1 Introduction

Intestinal absorption of cholesterol and plant sterols is regulated by various factors. Solubility in bile salt micelles is thought to be an essential factor affecting the intestinal absorption of cholesterol and plant sterols. Another important factor in the intestinal lumen is the releasability of monomer sterols from bile salt micelles before their incorporation into brush border membranes of intestinal epithelial cells. The affinity of sterols for bile salt micelles determines their releasability from these micelles. After sterols are released as a monomer, they are incorporated into intestinal epithelial cells through brush border membranes. Previously, it was thought that incorporation into intestinal cells was a passive diffusion process. Recently, it has become evident that the Niemann Pick C1 Like 1 (NPC1L1) protein has an important role in the incorporation of sterols. After incorporation of sterols into intestinal epithelial cells, it is thought that a proportion of sterols is excreted through the ATP-binding cassette transporter (ABC) G5/ABCG8 of intestinal cells. It is well established that plant sterols inhibit intestinal absorption of cholesterol and exert a hypocholesterolemic activity. Plant sterols are solubilized in bile salt micelles as cholesterol. Our study clearly showed that because the sterol-solubilizing capacity of bile salt micelles was limited, plant sterols solubilized in micelles reduced the solubility of cholesterol. This can be the major cause of inhibition of cholesterol absorption by plant sterols. Pancreatic cholesterol esterase accelerates intestinal absorption of unesterified cholesterol. Although it was suggested that cholesterol esterase accelerated esterification of cholesterol incorporated into intestinal cells and acted as a transporter at the surface of intestinal cells, our research revealed that the accelerated cholesterol absorption was caused by hydrolysis of phosphatidylcholine in bile salt micelles. It is thought that hydrolysis of phosphatidylcholine reduces the affinity of cholesterol for the micelles and accelerates the incorporation of cholesterol released from the micelles into intestinal cells.

Key words: ABCG5/ABCG8, cholesterol, cholesterol esterase, intestinal absorption, plant sterols
2 Absorbability of cholesterol and plant sterols
2.1 Differential absorption of cholesterol and various plant sterols and stanols

Various plant sterols and stanols are present in plant products, such as vegetables, fruits, and plant oils (Fig. 1). Sitosterol, campesterol, stigmasterol, and brassicasterol are major plant sterols. Plant stanols are not major constituents of plant products, but they are obtained by hydrogenation of plant sterols. It has been well established that plant sterols and stanols have hypocholesterolemic activity by inhibiting cholesterol absorption in the intestine. Plant sterols and stanols are generally less absorptive than cholesterol. Figure 2 shows lymphatic transport of cholesterol and sitosterol in rats cannulated thoracic duct. The 24 h lymphatic recovery of radiolabeled cholesterol was about 10-fold higher than that of radiolabeled sitosterol. Figure 3 shows the 24 h lymphatic recovery of various plant sterols and sitostanol. The recovery of campesterol was highest, whilst those of stigmasterol and sitostanol were lowest, and those of brassicasterol and sitosterol were intermediate. The mechanisms responsible for differential absorption of these plant sterols and cholesterol are not fully understood.

2.2 Are ABCG5/ABCG8 determinants of differential absorption of plant sterols?

Phytosterolemia is a rare autosomal recessive disease that leads to the deposition of plant sterols and stanols in the body. In 2000 and 2001, ABCG5 and ABCG8 were
Factors Affecting Intestinal Absorption of Cholesterol and Plant Sterols and Stanols

identified as the genes responsible for phytosterolemia\(^8\text{-}^9\). \(ABCG5\) and \(ABCG8\) are half-size ABC transporters that, together, act as a heterodimer and are thought to be involved in the excretion of sterols from brush border membranes of intestinal epithelial cells into the intestinal lumen, and from the liver canalicular membranes to the bile\(^6\text{-}^9\). Therefore, it is possible that mutation(s) in \(ABCG5\) or \(ABCG8\) could impair the function of sterol excretion in the intestine and the liver, and therefore, induce plant sterol deposition. Yu et al. showed that fractional absorption of cholesterol was comparable between \(ABCG5/ABCG8\) knockout and wild-type mice, but the rates of campesterol and sitosterol absorption in \(ABCG5/ABCG8\) knockout mice was 2–3-fold higher than in the wild-type mice\(^7\). This observation suggests that the \(ABCG5/ABCG8\) transporter in the intestine preferentially excretes plant sterols. Generally, the rate of intestinal cholesterol absorption is higher than that of campesterol, which is higher than that of sitosterol in healthy animals and humans\(^4\). The rank order of absorption rates of these three sterols was maintained in \(ABCG5/ABCG8\) knockout mice and phytosterolemic patients\(^9\). These results suggest that the differential absorption observed between cholesterol, campesterol, and sitosterol does not depend on \(ABCG5/ABCG8\).

