tocytes[25]. Fu *et al.*[26] reported the PPAR-α agonist ciprofibrate as an inhibitor of the expression of ACF, one of the responsible factors of apoB mRNA production; however, this inhibition was found only in the liver, not in the intestine of LDL-receptor knockout mice. This supports the idea that the factors involved in the regulation of apoB lipoproteins, including PPAR-α agonists, might differ between these two tissues, which leads to the need for more studies to understand the regulation of apoB in the small intestine.

MTP catalyzes the transfer of TG and cholesteryl esters to apoB and therefore has a main role in the assembly of apoB-containing lipoproteins. It has been reported that PPAR-α agonists increase MTP expression and apoB secretion in rodent liver but not in the intestine in spite of decreased plasma TG levels[27]. We found that MTP expression was not affected by fenofibrate in the intestine of CD36KO mice in the postprandial state, which also contributes to the idea that regulation of the production of apoB-containing lipoproteins in the intestine might be different from the liver.

Our results show fenofibrate to be an effective treatment for postprandial hyperlipidemia in CD36KO mice, and the reduction in the intestinal production of ApoB-containing lipoproteins as a new mechanism of action for this drug. Thus, since human CD36 deficiency is a genetic background of metabolic syndrome, as stated previously, we suggest that fenofibrate might play a similar role not only in CD36-deficient patients, but in MetS; this hypothesis, however, needs to be tested in further studies.

Conclusion

Fenofibrate reduces postprandial hypertriglyceridemia in CD36 knockout mice; this reduction is associated with the inhibition of intestinal apoB-48 production and the subsequent reduction of intestinal apoB-containing lipoproteins. This suggests a protective effect of fenofibrate against atherosclerosis in CD36KO mice as a monogenic model of metabolic syndrome.

Acknowledgments

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Review

Molecular Mechanisms of HDL-Cholesterol Elevation by Statins and Its Effects on HDL Functions

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Numerous large-scale clinical studies have revealed that the low-density lipoprotein cholesterol (LDL-C)-lowering effect of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) prevents coronary heart disease (CHD). Statins have not only LDL-C-lowering effects but also high-density lipoprotein cholesterol (HDL-C)-elevating effects, which differ among statins. In this article, we discuss the molecular mechanisms of HDL-C elevation by statins and its effect on HDL functions. We summarize the reports to date on the effects of statins on various proteins, enzymes and receptors involved in reverse cholesterol transport (RCT), which is one of the protective systems against atherosclerosis. Since statins increase the synthesis of apolipoprotein A-I (ApoA-I) and HDL neogenesis in the liver, the HDL-C-increasing effect of statins may reflect RCT activation. Moreover, HDL has pleiotropic effects, including anti-inflammatory and anti-oxidative effects, as well as RCT. In the future, it may be necessary to assess the functions of HDL elevated by statins, and select statins based on differences in their effects in clinical practice.

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Key words; Reverse cholesterol transport, Apolipoprotein A-I, ATP-binding cassette transporter A1, Adiponectin, Cholesteryl ester transfer protein

Introduction

HDL is a lipoprotein that plays a central role in reverse cholesterol transport (RCT). Hypo-HDL-cholesterolemia has long been recognized as a strong and independent risk factor for coronary heart disease (CHD). Epidemiologically, a low level of high-density lipoprotein cholesterol (HDL-C) is associated with an increased risk for CHD, even when the low-density lipoprotein cholesterol (LDL-C) level is low[1]. In addition, a low level of HDL-C is reportedly related to increased mortality[2, 3].

The main roles of HDL are to collect excess cholesterol stored in peripheral tissues, such as lipid-laden foam cells, and transport it to the liver for excretion into bile[4, 5]. Apolipoprotein A-1 (ApoA-I) produced in the liver and the small intestine, and free ApoA-I released during hydrolysis of triglyceride (TG)-rich lipoproteins bind cholesterols and phospholipids through ATP-binding cassette transporter A1 (ABCA1) to form pre-β HDL. Cholesterols extracted from peripheral cells are converted to cholesterol esters (CEs) by the action of lecithin:cholesterol acyltransferase (LCAT) to enter HDL particles and form HDL. Mature HDL produced after repeated cholesterol extraction and conversion to CE is returned to the liver through LDL receptors while CE is transported to VLDL (very low density lipoprotein) and LDL by cholesteryl ester transfer protein (CETP); however, HDL has recently been speculated to have various properties, including anti-inflammatory, anti-oxidative, anti-coagulation and vascular endothelial function-improving effects in addition to RCT (**Fig. 1**). These effects may comprehensively be involved in the prevention of atherosclerosis progression[6, 7].

Statins have the strongest LDL-C-lowering effect

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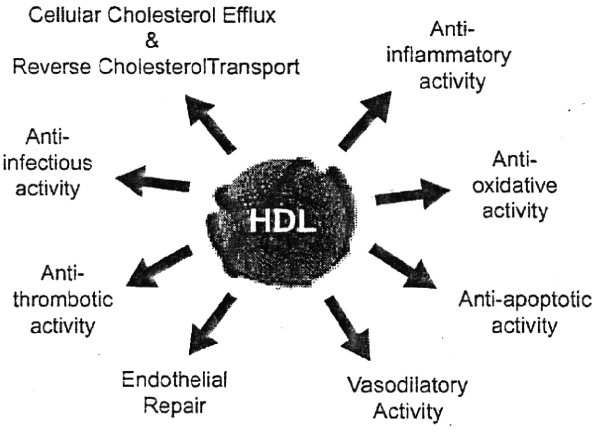

Fig. 1. Multiple anti-atherogenic actions of HDL.

HDL has various functions, including anti-inflammatory, anti-oxidative, anti-thrombotic and vascular endothelial repair activities.

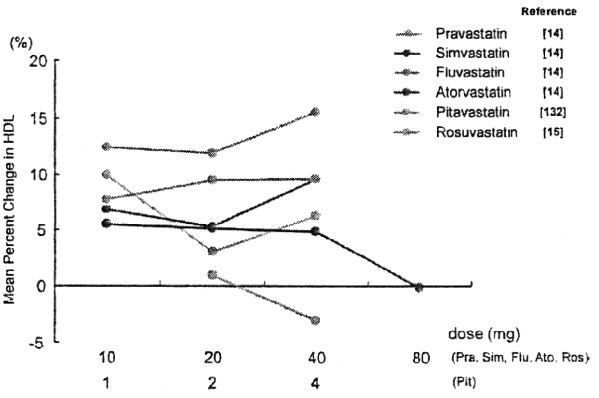

Fig. 2. Effects of each statin dose on HDL-C levels.

