Competitive Solubilization of Cholesterol/Cholesteryl Oleate and Seven Species of Sterol/Stanol in Model Intestinal Solution System

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Abstract: The addition of plant sterols/stanols (sterols or stanols) can reduce the solubilization of cholesterol in a model intestinal solution system. We studied the molecular structure of seven different sterols/stanols and the effect they had on the solubilization of cholesterol or cholesteryl ester in a model intestinal solution. The differences in the molecular structures of the sterol/stanol species influenced their abilities to reduce the solubility of cholesterol in the competitive solubilization experiments. Cholestanol whose molecular structure resembled cholesterol was the most effective at reducing the solubilization of cholesterol and cholesteryl ester, with the solubilities of cholesterol and cholesteryl oleate being 41% and 39% respectively of the values observed for the single solubilize systems. β-Sitosterol was also able to reduce the solubilities of cholesterol and cholesteryl oleate to 43% and 45% of those observed in a single solubilize system. Both, stigmasterol and brassicasterol have an unsaturated double bond in a steroid side chain and did not exhibit major cholesterol-lowering effects. These results were reflected by the Gibbs free energy change values ($\Delta G^\circ$) for solubilization, where the sterol/stanol species with cholesterol-lowering effects had similar or larger negative $\Delta G^\circ$ values than those observed for cholesterol.

Key words: bile salt, solubilization, cholesterol, sterol, stanol, model intestinal solution

1 INTRODUCTION

Excessive intake of cholesterol increases the risk of heart disease and arteriosclerosis. Plant sterols and stanols are able to decrease blood cholesterol level by inhibiting cholesterol absorption in the intestine. The one of the drop mechanism of the cholesterol absorption is originated from the plant sterol/stanol/sterol or stanol/is selectively solubilized for a bile acid micelle. Plant sterols/stanols are present in cereals and vegetable oil, where the sterol/stanol content in vegetable oils is less than 1%. The molecular structures of plant sterols/stanols resemble that of cholesterol, but these compounds are absorbed to a lesser extent than cholesterol. This difference in the quantity absorbed is due to the differences in molecular structures of cholesterol and the plant sterol/stanol. Correlations between the molecular structures of the plant sterols/stanols and their abilities to decrease the absorption of cholesterol have been reported. The cholesterol-lowering effects of sterols/stanols have been demonstrated after conducting human and animal studies. Using in vitro systems, the cholesterol-lowering effects of different sterols/stanols were studied. Results obtained from in vivo and in vitro systems were not identical, but they did show that several sterols/stanols could reduce cholesterol absorption or solubility.

We have previously performed competitive solubilization experiments in a system comprising bile salt, cholesterol, and competing compound and studied cholesterol-lowering effect by changing the molecular structure of the competing compound. The purpose of this study was to determine which sterol/stanol was most effective at decreasing the solubilization of cholesterol or cholesterol ester in a model intestinal solution. Furthermore, we discuss the characteristics of the molecular structure of the sterols/stanols that make these compounds suitable for decreasing the solubilization of cholesterol. We first determined the solubilities of seven species of sterol/stanol in the model intestinal solution. Furthermore, in a binary system of cholesterol/cholesterol ester and sterol/stanol crystals, we determined the maximum solubilities of both. As part of the
thermodynamic studies, the Gibbs energy change ($\Delta G^0$) values for the solubilization of the seven sterol/stanol species were compared with that of cholesterol.

Fig. 1  Molecular structures of sterols and stanols. The arrows indicate differences in comparison with a cholesterol molecule.

