The Role of Phytosterols as Cholesterol Lowering Agents

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INTRODUCTION

One of intervention medicine in lowering incidence of cardiovascular disease is by lowering the plasma cholesterol level. Various methods have been done, i.e. low fat diet. But low fat diet often gives disappointing result.[1] As happened in large scale low fat diet campaign of England, there was only 1-2% decrease of plasma cholesterol level compared to the last 25 years -average cholesterol level.[2]

Recently, one of diet methods that has been studied is diet supplementation of high lipid phytosterols.[1,2,3,4] Phytosterols are natural plant-sterols substances. Phytosterols have the same function as cholesterol in animal, that is as part of cell membrane.[2,3,4] Phytosterols are abundant in olive, corn, and sunflower seed oil, even though in general every vegetables contain Phytosterols.[1,2,3,4,5] Phytosterols are widely consumed as margarine, vegetable oil enrichment and in supplement capsule form.[1,3,4,5,6]

There are many advantages of phytosterols consumption. Phytosterols are more effective than low fat diet in lowering plasma cholesterol.[1,2] Phytosterols have equal effectiveness in lowering cholesterol as others anti lipid drugs other excluding statin.[1,2,3] Other advantages of phytosterols consumption are they did not cause any changes of taste or form in enriched diets. Phytosterols may also be used as adjuvant therapy to statin. Until now, phytosterols have no hazard to health.[2]

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The Structure of Plant Sterols and Plant Stanols

Phytosterols are natural chemical compounds which are present in plants, consist of plant sterols and plant stanols.[1,2,3,4,5,6] Both are abundant in vegetable oil as sunflower seed and olive oil.[1,3,4,5,6,7,8]

Plant sterols are sterols with carbonic group C-28 or C-29. It has similar structure with cholesterol contains C-27 carbonic chain.[3,4] Plant sterols consist of three group, i.e. : 4- desmethylsterols (has not any methyl), 4-monomethylsterols (with one methyl group) and 4,4-dimethylsterols (with two methyl groups). Majority of plant sterols are cytosterol, campesterol and stigmasterol. They are belong to 4- desmethylsterols.[1,2,3,4] Plant stanols are hydrogenation of plant sterols. Naturally, food contain very small amount of them than plant sterols.

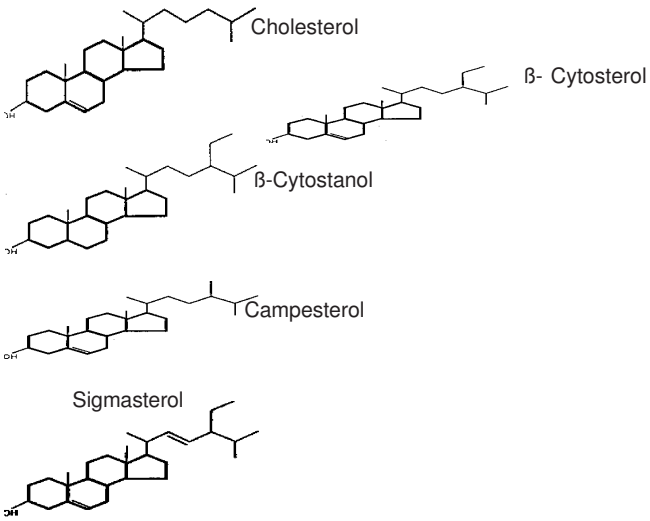

Figure 1. Structure of Plant Sterols, Cholesterol and Plant Stanols

Absorption and Metabolism

Plant sterols and plant stanols are hardly absorbed substance. Only about 1,5 – 5% of consumed cytosterols that may be absorbed.[1,2,3,4] While cholesterols are more absorbable than plant sterols. About 20 – 80% of consumed cholesterol will be absorbed in gastrointestinal tract. Consumption of 160 – 360 mg plant

sterols per day will give plasma cytosterol level by 0,3-1,7 mg/dl. Consumption of 3,24 g/dl plant sterols will increase cytosterol plasma level by 40% and campesterol by 70%. Difference of absorbance level is determined by the length of sterols, the longer sterol chain length is more difficult to be absorbed because of their hidrophobic nature. Plant stanols are less absorbable than plant sterols. Cytostanols absorbance rate range from 0-3 % and they can be detected in plasma. Likewise the most stanols, campestanols are least absorbable than campesterols.[4]