Percent changes in HDL-C by each statin dose. Pale blue, pravastatin (Pra); Violet, simvastatin (Sim); Pale violet, fluvastatin (Flu); Dark blue, atorvastatin (Ato); Lime green, pitavastatin (Pit); Light blue, rosuvastatin (Ros)

among the drugs clinically available at present and are used for the prevention of CHD worldwide; however, since the incidence of cardiovascular events has been shown to be relatively high in patients with low HDL-C levels even during statin treatment[8], the levels of HDL-C must also be paid attention during statin treatment. Concerning the effects of statins on levels of HDL-C, although statins clinically increase HDL-C levels, there is little evidence regarding their mechanism and the significance of their actions as compared to those pertaining to LDL-C. In this article, we discuss the usefulness of the statin-induced rise in HDL-C levels and review the findings to date on the effects on HDL functions and mechanisms of action, as well as the differences in effects among statins.

Clinical Significance of Statin-Induced HDL-Cholesterol-Elevating Effect

HDL levels during statin treatment are also important for predicting cardiovascular events. In the J-LIT study in which simvastatin was used, HDL levels below 40 mg/dL were associated with an increased risk for cardiovascular events[9]. Moreover, sub-analysis of the TNT study showed that the incidence of cardiovascular events tended to increase even in patients with HDL-C levels below 42 mg/dL, who had LDL-C levels below 70 mg/dL while receiving statin treatment[8]. These findings merit close investigation because it was unclear whether the changes were induced by low HDL-C levels in the subjects or by an insufficient HDL-C-elevating effect of the statins; however, several statin intervention studies have revealed the regression of coronary artery plaque volume to be related to the HDL-C-elevating effect of statins. The study of pravastatin showed that the decrease in plaque volumes correlated significantly with increased HDL-C levels[10]. The COSMOS study, in which rosuvastatin was administered to patients with stable CHD, showed that plaque regression occurred in 60% of patients, regardless of their LDL-C levels and that there was a significant correlation between changes in HDL-C levels, and changes in plaque volumes, suggesting that a rise in HDL-C level leads to regression of coronary artery plaques[11]. In the J-LIT study, the incidence of coronary events was decreased by 37.5% for primary prevention and 28.3% for secondary prevention, respectively, with each HDL-C elevation of 10 mg/dL[12, 13]. Therefore, the HDL-C-elevating effect of statins is expected to lead to the regression of coronary artery plaque volumes and prevention of coronary events.

HDL-C-Elevating Effects Differ Among Statins

Relationships between Statin doses and HDL-C-Elevating Effects

Fig. 2 summarizes the statin doses and HDL-C-elevating effects. The LDL-C-lowering effect, the main action of statins, is dose-dependent[14, 15]. Strong statins, including atorvastatin, pitavastatin and rosuvastatin, can reduce LDL-C levels by 40% or more[14-16]. On the other hand, statins that produce a greater percent change in LDL-C do not necessarily show a greater increase in HDL-C levels[17, 18]. Moreover, the dose-dependent effect is not apparent in the rise in HDL-C

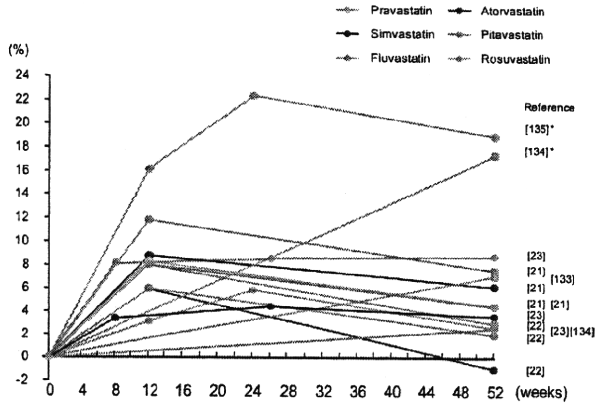

Fig. 3. Effects of each statin on HDL-C levels with long-term treatment.

Percent changes in HDL-C during 52-week treatment with each statin. Pale blue, pravastatin (Pra); Violet, simvastatin (Sim); Pale violet, fluvastatin (Flu); Dark blue, atorvastatin (Ato); Lime green, pitavastatin (Pit); Light blue, rosuvastatin (Ros). *: Changes in HDL-C levels of hypo-HDL cholesterolemic patients (HDL-C <40 mg/dL)

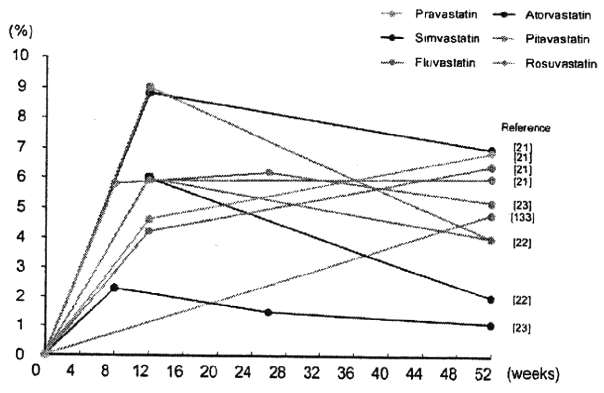

Fig. 4. Effects of each statin on ApoA-I levels with long-term treatment.

Percent changes in ApoA-I during 52-week treatment with each statin. Pale blue, pravastatin (Pra); Violet, simvastatin (Sim); Pale violet, fluvastatin (Flu); Dark blue, atorvastatin (Ato); Lime green, pitavastatin (Pit); Light blue, rosuvastatin (Ros)

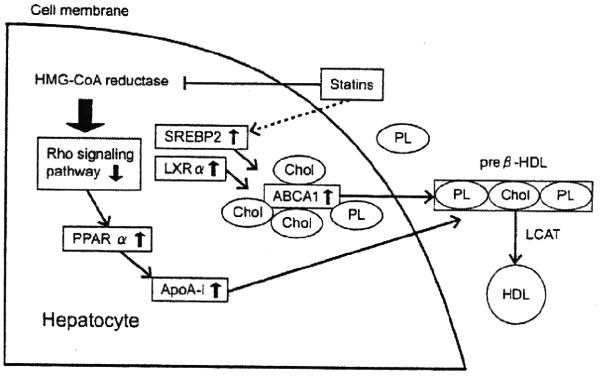

Fig. 5. Mechanisms of ApoA-1 and liver-type ABCA1 up-regulation by statins.