2 EXPERIMENTAL
2.1 Materials
Sodium glycocholate (NaGC, 97%), sodium glycochenodeoxycholate (NaGCDC, 97%), sodium glycodeoxycholate
(NaGDC, 97%), sodium taurocholate (NaTC, 97%), sodium taurochenodeoxycholate (NaTCDC, 97%), sodium taurodeoxycholate (NaTDC, 97%), oleic acid (99%), and 1-oleoyl-rac-glycerol (monolein, 99%) were of reagent grade (Sigma-Aldrich Co.). L-α-Phosphatidylcholine (PC, 99%) from egg yolk was also purchased from Sigma-Aldrich Co., and the average molecular weight was estimated to be 775 g mol⁻¹. Cholesterol (99%), cholesteryl oleate (98%), cholestanol (5α-cholestan-3β-ol, 95%), and β-sitostanol (95%) were also obtained from Sigma-Aldrich Co. β-Sitosterol, campesterol, brassicasterol, and stigmasterol were purchased from Tama Biological (Japan), and their purities (99%) were checked by gas chromatography. Fucosterol (90%) was purchased from Extrasynthese and directly used without further purification. The molecular structures of these sterol/stanol species are shown in Fig. 1. Naphthalene and pyrene of reagent grade (Wako Pure Chemical) were purified by recrystallizations repeatedly from the ethanol solution. Radioactive [4-¹⁴C]cholesterol (53 mCi/mmol) was obtained from ARC (USA).

The water used in the experiments was distilled once after ion-exchange treatment.

2.2 Preparation of the model intestinal solution

The experimental setup for the model intestinal solution was prepared with reference to dietary mixed micelle (DMM) and mixed intestinal lipid (MIL) systems. The composition of the bile salt mixture was the same as that suggested by Mel’nikov et al. The respective molar ratio is indicated as follows in the parenthesis: NaGC (6), NaGDC (6), NaGDC (4), NaTC (3), NaTCDC (3), and NaTDC (2). The standard composition and concentration for the model intestinal solution are as follows: mixed bile salt (10 mM), phosphatidylcholine (PC) (2 mM), oleic acid (4 mM), and monolein (2 mM). All solutions were prepared using 0.15 M NaCl solution and maintained at pH 7.5 with a 10 mM Tris-HCl buffer solution.

The procedure for preparing the model intestinal solution is described as follows: Stock solutions of PC, oleic acid, and monolein were dissolved in chloroform–methanol (1:1). The concentration of each solution was determined by weighing. The model intestinal solution was prepared volumetrically from each stock solution in a recovery flask. The chloroform–methanol solvent was evaporated from the vessel after 1 h using a rotary evaporator at 140 hPa and 308 K. The residual substances formed thin films on the inner wall. The mixed bile salt solution was continuously poured into a glass vessel. The suspension solution was sonicated for 30 min and then shaken for several minutes on the shaker at 308.2 K.

2.3 Solubilization

2.3.1 Aqueous solubility of fucosterol

A method for the determination of aqueous solubility of sterol/stanol has previously been reported by our group. Similar aqueous solubilities of sterol/stanol have also been reported by others. Therefore, the aqueous solubility of fucosterol at 308.2 K was measured using the method reported by Matsuoka et al. The average solubility was obtained after performing three measurements.

2.3.2 Solubilization method

3 ml of the model intestinal solution and excess solid (cholesterol or sterol/stanol or a mixed crystal of both (1:1) is sufficient to produce a saturated solution in a 10 mL injector tube. The system reached equilibrium within 24 h in most cases. The separation of solids was performed via filtration through a membrane filter with a pore size of 0.2 μm (Millipore Co., FGLP01300) by applying pressure on the injector in a thermostat controlled at 308.2 ± 0.3 K. The concentration of cholesterol or sterol/stanol in the filtrates was determined using an assay kit (L-type, Wako Diagnostics). The quantitative mechanism is based on the oxidation of sterols by sterol oxidases. The generated hydrogen peroxide changes to a pigment in proportion to the quantity of sterols. The sterols concentration was determined by a spectrophotometer (Shimadzu UV-1800), which measured the absorbance (wavelength of 550 nm at maximum absorbance). The averages were obtained from between 5 and 10 replicates, and the error bars in the corresponding figures indicate the standard deviations.