Igel et al. suggested that campesterol and sitosterol might be transiently incorporated into intestinal cells of mice, as is the case with cholesterol\(^2\). These authors administered plant oil containing deuterated cholesterol, campesterol, and sitosterol into mouse stomach \emph{in vivo}, and then incorporation of the sterols into the proximal jejunum was measured at 15, 30, 60, 120, and 240 min after administration. The ratio of incorporated sitosterol and campesterol to cholesterol was 0.73 and 0.90, respectively, at 15 min following which the ratios decreased rapidly. They suggested that sitosterol and campesterol are incorporated into intestinal cells as cholesterol at an early stage and then the former plant sterols are rapidly excreted from the cells through \(ABCG5/ABCG8\). They also observed that sitosterol was lost more rapidly from intestinal cells than was campesterol\(^11\), suggesting that the excretion of sitosterol through \(ABCG5/ABCG8\) is more rapid than that of campesterol. This can explain the differential absorption observed between cholesterol, campesterol, and sitosterol. We performed a similar experiment in Wistar rats, in which a bile salt micellar solution containing radiolabeled cholesterol and sitosterol was infused into the jejunal loop \emph{in situ}. The incorporation of radioactivity into the jejunal loop was measured at 5, 10, 20, 60, 120, and 240 min after the infusion of the micelles\(^9\). The incorporation of sitosterol into the jejunal loop was only one tenth to one fifth that of cholesterol at 5, 10, and 20 min after administration of micellar sterols into the loop. The ratio of sitosterol/cholesterol in the jejunal loop increased with time. In our study, the rapid incorporation of sitosterol into intestinal mucosal cells was not observed. Our results showed that incorporation of sitosterol into intestinal mucosa was considerably lower than that of cholesterol, and sitosterol was differentiated from cholesterol at the incorporation site of intestinal cells at an early stage of absorption. Cholesterol is quickly esterified, incorporated into chylomicrons in intestinal cells, and then secreted into the lymph fluid. In contrast, sitosterol is less esterified and less incorporated into chylomicrons and therefore, secretion to the lymph is delayed\(^5\). This means that incorporated sitosterol stays longer inside intestinal cells than cholesterol. Therefore, the observed increase in the ratio of sitosterol/cholesterol over time in intestinal mucosa may be due to the comparatively delayed secretion of sitosterol from the intestine to the lymph fluid. One reason for the inconsistencies between mice\(^12\) and rats\(^13\) might be due to differences in animal species and in the experimental conditions. However, it is unlikely that plant sterol absorption occurs by different mechanisms in mice and rats. Studies that are more precise are necessary in this respect.

2.3 Causes of deposition of plant sterols in stroke-prone spontaneously hypertensive rats (SHRSP)

Ratnayake et al. showed that stroke prone spontaneously hypertensive rats (SHRSP), which have been used worldwide as a model of hypertension with stroke, exhibited plant sterols deposited throughout the body\(^30\). We previously reported that WKY/NCrlCrlj, SHR/NCrlCrlj, and SHRSP/Sea animals deposited plant sterols in the body to a significantly greater extent compared with Sea:Wistar and WKA/Sea rats\(^31\). The SHRSP strain was genetically came...
from the SHR strain, which was derived from the WKY strain\textsuperscript{16, 17}. Lymphatic transport of radiolabeled sitosterol and cholesterol was higher in the SHRSP and WKY rats than in the WKA rats\textsuperscript{15}. Biliary secretion of plant sterols and cholesterol was lower in SHRSP than in WKA rats, although SHRSP animals had higher levels of plant sterols deposited in the liver than did WKA rats when fed on a high plant sterol diet\textsuperscript{15}. Therefore, we concluded that the deposition of plant sterols in SHRSP animals was due to the increased absorption and decreased biliary secretion of plant sterols\textsuperscript{15}.