Statins inhibit HMG-CoA reductase in hepatocytes to suppress Rho signals and increase ApoA-I through activation of PPAR α. Statins also elevate ABCA1 expression via activation of SREBP-2 and LXR. Subsequently, pre β-HDL forms from cholesterol and phospholipids extracted from ABCA1 by ApoA-I, and enlarges into HDL via the actions of LCAT.

Abbreviations: ApoA-I, Apolipoprotein A-I; ABCA1, ATP-binding cassette transporter A1; Chol, cholesterol; LXR, liver-X receptor; PL, phospholipids; PPAR α, peroxisome proliferator-activated receptor alpha; SREBP2, sterol regulatory element-binding protein 2.

levels. There is a report suggesting that atorvastatin at a higher dose has a less potent HDL-C-elevating effect[19, 20]. The degrees of HDL-C-elevating effects differ among the types and doses of statins.

Changes Over Time in HDL-C- and ApoA-I-Elevating Effects with Long-Term Statin Treatment

Fig. 3 and 4 summarize the percent changes in HDL-C and ApoA-I levels with long-term statin treatment. A study that compared rosuvastatin, pravastatin and simvastatin showed no significant differences in the effects of these statins on HDL-C and ApoA-I levels[21]. In contrast, rosuvastatin 10 mg/day showed a greater HDL-C-elevating effect than atorvastatin 10 mg/day at 52 weeks[22]. Pitavastatin 2 mg/day had significantly greater HDL-C- and ApoA-I-elevating effects than atorvastatin 10 mg/day[23]. Pitavastatin, even as a replacement for other statins, significantly increases HDL-C levels and maintains the effect for a long time[24, 25]. These differences may be associated with the differing effects of statins on various factors involved in HDL metabolism.

HDL Metabolism and the Influences of Statins

Production of HDL in the Liver

1) ApoA-I

ApoA-I, a main component protein of HDL, is synthesized in the liver and intestine and by the catabolism of mature HDL, and serves as the starting point of RCT. Administration of statins increases ApoA-I levels[23, 26]. Statins raise the amount of ApoA-I mRNA via suppression of the Rho signalling pathway and activation of peroxisome proliferator-activated receptor alpha (PPAR α) in HepG2 cells[27] (**Fig. 5**). Comparisons among simvastatin, atorvastatin and pitavas-

Table 1. Estimated distributions of statins in the liver

	Lovastatin	Rosuvastatin	Cerivastatin	Pravastatin	Atorvastatin	Pitavastatin	Simvastatin	Fluvastatin
Cmax (ng/mL)	2.7	19	3.2	45-66	13-67	41	6.9	200-440
Statin dose (mg)	40	40	0.3	40	40	2	40	40
Estimated Cmax (nM)[§]	6.7	39.5	6.9	106.2-155.8	23.3-120.1	97.6	164.8	487.2-1,072.0
Liver/plasma Distribution[§§]	-	25.0	173.7	21.5	108.7	53.9	38.9	29.5
Estimated human liver distribution (μM)[§§§]	-	1.0	1.2	2.3-3.3	2.5-13.1	5.3	6.4	14.4-31.6
T1/2 (hour)	-	20	3.2	1.8-2.0	7.8-21	13	3.5	0.8-2.4

Abbreviations: Cmax, human maximum concentration; T1/2, half-life.
§: Estimated Cmax (nM) was calculated from Cmax (ng/mL) and statin dose in reference 136.
§§: Liver/plasma distribution are calculated from reference 137-143.
§§§: Estimated human liver distributions were calculated from human blood levels and using liver/plasma ratios in rats.

tatin showed the lowest dose of pitavastatin to increase ApoA-I secretion from HepG2 cells[28]. The estimated distributions of each statin in the liver were 6.4 μM for simvastatin, 2.5 to 13.1 μM for atorvastatin and 5.3 μM for pitavastatin (**Table 1**). Pitavastatin was the only statin affecting ApoA-I secretion at a clinically used dose in the study by Maejima *et al.*[28]. This difference is assumed to be one of the explanations for HDL-C-elevating effects differing among statins.

2) ABCA1 in the Liver

ABCA1 in the liver transports cholesterols within cells to ApoA-I to form pre-β HDL. Statins increase the amount of ABCA1 mRNA in HepG2 cells[28, 29]. Although the mechanisms by which ABCA1 increases are not completely understood, pravastatin may activate ABCA1 gene expression in the liver via sterol regulatory element-binding protein 2 (SREBP-2) and the liver X receptor (LXR)[30] (**Fig. 5**).

3) LCAT

LCAT is involved in the maturation of pre-β HDL to HDL via the esterification of free cholesterol of pre-β HDL[31]. The influences of statins on LCAT activities are still controversial[32-40].

Cholesterol Efflux from Peripheral Cells

1) ABCA1 in Macrophages

ABCA1 in peripheral cells such as macrophages not only transports cholesterol within cells to ApoA-I to form pre-β HDL, but is also involved in cholesterol efflux from peripheral cells. A study showed that statins elevate the expressions of ABCA1 and ATP-binding cassette transporter G1 (ABCG1)[41], while other studies have found different effects[42-45]. The effects of statins on ABC transporters are assumed to change depending on the cholesterol content stored in cells[45].

2) ABCG1

ABCG1 in peripheral cells, such as macrophages, binds to HDL and pumps out cholesterol. Similar to ABCA1, the influences of statins on ABCG1 are controversial[41-45].

3) Peripheral Cell-Type Scavenger Receptor Class B Type I (SR-BI)

Although SR-BI expressed in peripheral cells, such as macrophages, recognizes ApoA-I to form HDL particles, it is unclear whether this is clinically involved in cholesterol efflux; however, the amount of SR-BI expression reportedly correlates with the amount of cholesterol efflux[46]. SR-BI may be one of the molecules determining HDL-C levels. Statins stimulate cholesterol efflux by increasing SR-BI expression in peripheral cells[47-49]. Since the regulation of SR-BI expression involves SREBP-1[50], statins are assumed to regulate SR-BI expression via SREBP-1.