2.3.2.1 Mixed solubilize system (cholesterol and sterol/stanol crystals)

The concentrations in a mixed solubilize system (cholesterol and sterol/stanol crystals) were determined as follows: First, the total concentrations of cholesterol and sterol/stanol (C_t) were evaluated by the enzymatic procedure. Secondly, crystals of radioactive cholesterol for determining the maximum solubility of cholesterol in a mixed solubilize system were prepared as follows: An ethanol solution of [4-¹⁴C]cholesterol (37 kBq/mL, 500 μL) was poured into a vessel with cholesterol crystals (50–60 mg). Pure ethanol solvent was added to the solution, while stirring, and the solution was dried in a P₂O₅ desiccator. The specific radioactivities of the labeled cholesterol crystals were precisely determined by a liquid scintillation counter (LSC-6100, Aloka) capable of measuring ¹⁴C radioactivity. The scintillation solution was prepared by mixing the cholesterol crystals (2–3 mg) and a liquid scintillator (14 mL of Aquasol-2, Perkin Elmer). The specific radioactivities of the cholesterol crystals were precisely determined each time (2–4 kBq mg⁻¹). Equimolar crystals of radioactive cholesterol and competitive sterol/stanol were mixed in the vessel. The method of solubilization in the mixed solubilize system was the same as that mentioned above, except for the quantitative procedure. The solubility of cholesterol (C_s) in the mixed system was determined from the specific radioactivity of the filtrates (100 μL) using the liquid scintillator. Finally, the concentrations of sterol/stanol in the
mixed solubilize system were obtained from $C_t - C_f$.

2.3.2.2 Mixed solubilize system (cholesteryl oleate and sterol/stanol crystals)

The mixed solubilize system include cholesteryl ester and free sterol/stanol crystal. The principle of the quantitative procedure is based on the molecular difference between ester and free form of sterol. An assay kit (E-type, Wako Diagnostics)\textsuperscript{22, 23} was used for this purpose. The cholesteryl ester in a sample is dissociated into cholesterol and a fatty acid by the action of the cholesteryl esterase. The principle used for the quantification of the sterols is the same as that described for the assay kit L-type (Wako Diagnostics) in section 2.3.2. Thus, the assay kit E-type (Wako Diagnostics) can be used to measure the total solubility of mixed solubilize system of sterol/stanol ($C_t$), while the assay kit L-type (Wako Diagnostics), can measure the solubility ($C_f$) of only the free form of sterol/stanol, since the kit doesn’t include an esterase. Therefore, we calculated the amount of cholesteryl oleate by calculating $C_t - C_f$.

2.3.2.3 Quantification method for aromatic compounds

The experimental procedure used for the polycyclic aromatic compounds naphthalene and pyrene was similar to that employed for the sterols/stanols, except for the quantitative part. The solubilities of naphthalene and pyrene were determined from the absorbance of the filtrates using the molar extinction coefficients of these compounds\textsuperscript{24}.

3 RESULT AND DISCUSSION

3.1 Competitive solubilization of cholesterol and aromatic compounds

We have studied competitive solubilization between cholesteryl and organic compounds (aromatic compounds, $n$-alkybenzene, and sterol/stanols) in pure bile salt systems\textsuperscript{14}. These studies showed that only sterol/stanol crystals were effective in reducing the solubility of cholesterol in the pure bile salt system. The cholesterol-lowering effect of competitive solubilizes could be influenced by steroid-steroid interaction in the solubilization system. In this study, the solubilization system was changed from single bile salts (steroid compound) to a model intestinal solution (dietary mixed micelle). The model intestinal solution is composed of mixed lipids and they mainly form mixed micelles and liposomes (radii of 30–200 nm) in the solution\textsuperscript{15, 18}. Thus, the sterol/stanol carriers are relative larger aggregates, which are composed of bulky steroids, fatty acids, and double acyl chains. The solubility of cholesterol was measured with the addition of the aromatic compounds naphthalene and pyrene in the model intestinal solution. Figure 2 shows the results from the competitive solubilization experiment performed with a model intestinal solution system (left panel) and with a 15 mM pure NaTDC solution (right panel). The solubility of cholesterol increased considerably when the aromatic compounds naphthalene and pyrene were added.

