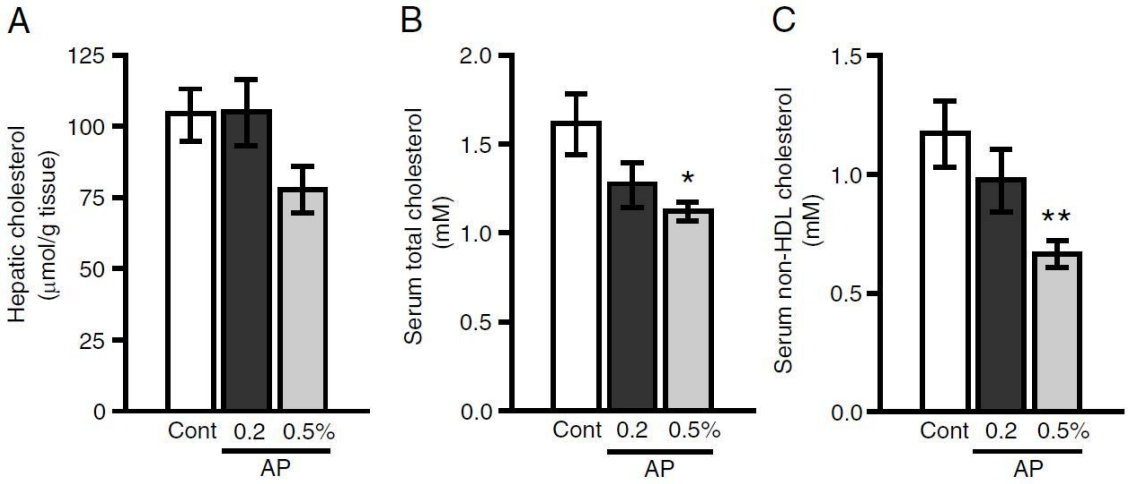

Fig. 1 Effects of dietary AP on cholesterol levels
Hepatic cholesterol (A), serum cholesterol (B), and serum non-HDL cholesterol (C) levels in rats fed the high cholesterol diet. Values are mean ± SEM, n = 8-9. Different from the control group, *p < 0.05, **p < 0.01.

