The Effect of Phytosterol on Serum Beta-Carotene Concentration of Hypercholesterolaemic Subjects

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Objective: Treatment of optic neuritic as recommended by the Optic Neuritic Treatment Trial (ONTT) was intravenous methylprednisolon followed by oral prednisone. This study aims to describe characteristics and response to intravenous methylprednisolon followed by oral prednisone treatment of optic neuritic patient in Sanglah General Hospital Denpasar.
Method: The study was a parallel clinical trial, forty subjects with hipercholesterolaemia were selected using certain criteria. The subjects were divided into two groups using block randomization. Twenty subjects in phytosterol group received phytosterol 2x0.6 g/day and dietary counseling (P), while twenty subjects in counseling group received only dietary counseling (C). Serum total cholesterol and β-carotene concentration were assessed on day 0 and 42. Before and after treatment, dietary intake were assessed with 2x24 hour recall methods.
Results: Seventeen subjects in P group and fourteen subjects in C group completed the study. There was no significant differences in baseline data both groups. After 42 days treatment, there was no significant differences in nutrients intakes in two groups except for β-carotene and phytosterol intakes in P group. There was a 9.17% significant reduction in serum total cholesterol concentration in P group while no change was observed in the C group ($p<0.05$). Serum β-carotene concentration was increased significantly in P group, 0.44±0.376 μmol/L to 0.536(0.21–1.95) μmol/L, while there was no significant increased in C group, 0.493±0.349 μmol/L to 0.56±0.33 μmol/L. There was a significant difference in serum β-carotene concentration after adjusted with serum total cholesterol concentration between two groups ($p<0.05$).
Conclusions: Dietary advice to consume an additionally of high β-carotene sources when consuming phytosterol 2x0.6 g/day for 42 consecutive days maintains serum β-carotene concentration while lowering serum total cholesterol concentration significantly in hypercholesterolaemic subjects, proved by statistical analysis.
Keywords: Hypercholesterolaemic, phytosterol, serum β-carotene concentration

INTRODUCTION

Cardiovascular disease (CVD) has been the leading cause of death. Coronary Heart Disease (CHD) is one of CVD that the most prevalent cause of death. Based on the epidemiologic research, there are several risk factors identified for the development of CHD. One of the most important risk factors for CHD is dyslipidemia, i.e. hypercholesterolemia.[1.2.3]

Along with increasing incidence of CHD among productive age group, many researches have been done for its prevention and treatment using food-based as well as using pharmacy-based approaches. One of food based is phytosterols or sterol isolated from plants and has a cholesterol-like structure but the action is differ from cholesterol.

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Phytosterols known lower blood cholesterol. Some of the phytosterol has been esterifies and made into food source such as margarines or milk. [4-6]

The prevalence of hypercholesterolaemia in Indonesia, increases at any age groups, start from 25 years of age. Many factors influence the decrease of CVD's prevalence, one of them is the increase of fruit and vegetables intakes as β-carotene sources. Several studies found the importance of β-carotene as antioxidant to reduce CVD's prevalence. Besides, the increasing fruit and vegetables intakes, NCEP ATP III, 2001 suggest to add phytosterol in daily diet to reduce blood cholesterol concentration. Consuming between 2 to 3 g per day lowers cholesterol by 9% to 20%. The mechanism for cholesterol lowering is by inhibiting absorption of dietary cholesterol. Based on previous studies, the cholesterol lowering effects of phytosterol could also lower serum β-carotene concentration. [7-10]

The aim of this study was to investigate the effects of phytosterol 2x0.6 g/day for 42

consecutive days on serum β-carotene concentration in hypercholesterolaemic subjects. Phytoserol in this study is properly solubilized in a partly vegetables oil-filled low fat milk, giving to free-living mildly hypercholesterolemia patients, along with their habitual diet.

MATERIALS AND METHODS

Subjects and sampling

This study was a parallel, clinical trial, matching, pre-post test design, random allocation, and open trial. The objective of the study was to investigate the effect of phytosterol by 2x0.6 g/day on serum β-carotene concentration in hypercholesterolaemic subjects. This study was conducted at Nutritional Departement of Faculty of Medicine, University of Indonesia, Jakarta, from July to December 2006. Inclusion criterias are female or male volunteers aged 20 years or over, serum total cholesterol 200-299 mg/dL, no medical history of metabolic disease (renal failure, diabetes mellitus, nephritic syndrome, or hypothyroidism). Exclusion criterias are ingesting of cholesterol lowering drug or drugs known to interfere with lipid profiles (hormone replacement treatment, diuretics, and β-blocker), and pregnant or nursing mother.