![Fig. 2](image)

**Fig. 2** The solubilities of cholesterol and the aromatic compounds naphthalene and pyrene in model intestinal solution system (molar fraction of crystal cholesterol/aromatic compounds = 1) and pure NaTDC solution system at 308.2 K.
Pyrene were added to the model intestinal solution (Fig. 2). A small increase in the solubility of cholesterol was also observed in the NaTDC solution when the aromatic compounds were included in the system. These results show that the aromatic compounds do not decrease the solubility of cholesterol in the model intestinal solution. This suggests that certain characteristics related to the molecular structure of the competing solubilizate are required to lower cholesterol in the model intestinal solution. In addition, a solubilization site for aromatic compounds may be different from site cholesterol in the aggregate.

3.2 Competitive solubilization of cholesterol and seven species of sterol/stanol

More than 30 species of plant sterols/stanols with different molecular structures are known to occur. Plant sterols/stanols present in vegetable oils are ingested as part of our diet. It is beneficial to determine the plant sterol/stanol which shows the most significant cholesterol-lowering effect, so that it can be used as part of the meal or as medicine for the prevention of heart disease. We have previously investigated competitive solubilization of cholesterol and six species of plant sterols/stanols in pure bile acid solution. As shown in Fig. 3 (right panel), cholestanol was the best at reducing the solubility of cholesterol. The addition of cholestanol can decrease the solubility of cholesterol in the cholesterol only system to 33%. In this study, we studied competitive solubilization of cholesterol and seven different species of plant sterols in a model intestinal solution. The purpose was to determine the molecular structure, which was the most effective in reducing the solubility of cholesterol. Figure 3 shows the results for the single solubilize system of sterol/stanol and the mixed solubilization system composed of cholesterol and sterol/stanol. The order of solubility in model intestinal solution was as follows: cholesterol > cholestanol > β-sitostanol = fucosterol = campesterol = β-sitosterol > stigmasterol > brassicasterol (Fig. 3). Thus, the solubilities of the seven different sterols/stanols were varied, where the maximum solubility was 1.1 mM for cholesterol, and the minimum was 0.16 mM for brassicasterol. The aqueous solubilities for the above-mentioned sterols/stanols are $10^{-3} \sim 10^{-5}$ M. In this study, the aqueous solubility of fucosterol was determined to be $4.66 \times 10^{-8}$ M, which is similar to the values obtained for the above mentioned plant sterols/stanols. A direct correlation for the order was not observed between the solubilities of the plant sterol/stanol in the model intestinal solution and their solubilities in water. The molecular structure of the sterol/stanol has a short side chain, and one without double bond is the structure that is advanta-

![Fig. 3](image_url)

**Fig. 3** The solubilities of cholesterol and seven species of sterol/stanol in model intestinal solution system (left panel) and pure NaTDC solution (15 mM) system (right panel) at 308.2 K.
geous to solubilization. On the other hand, the position of double bond at the side chain showed low quantity of solubilization as showing the above order.

Results from the competitive solubilization experiment conducted by mixing cholesterol and seven species of plant sterols in model intestinal solution are shown in Fig. 3. Addition of cholestanol and β-sitosterol reduced the solubility of cholesterol to ca. 40% in the model intestinal solution (Fig. 3). However, the cholestanol is not a plant-derived compound, so we cannot take in cholestanol from a meal directly. The effectiveness of β-sitosterol in lowering cholesterol has been also confirmed after animal and human studies\(^4,6\). The other plant sterols/stanols were not as effective as cholestanol and β-sitosterol in lowering the solubility of cholesterol. In particular, stigmasterol and brassicasterol had a minimal effect on lowering the solubility of cholesterol. Both have a double bond at the position of intramolecular C-22 and the limited flexibility of the bulky side chain could be disadvantageous for reducing solubility of cholesterol.