Phytosterols with lower absorbance level are preferable, because high plant sterols level in plasma will accelerate development of atherosclerosis and increase the risk of coronary heart disease.[1,2,4]

Action Mechanism of Phytosterols

Both plant sterol and plant stanols may lower the plasma cholesterol level by inhibiting cholesterol absorption, either diet cholesterol or endogen cholesterol. Up to now we are not certain about the mechanism of plant sterols and plant stanols in inhibiting cholesterol absorption. There are two theory which explain about possibility of cholesterol absorption inhibition by phytosterols, i.e.:[1]

1. Co- precipitation of cholesterol and plant sterols/ stanols
   In intestine lumen, cholesterol is found as solution together with other form of lipid. If fatty acid and monoglyceride were absorbed by the intestines, then concentration of hardly absorbed substances will be increase. If those substances have reached maximal level, then similiar substances will precipitate in solution. This may occur in cholesterol and plant sterols or plant stanols because of their similiarity in structure. Both cholesterol and plant sterols / stanols in free-form have low solubility in lipid or miselle. They will cause lower solubility level mutually. The greater amount of plant stanols or plant sterols, the lower solubility of cholesterol and the more precipitated cholesterol.
2. Competition of plant sterols / stanols and cholesterol in miselle
   Miselle is lipid dissolving detergent – lipid that present in the intestine. Miselle consist of gall salt, phospholipid, tri,di-and monoglyceride, fatty acid and free cholesterol. In absorption process, cholesterol in the miselle will be transported from intestine lumen through intestinal mucosa toward lymph drainage. Miselle has very limited cholesterol-carrying capability. There are other substances that have similiar structure as plant sterols or plant stanols that will compete with cholesterol to occupy the site in miselle. The larger amount of plant sterols or plant stanols, the less cholesterol in miselle then the less cholesterol that may be absorbed.

By mechanism as mentioned, cholesterol lowering effect of plant sterols or plant stanols may only be achieved if both of them are in large amount. The average amount of plant sterols or plant stanols in our food are not sufficient enough to achieve the cholesterol lowering effect in plasma. In order to achieve cholesterol lowering effect, we need 1 gram per day of plant sterols consumption.[1,2,3,4,5,6,7]

The Cholesterol Lowering Effect of Phytosterols

Since 50 years ago plant sterols are known as lowering plasma cholesterol agent. There has been a lot of long-term studies about cholesterol lowering effect of plant sterols and has been involving more than 1800 persons.[1] Since then, β sitosterol agent (Cytellin, Eli lily) has been sold out in United States for hypercholestrolemia therapy. Recently there are many margarine products which has been enriched by plant sterols or plant stanols.

Hendriks, et al found that avarage margarine consumption of European is about 20 gram per day. If this margarine is enriched by 8-10% plant sterols, then it will decrease the total plasma cholesterol and LDL cholesterol by 8 –13 %. This number is equivalent to consumption of plant sterols by 1.6 – 2.0 gram per day. Other study by Nguyen found that 24 gram margarine consumption per day, that has been enriched by 2-3 gram plant stanols esther will decrease total cholesterol more than 6.4% and LDL cholesterol by 10.1 %.[1,4]

The cholesterol lowering effect of plant sterols and plant stanols are dose dependent. The greater dose of plant sterols or plant stanols, the greater cholesterol lowering effect. But this only valid for doses up to 2.4 gram. Greater dose than 2.4 gram do not have greater cholesterol lowering effect. This is known from Hallikainen, et al study where 2.4 gram to 3.2 gram dose elevation did not increase any clinical significance.[8]

In the study about correlation between cholesterol lowering effect of plant sterols and plant stanols and age, we found that old age has greater cholesterol lowering effect than the young. In 50-59 years group with >2 gram dose per day we found LDL cholesterol decreased by 0.54 mmol/l. In 40-49 years, LDL cholesterol decreased by 0.43 mmol/l and in 30-39 years, LDL cholesterol decreased by 0.33 mmol/l.[2]