Few clinical results have been obtained on cholesterol efflux from peripheral cells before versus after administration of statins. It has only been reported that CE efflux capacity from macrophages was stable in an investigation of serum before and after simvastatin administration in patients with type 1 diabetes mellitus. In that study, HDL-C levels were elevated by simvastatin, pre-β HDL levels tended to decrease and CETP activity was reduced[51]. Further evaluation of CE efflux with statin treatment is therefore needed.

Transport of CE in HDL to the Liver

1) CETP

CETP facilitates the operation of the atherosclerosis prevention system via HDL and ApoA-I. Mature HDL produced by repeated cholesterol efflux and

conversion to CE is indirectly returned to the liver by LDL receptors after CE in HDL is transported to ApoB-containing particles (VLDL, IDL and LDL) by CETP. Many studies have shown that statins inhibit CETP mass and activities[51-55]. CETP gene expression is regulated by LXR[56] and reacts to changes in the cholesterol amount in cells along with plasma cholesterol levels[57]; therefore, suppression of cholesterol synthesis and decreased cholesterol amounts in cells may reduce LXR expression, leading to decreased gene expression of CETP; however, another study showed decreased CETP activity without reduced CETP mass[58], suggesting that statins have a direct inhibitory action on CETP activity. On the other hand, statins significantly decrease ApoB-containing lipoproteins and TG. Thus, decreased CETP activities may only reflect decreased lipoproteins that are substrates of CETP.

2) SR-BI in the Liver

CE in HDL is directly returned to the liver via SR-BI in the liver that recognizes ApoA-I to selectively take up CE. Atorvastatin did not significantly change SR-BI expression in the livers of dogs and mice[33, 59] and there are no reports on the effects of other statins on SR-BI expression.

Enzymes Involved in Catabolism of TG-Rich Lipoproteins and HDL

1) Lipoprotein Lipase (LPL)

LPL is produced in adipose tissue, skeletal muscles and the heart, and hydrolyzes TG-rich lipoproteins in blood on the surfaces of vascular endothelial cells to produce HDL[60]. A rise in LPL activities due to genetic factors is known to raise HDL-C levels[61, 62]. A clinical study showed that atorvastatin, simvastatin and pravastatin increased LPL mass or activities in patients with type II diabetes mellitus[63-65]. Basic research using 3T3-L1 preadipocytes revealed that simvastatin and pitavastatin elevated LPL activities[66]. It has been proposed, as a mechanism, that enhanced expressions of PPAR α and peroxisome proliferator-activated receptor gamma (PPAR γ) by statins increase the expressions of LPL mRNA and proteins[63].

2) Hepatic Lipase (HL)

HL hydrolyzes phospholipids and TGs to convert HDL2 into more dense HDL3[67, 68]. Atorvastatin dose-dependently shows HL activity-lowering effects exerted via an unknown mechanism (−11% at a dose of 10 mg/day and −22% at a dose of 80 mg/day)[69]. Thus, cholesterol in the HDL2 fraction may rise.

3) Endothelial Lipase (EL)

EL has phospholipase activity[70, 71] and hydrolyzes phospholipids in HDL particles[72]. HDL with decreased phospholipids is prone to decomposition and is metabolized, resulting in decreased HDL via the action of EL. EL is one of the factors promoting HDL catabolism. Pitavastatin decreases the serum EL mass through isoprenylation and suppression of RhoA[73]. Atorvastatin and simvastatin also suppress EL expression in macrophages[74, 75].

Other Factors Involved in RCT

1) Adiponectin

Adiponectin is one of the important molecules, secreted from adipocytes, that inhibits the progression of atherosclerosis. Adiponectin levels correlate positively with HDL-C levels[76]. Adiponectin promotes HDL production via ApoA-I production and increased ABCA1 expression in the liver[77, 78], and also in macrophages to activate RCT[79]. Pitavastatin and pravastatin elevate adiponectin expression in adipocytes[80]. Clinically, the adiponectin-elevating effects differ among statins[81-84], and pitavastatin reportedly increases adiponectin levels in hyperlipidemic patients with diabetes mellitus. Suppression of reactive oxygen species (ROS) production, and activation of SREBP1c and PPAR γ have been proposed as possible mechanisms[85].

Taken together, statins influence a variety of molecules involved in HDL metabolism and RCT. Thus, the effects of statins are illustrated and summarized in **Fig. 6**.

Effects of Statins on Properties and Functions of HDL Particles

Functional HDL and Dysfunctional HDL

As described above, HDL-C elevation by statins provides good clinical results; however, for some drugs, HDL-C elevation does not necessarily lead to a clinical benefit. Large-scale clinical studies, such as ILLUMINATE[86] and ILLUSTRATE[87], were conducted to prevent the occurrence and progression of atherosclerosis by raising HDL-C levels with the use of a CETP inhibitor, torcetrapib. Neither study showed inhibitory effects on atherosclerosis progression in coronary and carotid arteries. In fact, these studies were discontinued after only 1 year due to a significant increase in deaths from any cause, including cardiovascular death. It has been proposed that elevated blood pressure caused by increased aldosterone, induced during torcetrapib administration, was responsible for this excess mortality[88]. Other CETP

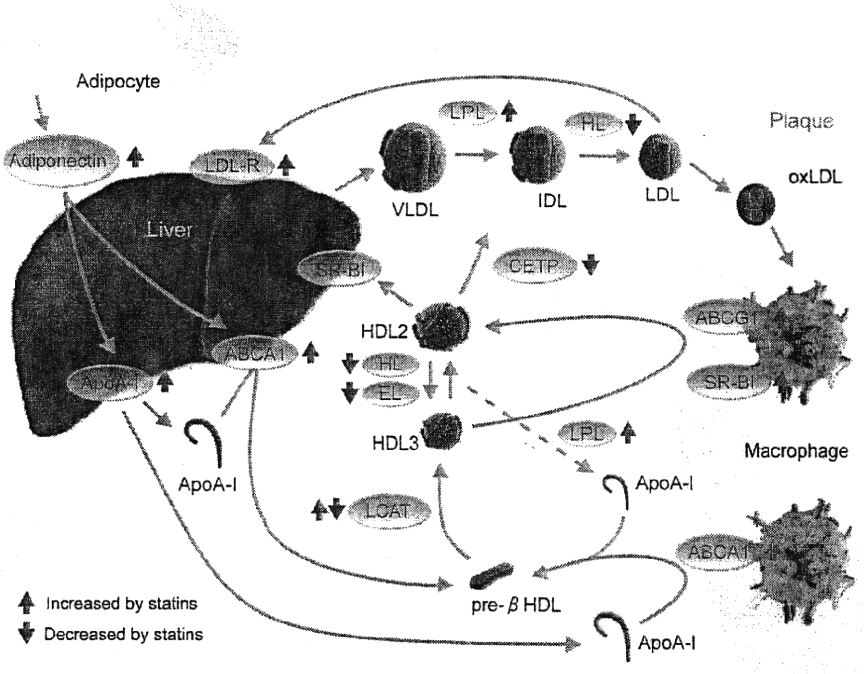

Fig. 6. Effects of statins on reverse cholesterol transport.