As shown in Fig. 3, both the model intestinal solution and the pure bile salt solution showed a similar trend of a reduction in the solubility of cholesterol upon the addition of plant sterol/stanol. This suggests that the difference in the structure of a cholesterol carrier (aggregate) does not have a major influence on the competitive solubilization. The total solubilities of cholesterol and sterols/stanols were higher in the model intestinal solution than in pure bile salt solution.

3.3 Competitive solubilization of cholesteryl oleate and seven species of sterol/stanol

A large percentage of cholesterol in food occurs in esterified form, and the major part of cholesterol is gradually converted to the free form of cholesterol \textit{in vivo}. However, a small percentage of cholesterol remain esterified form \textit{in vivo}\(^7\). We selected cholesteryl oleate, which is one of the most abundantly found cholesterol ester \textit{in vivo}\(^7\). In this section, we discuss whether as observed with cholesterol, the solubility of cholesteryl ester decreases upon the addition of the plant sterol/stanol.

The solubilities of cholesteryl oleate and seven species of sterol/stanol in model intestinal solutions (left panel) and pure 15 mM solutions of NaTDC (right panel) at 308.2 K are shown in Fig. 4. The competitive solubilization experiments were performed in model intestinal solution as well as pure bile salt solution. The solubility of cholesteryl oleate in model intestinal solution was 0.13 mM (Fig. 4), which was one-tenth of the solubility of free cholesterol in the same system (Fig. 3). Similarly, the solubility of cholesteryl

![Fig. 4](image-url) The solubilities of cholesteryl oleate and seven species of sterol/stanol in model intestinal solution system (left panel) and pure NaTDC (15 mM) solution system (right panel) at 308.2 K.
oleate in 15 mM NaTDC solution was one-eighth of the solubility of free cholesterol in the same solution. These results indicate that the solubility of esterified form of cholesterol is extremely low compared to the free form of cholesterol. There was a reduction in the solubility of cholesteryl oleate upon the addition of the plant sterol/stanol in both solutions. This trend was similar to that observed for free cholesterol (Fig. 3). Addition of cholestanol was the most effective, while brassicasterol was the least effective at lowering the solubility of cholesterol oleate. Again, this is similar to what was observed with the solubility of free cholesterol when cholestanol was added to the solution. Thus, the addition of plant sterol/stanol reduced the solubility of cholesteryl oleate in both solutions (Fig. 4). Even brassicasterol and stigmasterol, whose structures have double bonds in the side chain, lowered the solubility of cholesteryl oleate. The difference in the molecular structure of the sterol/stanol proved to be a major factor influencing the solubility of the cholesteryl ester.

3.4 Gibbs energy change for solubilization of cholesterol and seven species of sterol/stanol in the model intestinal solution

It is possible to determine the preferential solubilization of a certain sterol/stanol in the solubilization system by calculating the Gibbs free energy change ($\Delta G^\circ \beta$) values. The $\Delta G^\circ$ value obtained for the solubilization of cholesterol can be compared to that obtained for the other sterols/stanols. The $\Delta G^\circ$ value indicates the stabilization energy required for the transfer of the solubilize from the aqueous bulk phase to the large molecular aggregate phase. A large molecular aggregate is regarded as a separate phase if the partition equilibrium is reasonable for obtaining the Gibbs energy change ($\Delta G^\circ$). The selectivity of cholesterol and sterol/stanol in the binary system can be discussed based on the $\Delta G^\circ$ values relative to those obtained for a system consisting of only cholesterol or sterol/stanol. The molar fractions of the solubilizes in the aggregate phase and the aqueous phase are given by Eqs. (1) and (2), respectively:

$$X^A_R = \langle [R] \rangle / [(C - \text{cmc}) + \langle [R] \rangle]$$  \hspace{1cm} (1)

$$X^W_R = [R]/(55.5 + [R] + \text{cmc})$$  \hspace{1cm} (2)