LDL cholesterol level decrease by 0.5 mmol in

50-59 year and it was related to lower risk factors of heart disease by 25%. At the young age even they had lower cholesterol decrease but their risk factors of heart disease remained still. It was because of stronger correlation between cholesterol and heart disease in younger age. Hence, using margarine enriched with plant sterols or plant stanols hopefully will decrease heart disease risk factor by 25%.[1,2]

The cholesterol lowering effect of plant sterols or plant stanols is greater than low fat diet. Nowdays the result of low fat diet is very dissapointing even there has been large scale health campaign about low fat diet.[2,4] A survey in England revealed lowering cholesterol value only 1-2% compared to the last 25 years. A study investigated by Jones, comparing the effect of low fat diet and diet that had been enriched by 1.84 gram plant sterols and plant stanols per day in 50 hyper-cholesterolemia patient for 21 days. On 21st day, he found lowering LDL cholesterol result by 3.9% ($p<0.01$) in patients who had low fat diet, while in the patients who had plan sterols there were lowering LDL cholesterol by 12.9% ($p<0.001$), and in patients who had plant stanols there were 7.9% ($P<0.001$).[6] (figure 2 & 3)

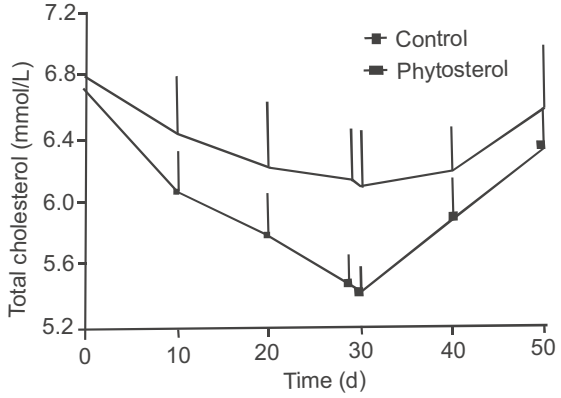

Figure 2. The Effect of Phytostrerols on Total Cholesterol Compared to Control in Jones's Study

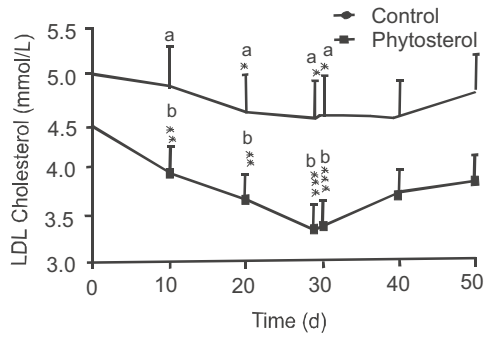

Figure 2. The Effect of Phytosterols on LDL Cholesterol Compared to Control in Jones's Study

The Effect of Phytosterols Against Other Lipids

The effect of plant sterols and plant stanols against other lipids are different from their effect against LDL cholesterol and total cholesterol. They have no significant effect against HDL cholesterol and triglyceride, The Jones's study found no significant alteration of HDL cholesterol in 50 hypercholesterolemia patient who had 1.84 gram plant sterols / stanols for 21 days.

Individual Response Against Phytosterols

Plant sterols and plant stanols are more effective in decreasing individual cholesterol which has high ability type in absorbing cholesterol and low for synthesis. The ability to decrase LDL cholesterol by plant sterols/stanols is equivalent to its ability in inhibiting cholesterol absorption. In other hand, individuals with high cholesterol synthetising and low absorption type, their lowering cholesterol effect of plant sterols/stanols are lower.This individual type may be estimated by measuring precursor sterols level in the synthesized cholesterol and by measuring the level of plasma plant stanols such as campesterols or cytosterols. But those measurement had never been done in clinical practice.

The Diet Effect Against Plant Sterols and Plant Stanols Mechanism

In the early study of plant sterols and plant stanols stated lowering cholesteol effect of plant sterols / stanols was related to high fat diet. Most of studies had used higher fat and cholesterol diet than avarage American diet. Hence there was a question whether plant sterols / stanols are effective enough in subject with low fat diet and cholesterol. Hallikainen and Uusitopa (1999) performed study in subject having diet appropriate wiht second step diet of National Cholesterol Education Program (NCEP), that is 26% average total fat, 6.9% saturated fat and cholesterol 146 mg / day. In this study, over 2 gram plant sterols per day may decrease LDL cholesterol over than 13.7% and decrease total cholesterol over 10.6%. Hence, the plant sterols still could decrease cholesterol in subject who had underwent low cholesterol diet (<250 mg/day). This fact strengthen the asumption that plant sterols / stanols may affect cholesterol absorption either diet or biliary cholesterol.