ApoA-I, a main component protein of HDL, is produced mainly in the liver. ApoA-I receives cholesterols and phospholipids through ABCA1 on the surfaces of hepatocytes and macrophages to form preβ-HDL. Adiponectin is secreted from adipocytes and promotes HDL production by increasing ApoA-I production and ABCA1 expression. Cholesterol present on preβ-HDL is esterified by LCAT to form a spherical HDL particle (HDL3). HDL3 pumps cholesterols via ABCG1 and SR-BI on macrophages, resulting in a large particle (HDL2). HDL2 is returned to HDL3, undergoing hydrolysis of TG and phospholipids by HL and EL. A spherical HDL particle occasionally supplies ApoA-I through catabolism by LPL. Cholesterol ester in HDL is transported to VLDL and LDL by CETP, and VLDL and LDL are decomposed by LPL and HL to finally be transported to the liver via LDL receptors. HDL2 is also taken up by the liver directly via liver-type SR-BI. Red arrows represent factors increased by statins and blue arrows factors reduced by statins.

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G1; ApoA-I, apolipoprotein A-I; CETP, cholesteryl ester transfer protein; EL, endothelial lipase; HL, hepatic lipase; LPL, lipoprotein lipase; SR-BI, scavenger receptor class B type I; TG, triglycerides.

inhibitors under development are reported to have no blood pressure-elevating effect[88]. It is unclear whether this result was attributable to a class effect of CETP inhibitors or an adverse effect specific to torcetrapib[86, 87]; however, these studies revealed not only a significant decrease in LDL-C levels but also a marked increase in HDL-C levels, suggesting that HDL-C elevation does not necessarily provide an anti-atherosclerotic effect. Recently, the prospective Framingham survey showed that the low plasma CETP activity group had a greater increased risk for cardiovascular disease than the high CETP activity group[89]; therefore, it cannot be ruled out that the mechanism of CETP inhibition may raise the risk for cardiovascular events, despite increasing HDL-C levels[90-92].

Probucol[93], known as a lipid-lowering drug, is also a potent antioxidant and possesses antiatherogenic capability, despite decreasing the plasma HDL-C level. We first reported that probucol treatment resulted in the regression of Achilles tendon xanthoma in patients with familial hypercholesterolemia (FH)[94]. We also demonstrated a positive correlation between the decrease in the plasma HDL-C level and the reduction rate of Achilles tendon xanthoma in patients with heterozygous FH. Probucol treatment reduced HDL particle size, inducing the promotion of cholesterol efflux from cells[95], and enhanced hepatic SR-BI expression in rabbits and a human hepatoma cell line[96], and

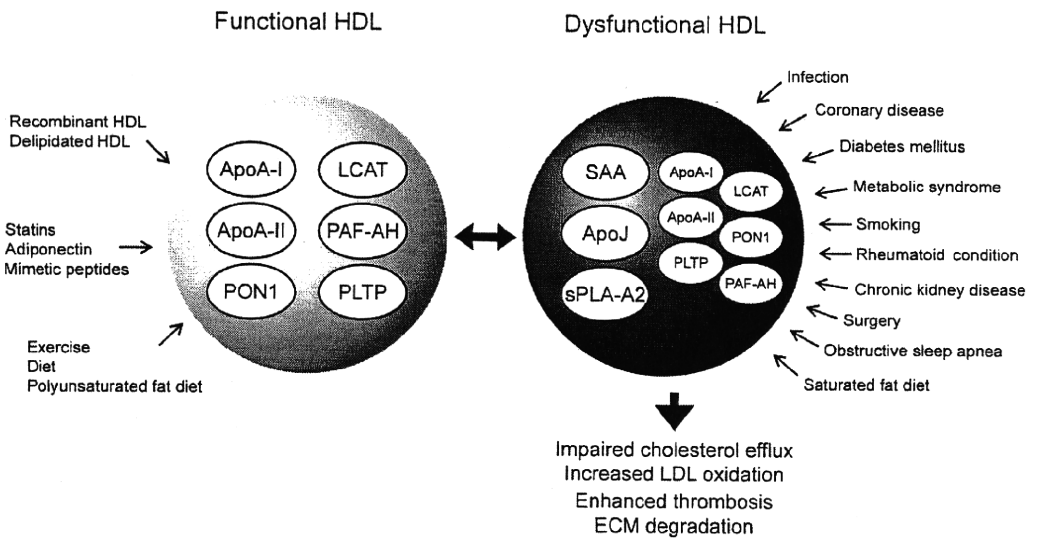

Fig. 7. Functional and dysfunctional HDL.

A model of functional HDL and dysfunctional HDL. Functional HDL exhibits anti-atherosclerotic actions involving ApoA-I, ApoA-II, LCAT, and PTLP that promote reverse cholesterol transport, PON1 with an anti-oxidative action and PAF-AH involving vascular adhesion molecules. However, when HDL is modified through inflammation or oxidation, the factors described above are reduced or inflammatory SAA, ApoJ and sPLA-A2 are elevated, leading to a change from functional to dysfunctional HDL, inducing arteriosclerosis via abnormal reverse cholesterol transport and increased oxidized LDL. Narrow arrows show various factors that affect HDL to be functional or dysfunctional.

Abbreviations: ApoA-I, apolipoprotein A-I; ApoA-II, apolipoprotein A-II; ApoJ, apolipoprotein J; ECM, extracellular matrix; PAF-AH, platelet-activating factor acetylhydrolase; PLTP, phospholipid transfer protein; PON1, paraoxonase 1; SAA, serum amyloid A; sPLA-A2, secretory phospholipase A2.

increased CETP activity[95], both leading to the acceleration of reverse cholesterol transport. To further assess the clinical efficacy of probucol treatment in clinical settings, the POSITIVE study (Probucol observational study illuminating therapeutic impact on vascular events) was performed, assessing whether long-term probucol treatment was associated with a lowered risk of cardiovascular events in a very high-risk population of patients with FH in Japan[97]. The study cohort included 410 patients with heterozygous FH. Probucol significantly lowered the event risk in the secondary prevention group, although not significant in primary prevention. These results suggest that long-term probucol treatment may prevent secondary cardiovascular events in a higher cardiovascular risk population of heterozygous FH, despite the reduction of HDL-C levels.