After completing a selection with inclusion and exclusion criterias, there were 40 subjects enrolled in this study. The subjects of this study were divided into two groups by randomization block, which the phytosterol group (P) received supplementation of phytosterol by 2x0,6 g/day with dietary counseling and the counseling group (C) received only dietary counseling for 42 consecutive days. During the intervention period, there were nine subjects dropped out, so at the end of the study there were 31 subjects completing the study, i.e., 17 subjects in the P group and 14 subjects in the C group.

Data were collected by interviewing the subjects for demography, daily activities, smoking habit, eating habit, and daily food intake, as well as physical examinations and laboratory determinations for serum total cholesterol and β-carotene concentrations. This study was already certified by the Ethical Clearance Research Committee of Faculty of Medicine University of Indonesia

Statistical analysis

Data were analysed using SPSS 11, numerical data were described using count and percentages. Numerical data were checked for normal distribution and described as mean and standard deviation if normally distributed and as median and minimum-maximum if not normally distributed. Statistical analysis was performed using independent t-test for normal distributed and Mann Whitney test for not normal distributed data. The level of significance was 5%.

RESULTS

The data show that there were no significant differences between the two groups including age and sex. Most of the subjects were non smoker and no significant differences in both groups. Anthropometric measurement before intervention showed no significant differences in body mass index (BMI) in both groups.

The beginning of intervention phase, there were no significant differences in nutrients intakes based on Food Frequency Questionnaire (FFQ) Semiquantitatif and daily food intakes based on recall 2x24 hours between two groups, but there were significant differences in β-carotene and phytosterol intakes during the intervention, especially in P group ($p<0.05$).

Laboratory findings after intervention showed that there were the decrease of serum total cholesterol concentration in P group ($p<0.05$), the increase of serum β-carotene in P group ($p<0.05$), and increased serum β-carotene after being adjusted with total cholesterol in P group ($p<0.05$), while there were not found in C group There were significant differences between the two groups on the percentage of change of laboratory findings before and after intervention. There was a significant decrease in serum total cholesterol concentration by 9.17±12.35% in P group, and an increase by 0.17±8.97% in C groups. There was a significant increase in serum β-carotene concentration by 132.32±153.40% in P group, and significant increase in serum β-carotene concentration after adjusted with serum total cholesterol concentration by 33.26±51.22% in C groups (Table 1).

Table 2 showed a changing and percentage of total cholesterol concentration , the results showing the reduction 9.17±12.35% in P group, meanwhile in K group there is an increased 0.71±8.97%. The changing and percentage between this two group, its show significantly the difference ($p<0.05$).

Beta-carotene concentration increased 132.32±153.40% in group P and 33.26±51.22% in group C. There is a significant different in changing and changed percentage in both groups ($p<0.05$). After adjusted with total cholesterol concentration, there is an increased 137.84 (-29.70–737.94)% in P group and 33.58±54.05% in C group (Table 2).

DISCUSSION

This study show a reduction in total colesterol concentration in P group 9.71% but in C group 0.71%. Some theries about lowering cholesterol mechanism is competition theory between phytosterol and cholesterol while entering into micel, lowering in chylomicron formation, and as a

Table 1 Laboratory Findings Before and After Intervention (42 Days)

Laboratory findings	P (n=17)	C (n=14)	*p value*
Total cholesterol concentration (mg/dL)			
Before	233.47±24.64	218.29±18.58	0.067
After	212(162-276)	219.57±24.29	0.293
p	0.010*	0.802	
Beta-carotene concentration (μmol/L)			
Before			
After	0.44±0.376	0.493±0.349	0.694
p	0.536 (0.21–1.95)	0.56±0.33	0.153
	0.04*	0.289	
Beta-carotene : total cholesterol concentration(μmol/mmol)			
Before			
After	0.074±0.062	0.087±0.06	0.546
p	0.151±0.096	0.099±0.058	0.076
	0.001*	0.306	

* significance at $p<0.05$

Table 2 Changing and Percentage Changes in β-Carotene Concentration After Adjusted with Total Cholesterol Concentration

Laboratory findings	P (n=17)	C (n=14)	*p*
Total cholesterol serum			
Changing (mg/dL)	-22.29±28.71	1.29±18.76	0.013*
Percentage	-9.17±12.35	0.71±8.97	0.018*
Beta-carotene serum			
Changing (μmol/L)	0.36±0.43	0.06±0.21	0.018*
Percentage	132.32±153.40	33.26±51.22	0.028*
Rasio β-carotene: total cholesterol serum			
Changing (μmol/mmol)	0.077±0.081	0.012±0.042	0.008*
Percentage	137.84 (-29.70–737.94)	33.58±54.05	0.012*