where $[R]$ denotes the total equivalent concentration of the solubilize, and the superscripts A and W refer to the aggregate phase and the aqueous phase, respectively. $[R]$ is the aqueous solubility of the solubilize, C denotes the total concentration of the solubilizer (mixed bile salt (10 mM), PC (2 mM), oleic acid (4 mM), and monoolein (2 mM)), and cmc denotes the mixed bile salt of the critical micelle concentration (2.2 mM). The molar fractions were calculated from the maximum solubilities obtained for various compounds, as shown in Fig. 3. The aqueous solubilities ($[R]$) at 308.2 K were obtained from previous reports; cholesterol $3.7 \times 10^{-8}$ M, campesterol $1.6 \times 10^{-8}$ M, brassicasterol $4.3 \times 10^{-8}$ M, $\beta$-sitosterol $2.8 \times 10^{-9}$ M, stigmasterol $1.9 \times 10^{-8}$ M, cholesteryl 2.3 × $10^{-8}$ M, $\beta$-sitostanol $5.8 \times 10^{-9}$ M, naphthalene $3.7 \times 10^{-4}$ M, and pyrene $1.1 \times 10^{-6}$ M. Moreover, as part of the current study, the aqueous solubility of luciferase was determined to be $4.66 \times 10^{-8}$ M. The chemical potential of each phase at temperature $T$ and pressure $P$ is expressed as follows:

$$\mu_R^A = \mu_R^0 + RT \ln X_R^A$$  \hspace{1cm} (3)

$$\mu_R^W = \mu_R^0 + RT \ln X_R^W$$  \hspace{1cm} (4)

where the subscript R refers to the solubilize, and the superscripts A and W refer to the aggregate phase and the aqueous phase, respectively. The superscript 0 implies the standard chemical potential at infinite dilution. These results yield the following relationship for the solubilize molecule at equilibrium:

$$RT \ln \left( \frac{X_R^A}{X_R^W} \right) = - (\mu_R^0 - \mu_R^0) = - \Delta G^\circ.$$  \hspace{1cm} (5)

From this equation, we can determine the change required for the transfer of a solubilize molecule from an aqueous phase to an aggregate phase.

Figure 5 shows $\Delta G^\circ$ values for solubilization of cholesterol and seven species of sterol/stanol in model intestinal solution at 308.2 K. The $\Delta G^\circ$ values of $\beta$-sitosterol, stigmasterol, cholesteryl, and $\beta$-sitostanol were more negative than that of cholesterol, however, the $\Delta G^\circ$ values of the other plant sterols/stanols were not a lot different from cholesterol. For example, the $\Delta G^\circ$ values for cholesterol, $\beta$-sitosterol, and brassicasterol in the model intestinal solution were $-46$, $-50$, and $-41$ kJ mol$^{-1}$, respectively, where the maximum difference was between cholesterol and brassicasterol (approximately 5 kJ mol$^{-1}$). Therefore, there was a reduction in the solubility of cholesterol by the addition of plant sterol/stanol. On the other hand, the $\Delta G^\circ$ values of naphthalene and pyrene (aromatic compounds) were $-25$ kJ mol$^{-1}$ and $-30$ kJ mol$^{-1}$, respectively, thus there was a remarkable difference compared to $\Delta G^\circ$ of cholesterol ($-46$ kJ mol$^{-1}$). Hence, the addition of aromatic compounds in model intestinal solution could not reduce the solubility of cholesterol. Several studies have reported the $\Delta G^\circ$ values for the solubilization of cholesterol or sterols/stanols in pure bile salt solution.$^{7,8,30}$ Armstrong and Carey reported that the solubilization of $\beta$-sitosterol in glycodeoxycholate micelles was more likely to be favored than that of cholesterol ($-1.8$ kJ mol$^{-1}$)$^{30}$. We have previously reported that the difference between the $\Delta G^\circ$ value for the solubilization of cholesterol and $\beta$-sitosterol in sodium deoxycholate solution (15 mM) was $-5.5$ kJ mol$^{-1}$. Thus, the results from the thermodynamic study of competitive solubilization can explain the cholesterol-lowering effects of sterols/stanols.
4 CONCLUSION