HDL particles exert various effects depending on their properties[6, 7, 98-100]. Ordinary HDL is "functional HDL" which exerts an anti-atherosclerotic effect through the inhibition of phospholipid oxidation in LDL[7] and down-regulates the expression of inflammatory cytokines and vascular adhesion molecules[101] in addition to enhancing RCT[4]; however, various factors change the properties of HDL particles. When the content of inflammatory serum amyloid A (SAA) and ApoJ increases in HDL particles, while ApoA-I and ApoA-II regulating RCT and paraoxonase with anti-oxidative action decrease, HDL particles are modified from "functional HDL" to "dysfunctional HDL" (**Fig. 7**). The factors involved in this transformation are speculated to be various complications, including infections[102], heart disease[103], diabetes mellitus[104], metabolic syndrome[105] and chronic renal disease[106]. Unlike CETP inhibitors, if the HDL elevating effect of statins prevents CHD occurrence, this suggests that statins affect HDL to function in an anti-atherosclerotic manner.

Effect of Statins on HDL Functions

1) RCT

The ability of HDL to efflux cholesterol from foam cells and transport CE back to the liver is the main function of HDL in the RCT system. Excessive expression of SR-BI in the liver suppresses atherosclerosis as well as reducing HDL-C levels[107], whereas

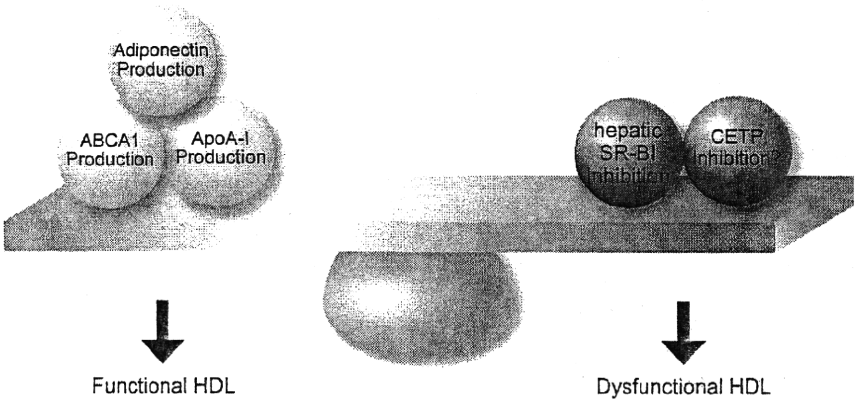

Fig. 8. Mechanisms underlying increases in functional and dysfunctional HDL by statins.

Statins elevate preβ-HDL-producing ability via the production of ApoA-I, ABCA1 and adiponectin to increase functional HDL. On the other hand, statins may produce dysfunctional HDL by CETP inhibition. Whether HDL produced by a statin is functional is determined by a balance between CETP inhibitory ability and pre-β-HDL-producing ability.
Abbreviations: ApoA-I, apolipoprotein A-I; ABCA1, ATP-binding cassette transporter A1; CETP, cholesteryl ester transfer protein.

atherosclerosis is enhanced in SR-BI knock-out mice[108], suggesting that inhibition of SR-BI in the liver results in RCT inactivation despite a marked increase in HDL-C levels. Debate about RCT functionality has focused mainly on CETP. HDL in patients with CETP deficiency has a decreased ability to extract CE from macrophages[109]; however, it was reported that a system containing whole serum from CETP-deficient patients for incubation showed an increased ability to extract CE[110]. Another study showed that macrophages with excessive ABCG1 expression had an increased ability to efflux cholesterol, even in HDL of CETP-deficient patients[111]. Additionally, patients with low CETP activity have an increased incidence of cardiovascular events[89], while another study found that the higher the serum CETP levels in patients, the more frequently they experienced calcification of coronary arteries and IMT thickening of the carotid arteries[112]; therefore, the relationship between CETP and RCT functionality is still controversial.

Because statins generally inhibit CETP activity[51-55], the ability to produce pre-β HDL by ApoA-I and ABCA1 is important for RCT activation (**Fig. 8**). Clinical results demonstrated that atorvastatin did not change the production rate (PR) or fractional catabolic rate (FCR) of ApoA-I[113]. Administration of atorvastatin to mice did not significantly change ApoA-I expression in the liver and reduced ABCA1 and CETP expressions in the liver, indicating that the HDL-elevating effect of atorvastatin may be mediated mainly by CETP inhibition[59]. Administration of rosuvastatin did not change ApoA-I, but significantly reduced the PR of ApoA-I and the FCR of ApoA-I and HDL particles that contain ApoA-I. This result suggested that rosuvastatin also raises HDL-C levels via CETP inhibition[114]. Rosuvastatin reportedly did not change pre-β HDL concentrations and reduced the activity and mass of CETP by decreasing the ability to efflux cholesterol from macrophages to plasma[55]. On the other hand, pravastatin elevated ApoA-I concentrations by increasing FCR and PR, although it inhibited CETP[115]. The ApoA-I synthesis rate and FCR by pitavastatin have not been clinically investigated; however, pitavastatin even at a low concentration (equivalent to a clinical blood concentration) promoted ApoA-I secretion and ABCA1 expression in HepG2 cells[28], and also clinically promoted maturation from pre-β HDL to HDL. Therefore, pitavastatin may be expected to exhibit an anti-atherosclerotic effect via RCT activation[53].

2) Anti-Inflammatory Action

As to the anti-inflammatory function of HDL, simvastatin treatment slowly improved the anti-inflammatory ability of HDL, as assessed by cell-free assay (CFA) in patients with CHD[103]. Administration of atorvastatin to patients with rheumatoid arthritis significantly reduced the anti-inflammatory ability of HDL[116]. Additionally, atorvastatin was reported to have a more potent blood SAA-lowering effect than simvastatin[117].