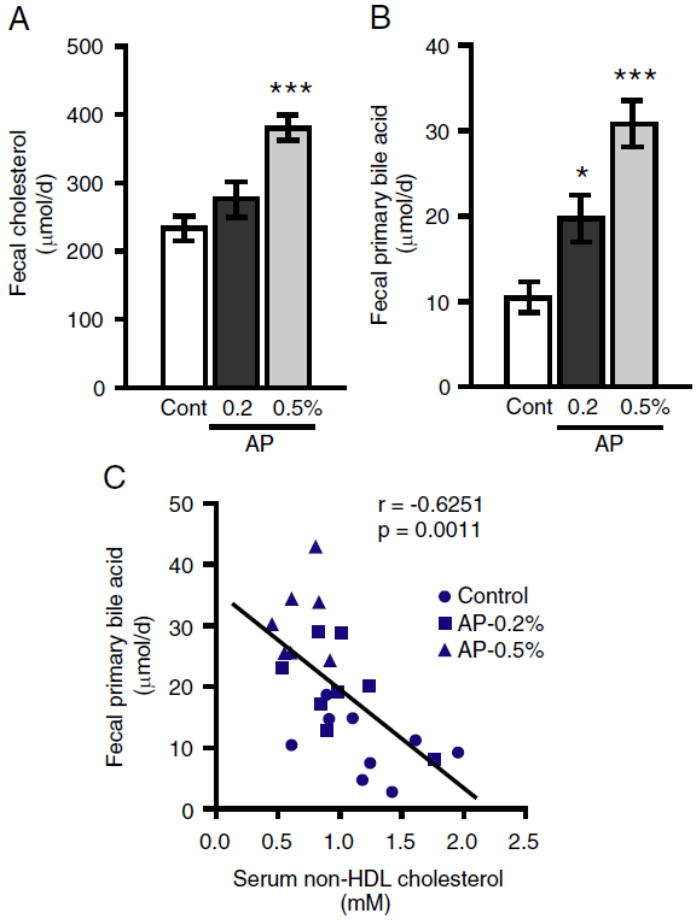

Fig. 2 Effects of dietary AP on fecal steroid levels
Fecal cholesterol (A) and primary bile acids (B) amounts in rats fed the high cholesterol diet. Values are mean ± SEM, n = 8-9. Different from the control group, *p < 0.05, ***p < 0.0001. C, Correlation between serum non-HDL cholesterol and fecal primary bile acids.

LDL receptor (LDLR) mRNA levels with intake of AP-0.5% were 1.59-fold higher than that of the control group (Fig. 3A, $p < 0.0001$). There was a significant negative correlation between serum non-HDL cholesterol and LDLR mRNA (Fig. 3B, $r = -0.6624$, $p = 0.0003$) and a significant positive one between fecal bile acid and LDLR mRNA (Fig. 3B, $r = 0.6130$, $p = 0.0014$). We failed to find a significant difference in hepatic HMG-CoA reductase (HMGCR) mRNA among the groups (Fig. 3A). The appearances of cholesterol biosynthesis genes other than HMGCR were analyzed by microarray, and results indicated no changes in the expressions of the ten probes including the transcription factor, sterol regulatory element binding protein 2 (SREBP-2), with intake of AP (Table 4).

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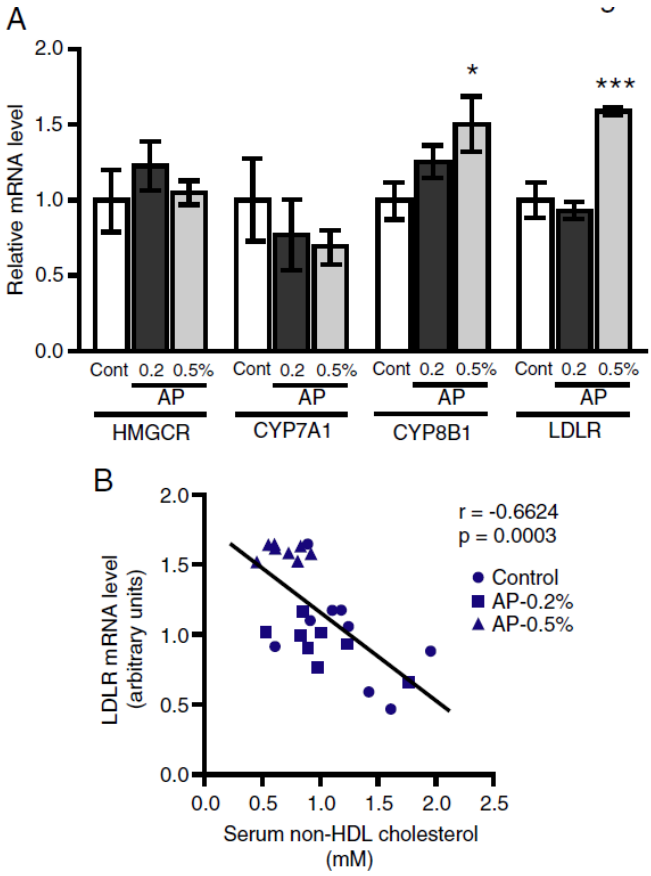

Fig. 3 Effects of dietary AP on expression of steroid metabolism genes
A, Expression levels of steroid metabolism genes in livers of rats fed the high cholesterol diet. Values are mean ± SEM, n = 8-9. Different from the control group, *p < 0.05, ***p < 0.0001. B, Correlation between serum non-HDL cholesterol and LDLR mRNA.