In a subsequent study, Yu \textit{et al.} revealed that the WKY/NCrlCrlj, SHR/NCrlCrlj, and SHRSP/Sea strains had the same missense mutation, Gly583Cys, in \textit{Abcg5}\textsuperscript{19}. In a more recent study, we showed that SHRSP/Izm, but not WKY/Izm, had plant sterols deposited in the body\textsuperscript{19}. We revealed that SHRSP/Izm has the same mutation in \textit{Abcg5} as WKY/NCrlCrlj, SHR/NCrlCrlj, and SHRSP/Sea, whereas the mutation was not found in WKY/Izm rats\textsuperscript{19}. These observations strongly support the hypothesis that the mutation in \textit{Abcg5} may be a cause of phytosterolemia in WKY/NCrlCrlj, SHR/NCrlCrlj, SHRSP/Sea, and SHRSP/Izm animals. However, when we compared lymphatic absorption of plant sterols in the SHRSP/Izm and WKY/NCrlCrlj strains, which have a mutation in \textit{Abcg5}, with that in WKY/Izm, no significant difference in lymphatic absorption was observed among the three strains\textsuperscript{19}. Only a tendency of slightly higher absorption of sitosterol was observed in mutant rat strains. Furthermore, when Wistar rats were used as a control rat strain, there was no significant difference in lymphatic absorption of sitosterol and campesterol between SHRSP/Izm and Wistar rats\textsuperscript{13, 20}. Biliary secretion of plant sterols when fed a plant sterol diet was almost the same between SHRSP/Izm and WKY/Izm rats\textsuperscript{19}. Our results suggest that rat strains carrying the \textit{Abcg5} mutation exhibit deposited plant sterols without a significant increase in absorption or decrease of excretion to bile. We concluded that disrupting the delicate balance between intestinal absorption of plant sterols and their biliary excretion induces gradual, but significant, plant sterol deposition in the rat strains that carry the \textit{Abcg5} mutation. It has been reported that plant sterol absorption was 2–3 times higher in ABCG5/ABCG8 knockout mice and phytosterolemics patients than in wild type mice and healthy humans, respectively\textsuperscript{15, 7}. The deposition of plant sterols in \textit{Abcg5}-mutant rat strains might be a unique case, but this case might provide an important example that enables us to understand the mechanisms of plant sterol deposition in phytosterolemia.

We also examined whether sitosterol is transiently incorporated into intestinal mucosa in SHRSP and compared with that in Wistar rats\textsuperscript{19}. The results from Wistar rats were described in section 2.2. No difference in the response was observed between SHRSP and Wistar rats. These results show that even in SHRSP animals, which have evidence of plant sterol deposition, sitosterol was incorporated into the intestinal mucosa to a lesser extent than was cholesterol; comparable to that observed in Wistar rats.

### 2.4 Determinants of differential absorption of plant sterols

The affinity of sterols for bile salt micelles was assessed by measuring the transfer of sterols from the micelles to triolein\textsuperscript{4}. Since sitosterol was transferred to triolein from micelles to a lesser extent than was cholesterol, it is possible that sitosterol is less releasable from micelles and therefore, it may have a higher affinity for micelles than does cholesterol\textsuperscript{2}. In the study, cholesterol was 3–4 times more releasable than sitosterol from bile salt micelles. We also showed that the rate of campesterol transfer was between those of cholesterol and sitosterol\textsuperscript{2}. Intestinal absorption of cholesterol, campesterol, and sitosterol occurs in the rank order cholesterol > campesterol > sitosterol\textsuperscript{2}. The results suggest that affinity for bile salt micelles may be a major determinant of sterol absorbability.