Table 2. Comparisons of kinetics and functions of HDL among statins

	HDL Production			HDL Catabolism			Anti-oxidation		Anti-Inflammation	
	ApoA-I/hepatic ABCA1		ApoA-I PR	CETP		ApoA-I FCR	PON1		HDL properties (CFA)/SAA	
	Basic (expression)	Clinical (concentration)	Clinical	Basic (expression)	Clinical (mass or activity)	Clinical	Basic (expression or activity)	Clinical (activity)	Basic	Clinical
Pravastatin	ABCA1 → [29]	ApoA-I ↑ [115]	↑ [115]	Unknown	↓ [149]	↑ [115]	→ [150] ↓ [151]	Unknown	Unknown	Unknown
Simvastatin	ApoA-I ↑ [28][155]	ApoA-I ↑ [154]	↑ [154]	Unknown	↓ [51]	→ [154]	↑ [127] ↓ [151]	↑ [125][127][153] → [129][152]	Unknown	CFA ↓ [103] SAA ↓ [117]
Fluvastatin	Unknown	ApoA-I ↑ [156]	Unknown	Unknown	Unknown	Unknown	↓ [150][151]	↑ [125]	Unknown	Unknown
Atorvastatin	ApoA-I ↑ [28] → [59] ABCA1 → [28] ↓ [59]	ApoA-I ↑ [145] → [113][144] ABCA1 → [144]	→ [113]	↓ [59]	↓ [54]	→ [113]	↑ [126]	↑ [125][126][129][148] → [128]	Unknown	CFA ↓ [116] SAA ↓ [117]
Pitavastatin	ApoA-I ↑ [28] ABCA1 ↑ [28]	ApoA-I ↑ [145]	Unknown	Unknown	↓ [53]	Unknown	↑ [124]	Unknown	Unknown	Unknown
Rosuvastatin	ApoA-I ↑ [146]	ApoA-I → [114][147]	↓ [114][147]	Unknown	↓ [53]	↓ [114][147]	Unknown	↑ [128]	Unknown	Unknown

Abbreviations: ApoA-I, apolipoprotein A-I; ABCA1, ATP-binding cassette transporter A1; CETP, cholesteryl ester transfer protein; CFA, cell-free assay; FCR, fractional catabolic rate; HDL, high density lipoprotein; PON1, paraoxonase 1; PR, production rate; SAA, serum amyloid A.

3) Anti-Oxidative Action

Statins may improve the anti-oxidative action of HDL via paraoxonase 1 (PON1). PON1 is an enzyme hydrolyzing peroxidized lipids, which is synthesized in the liver and is present in blood bound to ApoA-I of HDL particles[118]. PON1 exerts its anti-oxidative action by inhibiting the oxidation of LDL and even HDL itself, depending on the degree of its activity[119-122]. Pitavastatin was shown to increase the promoter activity and protein expression of PON1 *in vitro*. As for the mechanism, pitavastatin was revealed to promote PON1 expression via p44/42 MAP kinase-mediated phosphorylation of SREBP-2 and binding of Sp1 to PON1 DNA[123, 124]. The increased PON1 activity was clinically confirmed with the administration of fluvastatin, simvastatin, atorvastatin and rosuvastatin[125-128]. In a comparative study of simvastatin and atorvastatin, atorvastatin was superior in terms of increasing PON1 activity[129]. A study comparing atorvastatin with rosuvastatin showed no significant difference in changes in PON1 activity between the two, but rosuvastatin significantly increased PON1 activity after treatment[128].

4) Vascular Endothelial Function-Improving Action

Many sphingosine 1-phosphates (S1Ps) that activate endothelial nitric oxide synthase (eNOS) in vascular endothelial cells are also present in HDLs. S1P is thought to play an important role in the reactions of HDL to endothelial functions because S1P activates eNOS in vascular endothelial cells via S1P1 receptors[130]. Pitavastatin reportedly increased S1P1 receptor expression in cultured bovine vascular endothelial cells and also promoted eNOS phosphorylation induced by HDL[131]. Thus, statins may enhance vascular endothelial function through the activation of eNOS by S1Ps bound to HDLs.

Table 2 summarizes the effects of statins on HDL functions, including RCT, anti-inflammatory, anti-oxidative, and improving actions of vascular endothelial functions.

Conclusions

The HDL-C-elevating effects of statins differ among statins, due to differences in statin effects on various factors involved in HDL metabolism. On the other hand, studies should focus on HDL functionality as well as HDL-C levels. There are both functional and dysfunctional HDL molecules and it is important to promote functional HDL activities, including RCT, anti-oxidative and anti-inflammatory actions. It is possible that CETP inhibition may inactivate RCT and increase dysfunctional HDL. Whether HDL pro-

duced by statins is functional is determined by a balance between the ability of HDL neogenesis and CE transport activity into the liver. Although statins generally inhibit CETP, some statins that have a high ability to produce ApoA-I and ABCA1 in the liver are expected to exhibit RCT-mediated anti-atherosclerotic actions. Furthermore, statins may improve anti-inflammatory and anti-oxidative actions of HDL. Elevation of HDL-C by statin treatment may simultaneously improve HDL functionality, resulting in the prevention of coronary artery plaque progression and the occurrence of CHD. In the future, the effects of each statin treatment on HDL functions should be investigated in more detail to allow statin selection based on differences in their effects in clinical practice.

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Review

Current Therapy for Patients with Sitosterolemia–Effect of Ezetimibe on Plant Sterol Metabolism

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Sitosterolemia is a rare, autosomal recessive inherited sterol storage disease associated with high tissue and serum plant sterol concentrations, caused by mutations in the adenosine triphosphate-binding cassette (ABC) transporter ABCG5 or ABCG8 genes. Markedly increased serum concentration of plant sterols. such as sitosterol and campesterol, cause premature atherosclerosis and massive xanthomas. Hitherto known treatments for sitosterolemia, including a low-sterol diet, bile-salt binding resins, ileal bypass surgery and low density lipoprotein (LDL) apheresis have not yielded sufficient reduction of serum plant sterol levels and many patients show a sustained elevation of plant sterol levels, subsequently developing premature atherosclerotic cardiovascular diseases.
Ezetimibe, an inhibitor of intestinal cholesterol absorption through its binding to Niemann-Pick C1-like 1 (NPC1L1), has been widely used for decreasing serum LDL-cholesterol levels in patients with hypercholesterolemia. Ezetimibe also reduces the gastrointestinal absorption of plant sterols, thereby also lowering the serum concentrations of plant sterols. This pharmacological property of ezetimibe shows its potential as a novel effective therapy for sitosterolemia. In the current review, we discuss the current therapy for patients with sitosterolemia and present two Japanese adolescent patients with this disease, one of whom underwent percutaneous coronary intervention for accelerated coronary atherosclerosis. Ezetimibe administration in addition to conventional drug therapy successfully reduced serum sitosterol levels by 51.3% and 48.9%, respectively, in the two patients, demonstrating ezetimibe as a novel and potent treatment agent for sitosterolemia that could work additively with conventional drug therapy.