TABLE IV EFFECTS OF DIETARY AP ON EXPRESSION OF GENES RELATED TO CHOLESTEROL BIOSYNTHESIS IN LIVERS OF RATS

Affymetrix ID	Common name	Description	Fold change [a]	
			AP-0.2%	AP-0.5%
1367932_at	Hmgcs1	3-hydroxy-3-methylglutaryl-CoA synthase 1	1.06	1.40
1371979_at	Srebp2	sterol regulatory element binding protein 2	0.84	1.10
1372973_at	Lss	2,3-oxidosqualene: lanosterol cyclase	1.06	1.04
1370310_at	Hmgcs2	3-hydroxy-3-methylglutaryl-CoA synthase 2	1.14	1.04
1368189_at	Dhcr7	7-dehydrocholesterol reductase	1.45	1.04
1367839_at	Fdft1	farnesyl diphosphate farnesyl transferase 1	1.06	1.01
1373144_at	Fdps	Farensyl diphosphate synthase	1.13	0.99
1386990_at	Ebp	phenylalkylamine Ca^{2+} antagonist (emopamil) binding protein	1.03	0.97
1367667_at	Fdps	Farensyl diphosphate synthase	0.94	0.92
1367767_at	Hmgcl	3-hydroxy-3-methylglutaryl CoA lyase	1.08	0.89

[a] Fold changes relative to the control group.

Regarding bile acid synthesis, as shown in Fig. 3A, among the groups, there was no significant change in cholesterol 7α-hydroxylase (CYP7A1) mRNA, a rate-limiting enzyme of bile acid synthesis. On the other hand, sterol 12α-hydroxylase (CYP8B1) mRNA was significantly increased with intake of AP-0.5% (Fig. 3A, 1.50-fold vs. control, p = 0.0278).

Farnesoid X receptor (FXR, NR1H4), a hepatic transcription factor related to steroid metabolism, was up-regulated by AP-0.5% intake (Fig. 4A, 1.56-fold vs. control, p = 0.0021). There were a negative correlation between expression of CYP7A1 and

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FXR (r = -0.3756, p = 0.0850) and a significant positive one between fecal primary bile acids and expression of FXR (Fig. 4B, r = 0.5765, p = 0.0040).

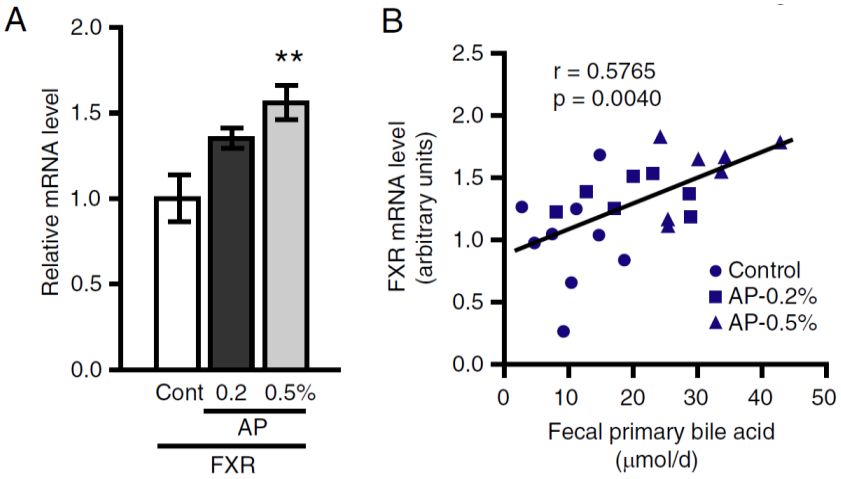

Fig. 4 Effects of dietary AP on expression of FXR
A, Expression levels of FXR in livers of rats fed the high cholesterol diet. Values are mean ± SEM, n = 8-9. **Different from the control group, p < 0.01.
B, Correlation between fecal primary bile acids and FXR mRNA.