Recently, we examined the relationship between the lymphatic absorption and affinity, and micellar solubility of various plant sterols, which were brassicasterol, campesterol, stigmasterol, sitosterol, and sitostanol\textsuperscript{2}. When the releasability of sterols from bile salt micelles was assessed, the order of releasability was as follows: brassicasterol > campesterol > stigmasterol > sitosterol > sitostanol. The solubility in bile salt micelles was higher for sitosterol, campesterol, and sitostanol and lower for brassicasterol and stigmasterol. Out of sitosterol, campesterol, and sitostanol, the rank order of solubility was as follows: sitosterol > campesterol > sitostanol. Although no significant correlation was observed between lymphatic recovery of plant sterols and their micellar solubility, or the transfer rate from the bile salt micelle, we found a highly positive correlation between their lymphatic absorption and the multiplication value of micellar solubility and transfer rate (\( r = 0.88 \)). This observation suggests that solubility in, and affinity for, the bile salt micelle of plant sterols are important determinants of their intestinal absorption in rats\textsuperscript{20}. Our observation was derived from \textit{in vitro} studies. Further studies in \textit{vivo} are necessary to complement our results.

### 2.5 Is NPC1L1 a determinant of differential absorption of plant sterols?

It has been demonstrated that NPC1L1 is responsible for the intestinal absorption of cholesterol and plant sterols\textsuperscript{2}. Since ezetimibe, an inhibitor of NPC1L1, reduces intestinal absorption of both cholesterol and plant sterols, this suggests that NPC1L1 is involved in intestinal absorption of both cholesterol and plant sterols. Recently, Zhang \textit{et al.} found that the N-terminal domain of NPC1L1 bound cholesterol and that this can signal the initiation of endocytosis of NPC1L1-flotillin-cholesterol membrane microdomains\textsuperscript{41}.
Furthermore, they showed that the N-terminal domain of NPC1L1 did not bind sitosterol or stigmasterol. This may explain why sitosterol and stigmasterol are less absorptive than cholesterol. However, the mechanisms responsible for the differential absorption rates among various plant sterols and stanols are unclear and require further study.

3 Role of plant sterols on cholesterol absorption
3.1 Inhibition of cholesterol absorption by plant sterols

It is well established that dietary plant sterols have hypocholesterolemic activity in experimental animals and humans. Because being fed a diet of plant sterols increases fecal excretion of cholesterol and its metabolites, it is thought that plant sterols inhibit cholesterol absorption in the intestine. Inhibition of cholesterol absorption by plant sterols was confirmed by directly measuring the lymphatic absorption of cholesterol. Lymphatic recovery of radiolabeled cholesterol in the thoracic duct-cannulated rats administered an emulsion containing cholesterol plus sitosterol, was significantly reduced when compared to that from rats administered cholesterol only.

3.2 Mechanisms of cholesterol absorption inhibition by plant sterols
3.2.1 Suppression of micellar solubilization of cholesterol

Previously, we measured the effects of sitosterol on the solubility of cholesterol in bile salt micelles. In vitro condition, the extent of cholesterol incorporation in the micellar incorporation reached a plateau above a certain level of cholesterol. Sitosterol was also solubilized in bile salt micelles at almost the same level as cholesterol. These data suggest that incorporation of sterols into bile salt micelles depends on the sterol-solubilizing capacity of the micelles. Therefore, when both cholesterol and sitosterol were solubilized in micelles above a certain level, this co-solubilization reduced that of cholesterol. However, when sterols were added at concentrations below the level at which the micelles were not saturated with the sterols, the solubility of cholesterol was not reduced. These data were obtained from an in vitro study. We were interested in whether bile salt micelles have a limited solubilizing capacity of sterols in vivo. Rats were fed 0.5% cholesterol (C) or 0.5% cholesterol plus 0.5% sitosterol (C+S) diet and then the sterol content in the aqueous (micellar) phase of intestinal content was measured. The cholesterol content in the aqueous phase of the C+S diet fed group was significantly lower than that of the C group (Fig. 4A). Since sitosterol was also solubilized in the aqueous phase in the C+S group, the total sterol in the aqueous phase was similar between the C and the C+S groups (Fig. 4A). We also showed that the limited solubility of cholesterol in the C+S group was directly reflected in the incorporation of radiolabeled cholesterol into intestinal mucosa (Fig. 4B).

These results suggest that the limited micellar solubility of

Fig. 4 Effects of dietary sitosterol on micellar solubility of cholesterol and plant sterols (A) and mucosal incorporation of $^{14}$C-cholesterol (B) in rats.