Plant Sterols/Stanols and Anti Lipid Drugs

The lowering cholesterol ability of plant sterols/ stanols by 0.5 mmol/L equivalent to all anti lipid drugs except statin. Statin has three times greater ability in lowering cholesterol than plant sterols / stanols.

Combination of plant sterols / stanols and statin have

Table 1. The Study Result Summary of Plant Stanols Esthers (PSE)

Study	Subjects	PSE intake	Baseline Cholesterol	Dietary Cholesterol	Duration of use	TC reduction	LDL-C reduction[2]
	N	G/d	Mmol/L	Mg/d	Wk	%	
Vanhanen et al 1984	7 (8)	0.8	5.5	265	6	5	7
Miettinen and Vanhanen 1994	7 (8)	0.8	5.5	294	9	5[3]	8[3]
Miettinen et al. 1995	51 (5)	1.8	6.0	308	52	9[3]	11[3]
Vanhanen 1994	7 (8)	2.0	5.5	265	6	9[3]	16[3]
Ninikoski et al 1997	12 (12)	2.2	5.0	NR	5	10[3]	13[3]
Halkainen and Uusitupa 1999	20 (17)	2.2	6.1	141	8	8[3]	9[6]
Halkainen and Uusitupa 1999	18 (17)	2.4	6.6	162	8	11[3]	14[6]
Westrate and Meijer 1996	100 (100)	2.5	5.2	243	3.5	7[3]	13[6]
Miettinen et al 1995	51 (51)	2.6	6.1	340	52	10[3]	14[6]
Gylling and Miettinen 1994	114 (11)	3.0	6.0	357	6	5[3]	9[3]
Gylling and Miettinen 1994	84 (8)	3.0	6.6	233	7	11[3]	14[6]
Gylling et al 1997	115 (11)	3.0	6.0	207	7	6[3]	15[6]
Gylling et al 1995	156 (15)	3.0	7.7	114	6	11[3]	15[6]
Vanhanen et al 1993	34 (33)	3.4	5.9	270	6	8[3]	10[6]
Plat et al 1998	34 (42)	3.7	5.0	230	8	8[3]	12[6]
Plat et al 1998	36 (42)	3.8	5.0	247	8	8[3]	13[6]

1. Adult men and women in PSE group unless indicated; n for control group in parenthesis
2. Difference between control and PSE group in % total cholesterol or LDL cholesterol change from baseline to end of study
3. $P < 0.05$ vs control
4. Type 2 diabetics
5. Postmenopausal women
6. Familial hypercholesterolemia children.

a synergic effect, this combination especially appropriate to individual with low cholesterol synthesizing type and high absorption ability. This combination is believed that it may lower the heart disease incidence. The ischemic heart disease patient, plant sterols or stanols still may not substitute the statin. Statin has higher ability in lowering cholesterol.

The study about combination of plant sterols / stanols and statin was done by University of New South Wales Lipid Research Department, St Vincent's Hospital, Sydney, Australia. This study was done by double-blind method in 236 adult subjects with hypercholestrolemia. Participant were divided into four groups with four different treatement, i.e. :

1. 400 mg cerivastatin and 25 gram of ordinary margarine per-day.
2. 400 mg cerivastatin and 25 gram of enriched margarine by sterol esther per-day
3. Placebo and 25 gram of ordinary margarin per-day
4. Placebo and 25 gram enriched margarine by sterol esther per-day

The result of this study revealed 32% decrease of LDL cholesterol in cerivastatin consumption compared to placebo. In margarine consumption that had been enriched by sterol esters, there were 8% decrease of LDL cholesterol compared to ordinary margarine. The combination of statin and margarine that had been enriched by sterol esther developed LDL cholesterol decrease by thirty nine percent. This amount is equivalent to double dose statin. Hence, combination of plant sterols or plant stanols and statin may be an alternative to high-dose statin patients.