In microarray analysis, 7445 probes were available. Administration of AP-0.2% and AP-0.5% increased 104 (1.4%) and 149 (2.0%) probes by 1.5 times or more, respectively. Administration of AP-0.2% and AP-0.5% decreased 71 (1.0%) and 109 (1.5%) probes by 1.5 times or more, respectively. Microarray analysis revealed that steroid 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase relating to steroid metabolism were up-regulated by dietary AP in a dose-dependent manner (Table 5). Three probes of hepatocyte nuclear factor (HNF), containing HNF-3β and HNF-6, were also up-regulated by dietary AP in a dose-dependent manner (Table 5).

TABLE V SELECTED UP-REGULATED GENES BY DIETARY AP IN LIVERS OF RATS FED THE HIGH CHOLESTEROL DIET

Affymetrix ID	Common name	Description	Fold change [a]	
			AP-0.2%	AP-0.5%
1371034_at	Hnf6	one cut domain, family member 1 (HNF-6)	1.64	2.31
1387760_at	Hnf6	one cut domain, family member 1 (HNF-6)	1.66	1.91
1368711_at	Hnf3b	Forkhead box A2 (FOXA2, HNF-3β)	1.41	1.86
1393902_at	-	aldo-keto reductase family 1, member C6 (17β-hydroxysteroid dehydrogenase)	1.43	1.82
1371076_at	Cyp2b15	cytochrome P450, 2b19 (Steroid 17α-hydroxylase)	1.28	1.65

[a] Fold changes relative to the control group.

IV. DISCUSSION

It has been shown that AP promotes the excretion of cholesterol via reductions in the micellar solubility of cholesterol [14]. Another report shows that AP may improve cholesterol levels by lowering intestinal cholesterol esterification and lipoprotein secretion [17]. In this study, we show that AP promoted fecal primary bile acids as well as cholesterol, and there was a negative correlation between blood non-HDL cholesterol and fecal primary bile acids. On the basis of these results, we assume that AP functions by affecting cholesterol metabolism. Therefore, we assessed hepatic gene expressions involved in cholesterol metabolism such as cholesterol biosynthesis, bile acid biosynthesis, and LDLR.

We found that dietary AP-0.5% increased the levels of LDLR mRNA with little variation, and there was a negative correlation between serum non-HDL cholesterol and LDLR mRNA. It has been reported that a genomic LDLR deficiency plays a causal role in familial hypercholesterolemia [18]. LDLR is expressed at the hepatic cell surface and recognizes apolipoprotein B, which is composed of lipoproteins such as LDL and very low density lipoprotein (VLDL). Removal of LDL or VLDL from blood circulation via LDLR is one of the mechanisms that modulate blood cholesterol concentrations. In contrast, previous studies have shown the relationship between the up-regulation of LDLR expression and food ingredient-related flavonoids in vitro [19-21]. The bioavailability of AP has been reported previously [8]. It is possible that absorbed AP or its metabolites could directly up-regulate LDLR in hepatic cells in vivo.

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Statin, used for the prevention and treatment of hypercholesterolemia, improves cholesterol levels via the inhibition of cholesterol synthesis [2]. HMGCR is a rate-limiting enzyme involved in cholesterol biosynthesis. In this study, neither HMGCR expression changes in an AP dose-dependent manner nor correlations between HMGCR mRNA and serum cholesterol levels (data not shown) were detected. Additionally, microarray data revealed that intake of AP did not change expressions of other genes related to cholesterol biosynthesis. Moreover, we failed to observe appearance of expression changes in transcription factor SREBP-2, which has been reported to promote the expression of genes related to cholesterol biosynthesis, such as HMGCR [22]. Results show that intake of AP did not affect the expression of genes related to cholesterol biosynthesis.