A: Rats were meal-fed a 0.5% cholesterol or 0.5% cholesterol + 0.5% sitosterol diet for 1 hr. Two hr after withdrawal of the diet, intestinal content was collected and the micellar phase was separated.

B: Rats were meal-fed a diet similar to that in Exp. A for 1 hr, except that $^{14}$C-cholesterol was added in the diet. Two hr after withdrawal of the diet, the intestinal mucosa was collected. Drawn from the results in Ref. 22.
cholesterol by sitosterol can be a major cause of cholesterol absorption inhibition. Results from several studies support these observations.\textsuperscript{20, 24}

3.2.2 The effect of plant sterols on post-micellization processes of cholesterol absorption

We studied the effects of sitosterol solubilized in bile salt micellar solution on the intestinal absorption of cholesterol. We found that micellar sitosterol did not influence the incorporation of micellar cholesterol into brush border membranes\textsuperscript{6} or the rat jejunal loop \textit{in situ}\textsuperscript{22}. When rats with cannulated lymph and bile ducts were intraduodenally infused with bile salt micelles containing cholesterol alone or cholesterol plus sitosterol for 24 h, lymphatic transport of micellar cholesterol was not influenced by the sitosterol solubilized in bile salt micelles\textsuperscript{6}. Therefore, we concluded that sitosterol does not influence post-micellization processes of cholesterol absorption.

Recently, Smet \textit{et al.} summarized the proposed mechanisms responsible for the effects of plant sterols and stanols on intestinal cholesterol metabolism\textsuperscript{5}. Although many other interesting mechanisms regulating cholesterol absorption by plant sterols have been suggested, the authors concluded that suppression of micellar solubility of cholesterol by plant sterols is the only equivocally established effect.

4 Mechanisms responsible for the acceleration of cholesterol absorption by pancreatic cholesterol esterase

4.1 Background

Cholesterol esterase, secreted from the pancreas, hydrolyzes cholesterol ester to unesterified cholesterol and free fatty acids. Since cholesterol esters are not directly absorbed by the intestine, they must be hydrolyzed by cholesterol esterase. Therefore, cholesterol esterase aids the effective absorption of cholesterol esters. Cholesterol esterase is sometimes referred to as bile salt-stimulated lipase, and carboxy ester lipase. Therefore, we concluded that sitosterol does not influence post-micellization processes of cholesterol absorption.

It was suggested that cholesterol esterase accelerates intestinal absorption and esterification of unesterified cholesterol\textsuperscript{26, 27}. Gallo \textit{et al.} reported that depletion of cholesterol esterase in the intestinal lumen following antibody administration led to a reduction of lymphatic cholesterol absorption in rats\textsuperscript{28}. Furthermore, they observed that the addition of cholesterol esterase to culture medium increased cholesterol esterification of isolated intestinal cells\textsuperscript{27}. Subsequently, these authors showed that pancreatic cholesterol esterase is localized inside rat intestinal mucosal cells\textsuperscript{29}. They suggested that cholesterol esterase might be involved in intestinal absorption of unesterified cholesterol by accelerating the esterification of cholesterol in intestinal mucosal cells\textsuperscript{20}. Bhat and Brockman also showed that cholesterol esterase accelerated cholesterol incorporation into rat intestinal sacs\textsuperscript{30}, and suggested that it acts as a cholesterol transporter when bound to the brush border membranes of intestinal epithelial cells.

In contrast, several reports found that cholesterol esterase had no effect on cholesterol absorption\textsuperscript{31-33}. For example, Howles \textit{et al.} showed that cholesterol absorption in cholesterol esterase-knockout mice was the same as that in wild type mice, and in contrast, the absorption of cholesterol esters was dramatically suppressed in the knockout mice\textsuperscript{31}. Field suggested that cholesterol esterase within intestinal epithelial cells could be contamination from the enzyme that exists in the intestinal lumen\textsuperscript{34}. Despite numerous studies, the role of cholesterol esterase in cholesterol absorption has yet to be determined. Therefore, we attempted to identify the reason for the discrepancies.