The Effect of Plant Sterols/Stanols Against Absorption of Lipid-soluble Vitamin

Some of studies that had been held by Hallikainen, et al ; Hendrik,et al; Westrstrate and Meijer, conclude that plant sterols / stanols may reduce absorption of β-

carotene (pro-vitamin A), lycopene, and β-tocopherol (vitamin E). In study carried by Law (2000) found that plant sterols / stanols reduce concentration of plasma β-carotene by 25 %, β- carotene by 10 %, vitamin E by 8 %.

The function of those mentioned vitamins is to prevent oxidation of LDL cholesterol. But decrease of those vitamins by plant sterols/stanols consumption was not clinically significant. Decrease of those vitamins was balanced by decrease of plasma LDL cholesterol by plant sterols/stanols Hendrik et al considered absorption inhibition of lipid-soluble vitamin, they suggested dose limitation of plant sterols/stanols every day by 1.6 gram. At this dose there was no significant reduce of plasma carotene level.

The Side Effect of Plant Sterols and Plant Stanols

Studies of phytosterols have been conducted since 1950 and have involved over 1800 subject with dose up to 25 gram per day with duration of study over than 3 years. So far there is no dangerous side effect.

Studies about absorption, distribution metabolism and excretion of plan sterols and plant stanols found that plant stenols are very low absorbed in intestine. It is only about 1-10 %. Then it will be immediately eliminated through the gall. Study of rats fed by plant sterols with 3900 mg / kg BW / day dose, there was no side effect. Similar study with plant stanols esther by 1.78% dose equivalent to 1.0 % free plant stanols was reported with no side effect.

Some of literatures state that plant sterols are estrogenic in its nature. But in some of in vitro studies using plant sterols, it seems that plant sterols were not bound to immature human or rats estrogen receptor. Also in in vivo studies on rats there was no estrogenic nature of plant sterols. Plant stanols were also not proven to have estrogenic nature either in vitro (proliferation of MCF-7 cells) or in vivo studies. In toxicity test of reproduction that was performed by Waalken-Berendens (1999), we found that plant sterols and plant stanols are safely consume up to 4000 mg/kgBW/day.

In some of studies that was performed by Weststrate; Hendriks; Ayesh using 8.6 gram plant sterol per- day for 4 weeks period, there was no hematological nor intestinal micro-flora alteration. The general condition evaluation of subject has also no hazardous side effect.

There is one condition that rarely found, known as Phytosterolaemia (cytosterolemia) that is a defect of Phytosterol metabolism. Until now there are only 34 cases over the world. Phytosterolaemia is autosomal recessive disorder which is signed by high plant sterols absorption level and decreasing of plant sterols excretion. The clinical manifestation are tendon xanthoma and early atherosclerosis development.

CONCLUSION

The term " phytosterols " covers plant sterols and plant stanols. Phytosterols are naturally occurring substances present in the diet as minor components of vegetable oils. Plant stanols, occurring in nature at a lower level, are hydrogenation compounds of the respective plant sterols.

An elevated level of blood cholesterol is one of the well established risk factors for coronary heart disease. Both plant sterols and plant stanols are effective in lowering plasma total and LDL cholesterol and this occurs by inhibiting the absorption of cholesterol from the small intestine. In order to achieve a cholesterol lowering benefit approximately 1 gram/day of plant sterols or plant stanols need to be consumed, while plant sterols or plant stanols in common diet is averaging from 200-400 mg per day.

Supplementation of plant sterols esther or plant stanols esther into diet such as margarine is developed in order to lower the cholesterol level. The average margarine consumption of 20 gram per day that has been enriched by 8-10 % plant sterols or plant stanols will decrease the total cholesterol and LDL cholesterol up to 8-13 %.

Both plant sterols and plant stanols have equal ability with anty lipid drugs in lowering cholesterol, except statin. Statin has three times ability in lowering cholesterol compare to plant stanols and plant sterols. Statin may be used together with plant sterols and plant stanols. Combination of plant sterols or plant stanols and statin causes equivalent lowering cholesterol effect as double dose statin.

Plant sterols and plant stanols appear to be without hazard to health in large number of human studies. There are no evidence of toxicity even at high dose plant sterols and plant stanols.

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