Small differences in the molecular structures of the plant sterols/stanols lead to large differences in their solubilities in the model intestinal solution. It is of both medical and academic interest to determine which sterol has the most significant effect on lowering cholesterol and cholesteryl oleate. In the competitive solubilization experiments, cholestanol and \( \beta \)-sitosterol were most effective at decreasing the solubility of cholesterol and cholesteryl oleate. Moreover, the Gibbs energy change value \( \Delta G^0 \) for the solubilization of \( \beta \)-sitosterol in the model intestinal solution was the lowest. However, cholestanol does not occur in plants, and is biosynthesized from cholesterol in vivo; hence, it cannot be ingested as part of the daily diet to prevent heart disease; while \( \beta \)-sitosterol is widely present in plants, and can be ingested. Both, brassicasterol and stigmasterol have an unsaturated bond in a side chain and were found to be unsuitable for lowering cholesterol and cholesteryl oleate in a model intestinal solution. Results from the in vitro experiment indicated that a small difference in the molecular structure of the plant sterols could greatly influence their ability to reduce the solubilization of cholesterol.

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References

1) Murray, C. J.; Lopez, A. D. Mortality by cause for eight regions of the world: Global Burden of Disease Study. Lancet \textbf{349}, 1269-1276 (1997).
2) de Jong, A.; Plat, J.; Mensink, R. P. Metabolic effects of plant sterols and stanols. \textit{J. Nutr. Biochem.} \textbf{14}, 362-369 (2003).
3) Katan, M. B.; Grundy, S. M.; Jones, P.; Law, M.; Miettinen, T.; Paoletti, R. Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. \textit{Mayo Clin. Proc.} \textbf{78}, 965-978 (2003).
4) Miettinen, T.; Gylling, H. Plant stanol and sterol esters in prevention of cardiovascular diseases. \textit{Ann. Med.} \textbf{36}, 126-134 (2004).
5) O’Neill, F. H.; Sanders, T. A. B.; Thompson, G. R. Comparison of efficacy of plant stanol ester and sterol ester: short-term and longer-term studies. \textit{Am. J. Cardiol.} \textbf{96}, 29D-36D (2005).
6) Trautwein, E. A.; Duchateau, G. S. M. J. E.; Lin, Y.; Mel’nikov, S. M.; Moluizen, H. O. F.; Ntanios, F. Y. Proposed mechanisms of cholesterol-lowering action of plant sterols. \textit{Eur. J. Lipid Sci. Technol.} \textbf{105}, 171-185 (2003).
7) Matsuoka, K.; Kajimoto, E.; Horiuchi, M.; Honda, C.; Endo, K. Competitive solubilization of cholesterol and six species of sterol/stand in bile salt micelles. \textit{Chem. Phys. Lipids} \textbf{163}, 397-402 (2010).
8) Nagadome, S.; Okazaki, Y.; Lee, S.; Sasaki, Y.; Sugihara, G. Selective solubilization of sterols by bile salt micelles in water: A thermodynamic study. \textit{Langmuir} \textbf{17}, 4405-4412 (2001).
9) Kochhar, S. P. Influence of processing on sterols of edible vegetable oils. \textit{Prog. Lipid Res.} \textbf{22}, 161-188
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Hayes, K. C.; Pronczuk, A.; Perlman, D. Nonesterified phytosterols dissolved and recrystallized in oil reduce plasma cholesterol in gerbils and humans. J. Nutr. 134, 1395-1399 (2004).

Vahouny, G. V.; Connor, W. E.; Subramaniam, S.; Lin, D. S.; Gallo, L. L. Comparative lymphatic absorption of sitosterol, stigmasterol, and fucosterol and differential inhibition of cholesterol absorption. Am. J. Clin. Nutr. 37, 805-809 (1983).

Hamada, T.; Goto, H.; Yamahira, T.; Sugawara, T.; Imazumi, K.; Ikeda, I. Solubility in and affinity for the bile salt micelle of plant sterols are important determinants of their intestinal absorption in rats. Lipids 41, 551-556 (2006).

Ikeda, I.; Tanabe, Y.; Sugano, M. Effects of sitosterol and sitostanol on micellar solubility of cholesterol. J. Nutr. Sci. Vitamínol. 35, 361-369 (1989).