4.2 Does cholesterol esterase accelerate intestinal absorption of unesterified cholesterol?

As described above, some studies found no effect of pancreatic cholesterol esterase on the acceleration of cholesterol absorption. Therefore, we performed some experiments to confirm whether cholesterol absorption is accelerated by cholesterol esterase. First, we performed a study using rat intestinal brush border membranes, and a bile salt micellar solution containing unlabeled cholesterol, radiolabeled cholesterol, phosphatidylcholine (PC), and sodium taurocholate (PC-containing micelle). When the brush border membrane fraction obtained from rat jejunum was incubated with the PC-containing micelle, the addition of cholesterol esterase dose-dependently accelerated the uptake of cholesterol\textsuperscript{35}.

In the second study, differentiated Caco-2 cells were used to confirm the effect of cholesterol esterase. Caco-2 cells were grown to confluency on the upper side of pre-soaked membrane filters. After the maturation of brush border membranes in Caco-2 cells was confirmed, the PC-containing micelle was added to the apical side of differentiated Caco-2 cells. The incorporation of cholesterol into the Caco-2 cells was dose-dependently accelerated by the addition of cholesterol esterase\textsuperscript{35}.

Finally, the effect of cholesterol esterase was confirmed using thoracic duct-cannulated rats drained of pancreatic juice and bile. After surgery, the PC-containing micelle without radiolabeled cholesterol was administered continuously to the intestinal lumen \textit{via} a tube inserted into the duodenum. The next morning, the PC-containing micelle containing radiolabeled cholesterol was administered to the intestinal lumen and lymph fluid was collected over 24 h. In the cholesterol esterase(+) group, cholesterol esterase was added to the PC-containing micelle with radiolabeled cholesterol. Lymphatic recovery of radioactivity was
significantly higher in the cholesterol esterase (+) group compared to the cholesterol esterase (−) group. Taken together, these data suggest that cholesterol esterase accelerates the intestinal cholesterol absorption.

4.3 Effect of cholesterol esterase bound to intestinal brush border membranes on cholesterol absorption

Bhat and Brockman and Lopez-Candales suggested that cholesterol esterase binds to intestinal brush border membranes, and that it might function as a cholesterol transporter. We studied the influence of cholesterol esterase existing in rat intestinal brush border membranes on the incorporation of cholesterol from the PC-containing micelle. We detected cholesterol esterase activity in brush border membranes when pancreatic juice was drained from rats overnight, the activity of cholesterol esterase in brush border membranes was reduced to almost a half compared to that in rats that did not have their pancreatic juice drained. Incorporation of micellar cholesterol into brush border membranes obtained from pancreatic juice drained rats was the same as that from normal rats. In another study, when brush border membranes from normal rats were incubated with bovine cholesterol esterase, the activity of cholesterol esterase in brush border membranes almost doubled. Under these conditions, incorporation of micellar cholesterol into brush border membranes was not altered. Therefore, at least in our experimental conditions, there was no effect of cholesterol esterase bound to brush border membranes on cholesterol incorporation. Although we detected cholesterol esterase activity in rat brush border membranes, the level was extremely low. Therefore, the existence of cholesterol esterase can derive from contamination from the intestinal lumen rather than it being bound to brush border membranes.

4.4 Absorption of cholesterol contained in the PC-depleted micelle was not accelerated by cholesterol esterase

Various bile-salt micelles have been utilized in studies investigating the effects of cholesterol esterase on intestinal cholesterol absorption. Although we used the PC-containing micelle, some researchers used monoacylglycerol instead of PC as a component of micelles. Since bile contains PC, the PC-depleted micelle is not physiologically relevant. We attempted to examine the effects of cholesterol esterase on cholesterol absorption using the PC-depleted micelle. As a result, in the PC-depleted micelle, cholesterol esterase did not accelerate the incorporation of cholesterol into Caco-2 cells, and its lymphatic absorption in rats drained of bile and pancreatic juice. These observations suggest that PC in bile salt micelles is an important factor in the acceleration of cholesterol absorption by cholesterol esterase.