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Key words; Sitosterolemia, ABCG5, ABCG8, Plant sterol, Xanthoma, NPC1L1, Ezetimibe

Introduction

Sitosterolemia is a very rare autosomal recessive inherited disease associated with marked increase of the serum and tissue concentrations of plant sterols, such as sitosterol and campesterol[1-3]. Sitosterolemia was first described in two sisters by Bhattacharyya and Connor in 1974[4]. Only 45 patients with sitosterolemia have been reported worldwide until date in the medical literature[5]. Sitosterolemia has been mapped to a special gene locus on human chromosome 2p21, involving two genes, the adenosine triphosphate-binding cassette (ABC) transporters ABCG5 and ABCG8[6-8]. ABCG5 and ABCG8 are half- transporters that form heterodimers, one of which is defective in patients with sitosterolemia. Interestingly, no cases have been identified with concurrent mutations in both transporter sequences. ABCG5 and ABCG8 are expressed at the apical membranes of intestinal mucosal cells and the bile canalicular membrane of hepatocytes, and regulate the outbound secretion of sterols. Normally, plant sterols are poorly absorbed from the gastrointestinal tract; fewer than 5% of plant sterols

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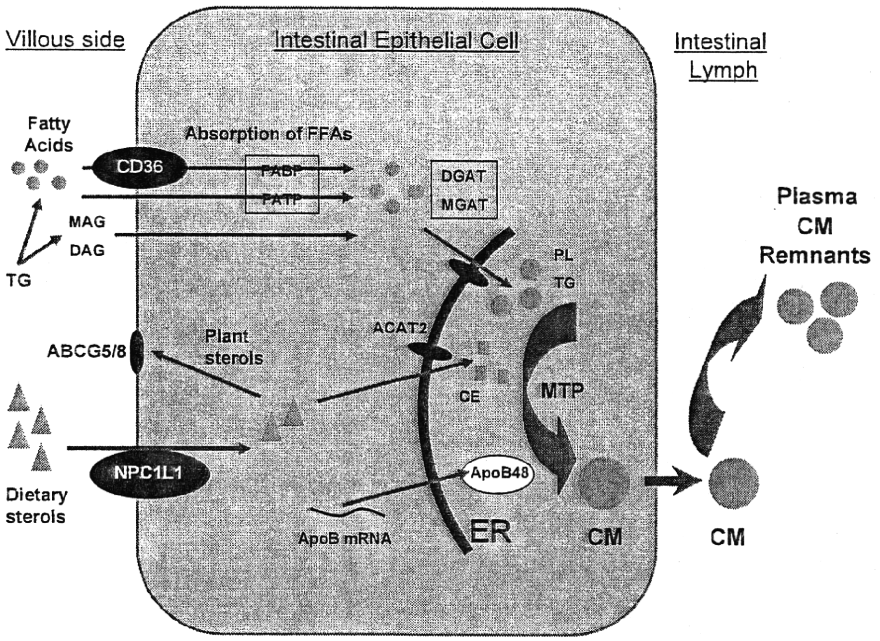

Fig. 1. Molecular mechanisms of cholesterol and plant sterol absorption.

CE: Cholesteryl Esters, CM: Chylomicrons, FFA: Free Fatty Acid, TG: Triglycerides, MAG: Monoacylglycerol, DAG: Diacylglycerol, MGAT: Monoacylglycerol acyltransferase, DGAT: Diacylglycerol acyltransferase, MTP: Microsomal triglyceride transfer protein, ACAT2: Acyl-CoA: cholesterol acyltransferase-2, FABP: Fatty acid binding protein, FATP: Fatty acid transport protein.

are absorbed as compared with approximately 40% of cholesterol. In contrast, the absorption of sitosterol is increased by up to 15–63% in patients with sitosterolemia[9-11].

As shown in **Fig. 1**, after absorption of sterols by the intestinal epithelium via NPC1L1, cholesterol is esterified by acyl-CoA: cholesterol acyltransferase-2 (ACAT-2) and both cholesteryl esters and triglycerides are assembled with apolipoprotein B-48 by microsomal triglyceride transfer protein (MTP) to form chylomicrons. Chylomicrons are then secreted into the intestinal lymph to enter the systemic circulation. Excess cholesterol and plant sterols are re-secreted into the intestinal lumen for excretion by ABCG5 and ABCG8. Deficiency of these transporters impairs plant sterol excretion from the enterocytes and hepatocytes, resulting in the accumulation of plant sterols in the plasma and tissues. Sitosterolemia is clinically characterized by massive xanthomatosis and premature atherosclerosis, which often causes the early onset of cardiovascular diseases and fatal myocardial infarction[12-17]. Some patients with sitosterolemia also exhibit other clinical findings, including hemolysis, platelet abnormalities and hypersplenism[18-20]. Increased amounts of plant sterols, particularly sitosterol, stigmasterol, campesterol, and their 5-alpha derivatives are deposited on the arterial walls and in xanthomas in patients with sitosterolemia[21]. The unusually high content of plant sterols in the circulatory lipoproteins has been postulated to possibly promote the deposition of these sterols in the arterial walls[22-25]. Thus, treatment to reduce serum plant sterol levels in patients with sitosterolemia is crucial for preventing life-threatening cardiovascular events. To date, dietary, pharmacological and surgical therapies have been used to lower serum plant sterol levels. In the current paper, we review the relation of plant sterols to cardiovascular disease and briefly summarize the current therapy for patients with sitosterolemia, especially the effect of the intestinal cholesterol absorption inhibitor, ezetimibe, on plant sterol levels.

Plant Sterols and Cardiovascular Diseases

Patients with sitosterolemia often have hypercholesterolemia. The reason suggested by Mi-Hye Lee *et al.* is that the net effect of the complete loss of function of either 'sterolin', two genes transcribed in opposite directions and encoding ABCG5 and ABCG8, results in increased cholesterol and sitosterol absorp-