Matsuoka, K.; Hirosawa, T.; Honda, C.; Endo, K.; Moroi, Y.; Shibata, O. Thermodynamic study on competitive solubilization of cholesterol and β-sitosterol in bile salt micelles. Chem. Phys. Lipids 148, 51-60 (2007).

Matsuoka, K.; Ebisawa, R.; Yu, S.; Honda, C.; Endo, K. Competitive solubilization of cholesterol and β-sitosterol with changing biliary lipid compositions in model intestinal solution. Chem. Phys. Lipids 165, 7-14 (2012).

Mazer, N. A.; Benedek, G. B.; Carey, M. C. Quasielastic light-scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. Biochemistry 19, 601-615 (1980).

Montet, J. C.; Dervichian, D. G. Micellar solubilization of cholesterol by biliary salts and lecithins extracted from human bile. Biochimie 53, 751-754 (1971).

Mel'nikov, S. M.; Seijen ten Hoorn, J. W. M.; Eijkelenboom, A. P. A. M. Effect of phytosterols and phytostanols on the solubilization of cholesterol by dietary mixed micelles: an in vitro study. Chem. Phys. Lipids 127, 121-141 (2004).

Staggers, J. E.; Hernell, O.; Stafford, R. J.; Carey, M. C. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. I. Phase behavior and aggregation states of model lipid systems patterned after aqueous duodenal contents of healthy adult human beings. Biochemistry 29, 2028-2040 (1990).

Matsuoka, K.; Kuranaga, Y.; Moroi, Y. Solubilization of cholesterol and polycyclic aromatic compounds into sodium bile salt micelles (Part 2). Biochim. Biophys. Acta 1580, 209-214 (2002).

Matsuoka, K.; Nakazawa, T.; Nakamura, A.; Honda, C.; Endo, K.; Tsukada, M. Study of thermodynamic parameters for solubilization of plant sterol and stanol in bile salt micelles. Chem. Phys. Lipids 154, 87-93 (2008).

Allain, C. C.; Poon, L. S.; Chan, C. S.; Richmond, W.; Fu, P. C. Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470-475 (1974).

Richmond, W. Preparation and properties of a cholesterol oxidase from Nocardia species and its application to the enzymic assay of total cholesterol in serum. Clin. Chem. 19, 1350-1356 (1973).

Sugioka, H.; Moroi, Y. Micelle formation of sodium cholate and solubilization into the micelle. Biochim. Biophys. Acta 1394, 99-110 (1998).

Matsuoka, K.; Ishii, S.; Honda, C.; Endo, K.; Saito, A.; Moroi, Y.; Shibata, O. NMR study on solubilization of sterols and aromatic compounds in sodium taurodeoxycholate micelles. Bull. Chem. Soc. Jpn. 80, 2334-2341 (2007).

Ishizaki, T. Application of phytosterolesters to food . FOSHU Pure Select SARALEAR . Foods Food Ingre. J. Jpn. 210, 512-518 (2005).

Rasmussen, H. E.; Guderian, D. M. Jr.; Wray, C. A.; Dussault, P. H.; Schlegel, V. L.; Carr, T. P. Reduction in cholesterol absorption is enhanced by stearate-enriched plant sterol esters in hamsters. J. Nutr. 136, 2722-2727 (2006).

Doi, Y.; Kawashima, Y.; Matsuoka, K.; Moroi, Y. O/W Emulsion of n-Alkylbenzene/Ionic Surfactant/Water Systems. J. Phys. Chem. B 108, 2594-2599 (2004).

Wauchope, R. D.; Getzen, F. W. Temperature dependence of solubilities in water and heats of fusion of solid aromatic hydrocarbons. J. Chem. Eng. Data 17, 38-41 (1972).

Armstrong, M. J.; Carey, M. C. Thermodynamic and molecular determinants of sterol solubilities in bile salt micelles. J. Lipid Res. 28, 1144-1155 (1987).