4.5 Role of PC on accelerated absorption of cholesterol by cholesterol esterase

As described, cholesterol esterase is also referred to as carboxy ester lipase, and as the name suggests, it can hydrolyze phospholipids. Since the PC-containing micelles include PC, PC must be hydrolyzed by cholesterol esterase. Therefore, assuming that part of the PC was hydrolyzed to lysoPC and fatty acid, a part of PC in the PC-containing micelle was replaced by lysoPC + fatty acid and then lymphatic absorption of cholesterol was examined in thoracic duct-cannulated rats that had been drained of bile and pancreatic juice. Lymphatic recovery of radiolabeled cholesterol was increased following decreased PC, and the increase of LPC and fatty acid in bile salt micelles infused to duodenum. In Caco-2 cells, when part of the PC in bile salt micelles was replaced by LysoPC + fatty acid, incorporation of cholesterol was accelerated. These observations suggest that acceleration of cholesterol absorption by cholesterol esterase is induced by the hydrolysis of PC in the PC-containing micelle.

4.6 Effect of phospholipase A2 on cholesterol absorption

Phospholipase A2 hydrolyzes PC to LysoPC and free fatty acid. Therefore, phospholipase A2 is predicted to accelerate cholesterol absorption. Phospholipase A2 accelerated lymphatic recovery of cholesterol from the PC-containing micelle in thoracic duct-cannulated rats that were drained of bile and pancreatic juice and in Caco-2 cells. PC has been shown to suppress the intestinal absorption of cholesterol. Homan and Hamelehle previously reported that phospholipase A2 relieves PC inhibition of micellar cholesterol absorption and transport in Caco-2 cells. Richmond et al. reported that cholesterol absorption in phospholipase A2-deficient mice was accelerated by compensatory phospholipid digestion. Therefore, evidence suggests that the hydrolysis of PC in intestinal lumen is involved in the acceleration of cholesterol absorption.

4.7 Why does the hydrolysis of PC accelerate cholesterol absorption?

We previously reported that the affinity for bile salt micelles of sterols is an important determinant for their intestinal absorption as described at Section 2.4. Therefore, we measured the effects of cholesterol esterase on the releaseability of cholesterol from the PC-containing micelle. As a result, the addition of cholesterol esterase to the PC-containing micelle dose-dependently accelerated the release of cholesterol, suggesting that PC hydrolysis decreases the affinity of cholesterol for the micelle and accelerates the incorporation of cholesterol released from the micelle into intestinal cells. In contrast, in the PC-depleted micelle, no acceleration was induced by the addition of cholesterol esterase.
4.8 Cause of increased esterification of cholesterol in intestinal cells by cholesterol esterase

When cholesterol esterase with the PC-containing micelle was added to the apical side of Caco-2 cells, and the incorporation of micellar cholesterol was increased, the esterification of incorporated cholesterol also increased\(^{35}\). The rate of esterification was increased with increasing incorporation of cholesterol. The increased esterification of cholesterol in Caco-2 cells was also observed when part of PC in the PC-containing micelle was replaced by LysoPC and fatty acid, and cholesterol incorporation was accelerated\(^{35}\). The same phenomenon was observed when cholesterol incorporation was accelerated by phospholipase A\(_2\) in Caco-2 cells\(^{35}\). The results indicate that accelerated esterification of cholesterol is not caused by cholesterol esterase, but it is induced by increased incorporation of cholesterol into Caco-2 cells. When the PC-containing micelles including various amounts of cholesterol were added onto the apical side of Caco-2 cells, the incorporation of cholesterol into the cells was increased with the stepwise increase in micellar cholesterol\(^{35}\). The esterification of cholesterol was increased with the increase of incorporated cholesterol. A highly positive correlation between incorporated cholesterol and the esterification rate was observed\(^{35}\). It has been shown that acylCoA cholesterol acyltransferase (ACAT)\(_2\) plays a central role in cholesterol esterification in intestinal absorptive cells, and that this enzyme is activated by the substrate, cholesterol\(^{35}\). Therefore, it is thought that in Caco-2 cells, the increase of uptake of micellar cholesterol by cholesterol esterase activated ACAT\(_2\), and then, esterified cholesterol was increased. We concluded that increased esterification of cholesterol, by cholesterol esterase is not caused by the contamination of intestinal epithelial cells by this enzyme\(^{35}\).

References


17) Yamori, Y.; Nagaoka, A.; Okamoto, K. Importance of genetic factors in stroke: An evidence obtained by selective breeding of stroke-prone and –resistant SHR.
Factors Affecting Intestinal Absorption of Cholesterol and Plant Sterols and Stanols