The elimination of cholesterol is achieved via a major metabolic pathway in which cholesterol is converted into bile acid in the liver by modification of the cholesterol structure, followed by oxidation and cleavage of the side chain. CYP8B1 catalyzes 12α-hydroxylation, which is related to the formation of CA [23]. In this study, CYP8B1 mRNA was increased by dietary AP-0.5%. This result may partially explain why AP accelerated cholesterol catabolism and bile acid biosynthesis.

CYP7A1 plays an important role in cholesterol catabolism and bile acid biosynthesis. It catalyzes 7α-hydroxylation, which is related to the conversion of cholesterol to 7α-hydroxycholesterol, a rate-limiting step of the bile acid synthesis pathway [24], and the genomic CYP7A1 deficiency becomes a hypercholesterolemic phenotype [25]. Previous reports indicate that the effect of lowering cholesterol is related to the up-regulation of CYP7A1 [26-28]. On the other hand, it has also been shown that CYP7A1 is down-regulated due to feedback inhibition by bile acid [29]. Previous reports showed that improvement of cholesterol homeostasis was not always associated with up-regulation of CYP7A1 gene expression [30, 31]. Our study demonstrated that there was a decreasing trend in expression levels of CYP7A1 and there was a significant increase in bile acid by intake of AP. It is possible that CYP7A1 was down-regulated due to increases in bile acid pool size by AP, and results of improvement of cholesterol level and low level of CYP7A1 were not contradictory.

Intake of AP increased the expression of FXR, involved in the regulation of cholesterol and bile acid metabolism [32]. Primary bile acids, such as CDCA, act as physiological ligands of FXR [33, 34]. FXR activated by bile acid represses CYP7A1 expression on a long-term basis at the transcriptional level [35, 36]. In this study, dietary AP increased both fecal primary bile acids and hepatic FXR mRNA levels. Additionally, there was a positive correlation between primary bile acids excretion levels and FXR mRNA levels. Although the amount of bile acid pools in the liver, gallbladder and enteric canal was not measured in this experiment, our data suggested that hepatic CYP7A1 may be regulated cooperatively by FXR and bile acid, which were increased by AP.

Microarray analysis revealed that dietary AP up-regulated probes encoding steroid 17α-hydroxylase and 17β-hydroxysteroid dehydrogenase. These genes were reported to be involved in the pathway of steroid hormone synthesis [37]. Furthermore, HNF-3β and HNF-6 were up-regulated by dietary AP. HNF-3β and HNF-6 are nuclear transcription factors related to carbohydrate and lipid metabolism. HNF-3β, known as FOXA2, is involved in hepatic bile acid homeostasis [38, 39], and HNF-6 has been reported to enhance HNF-3β transcriptional activity [40]. These expression changes may confirm that intake of AP affected steroid and bile acid metabolism.

AP has been previously reported to be safe in a 90-day consecutive oral administration test [41, 42]. In this study, serum total cholesterol levels in rats fed diets containing cholesterol and AP were comparable with those in rats fed noncholesterol-containing diets. This suggests that the hypocholesterolemic effect of AP is safe. Since AP is contained in the edible part of the fruit, it is easy to ingest routinely and is a safe food factor. Apple polyphenol may be promising for preventing hypercholesterolemia.

V. CONCLUSIONS

In this study, dietary AP was found to improve blood cholesterol levels and increase LDLR mRNA. Dietary AP increased the excretion of cholesterol and primary bile acids, as well as the expression of several steroid catabolism genes such as CYP8B1, without elevations in the expression of CYP7A1, the rate-limiting enzyme of bile acid biosynthesis. Expression of FXR, which is involved in the regulation of bile acid biosynthesis, was up-regulated by AP, and it correlated positively with bile acid excretion. Dietary AP did not affect the expression of genes related to cholesterol biosynthesis, and was different from statin function. In conclusion, these results suggest that dietary AP improved blood cholesterol levels with adequate intake of LDL by increasing hepatic LDLR levels, and promoting the production of bile acid while up-regulating the expression of genes related to steroid catabolism enzymes and regulators.

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