* significance at p<0.05

ligand to activate Liver X Receptor (LXR), to form heterodimer with Retinoid X Receptor (RXR), for the next steps is gene modulating and protein synthesis that play role in absorption, metabolism, and cholesterol secretion.[5,7,8]

This study shows a reduction of total cholesterol concentration 9.17% (amount of phytosterol 1.2 g/day), this result is much greater than the finding of Thomsen *et al*[6], which found a reduction of total cholesterol concentration 4.73% (for dose of 1.2 g/day) and 7.09% (for dose of 1.6 g/day) during four weeks. The greater reduction was probably caused by phystosterl action itself, not only because of hypercholesterolemic diet as suggestion in this study, and duration of this study (six weeks).

This study used β-carotene concentration as parameter to look for its role to maximising phytosterol action, and yet to see β-carotene as safety parameter. Previous study,Westrate dan Meijer[11] mentioned that β-carotene as sensitive parameter to control the influenced lowering lipid soluble vitamins absorption in phytosterol comsuption. Another meta-analysis study by Katan *et al*[12] to find in 18 studies showing significant reductions in β-carotene concentration after adjusted by total cholesterol concentration but not happened with other carotenoid or lipid soluble vitamins. Richelle *et al*[5] and Traber[13] showed a

theory of reduction in β-carotene alone, the reduction caused by lowering absorption of β-carotene, lowering lipoprotein concentration as β-carotene transportation in the circulation, and the recent theory is increasing of β-carotene as a ligand that activated RXR. Korpela *et al*[14], using β-carotene as a safety parameter in phytosterol consumption.

This study showed an increased of β-carotene concentration along with higher consumption of β-carotene sources in P group. Result of this study showed the same result as Raeni-Sarjaz *et al*[15] study that showed an increased of β-carotene concentration 16,2% in phytosterol group compare control group. The result of the study showed the increasing of β-carotene concentration becaused of healthy food consumption which is raising consumption of fruits and vegetables especially yellow-orange fruits and green leafy vegetables.
Noakes *et al*[16] showed a significant increased of β-carotene concentration 13% in control group along with increasing consumption β-carotene sources 72%. Meanwhile in phytosterol group there was no significant changed in β-carotene concentration, even there was an increased of . consumption β-carotene sources. Noakes *et al*[16] suggestion that to eat more that five portion per day of high sources of β-carotene.

This study, giving 1.2g/day (2x0.6g) of phytosterol was the same dose with Hendriks *et al*[4] study that suggest phytosterol with optimal dose to give cholesterol lowering effect, without affecting β-carotene concentration. The best dose is 1.6 g/day compare with lowest dose (0.83 g/day) atau highest (3.24 g/day). This effects caused by the smallest dose which could lowering cholesterol concentration lower with higher effect of lowering β-carotene, the theory was that the smallest dose did not caused adaptation mechanism to cholesterol concentration in the body resulting decreasing cholesterol total not as much as predictive but still using β-carotene as a ligand RXR approximately. This mechamism could caused the lowest of β-carotene concentration in the circulation of the body. Giving the biggest dose, lowering effect of cholesterol could happened along with lowering β-carotene concentration that as higher as lowering effect of phytosterol to cholesterol concentration.
Phytosterol product in low fat milk could lowering effect of losing β-carotene, Richelle *et al*[5] showed that free phytosterol caused the lowest β-carotene effect that ester phytosterol. Ester form of phystosterol could join the micel easily than free phytosterl, so the reduction of β-carotene could minimalize. The processed to cook β-carotene sources should be thought too, concumption β-carotene sources without fat could reduced its absorption, subject usually drink fresh carrot juice or raw vegetables, the absorption of this product could lowering its absorption, so in this study we tought the subjects to provide β-carotene sources with the right processing through diet counseling.
The increased of β-carotene carotene in this study probably becaused of increasing of β-carotene sources in P group, but not happened in C group. The theory becaused of phytosterol action that need β-carotene as a ligand to form a heterodimer to activated gene expression in cholesterol metabolism. This could answer why β-carotene concentration seem not significant in P group compare with C group even the rising of consumption much more.

Complaint from the subject in P group were nausea in the beginning drinking phystosterol's milk and diarrhea, but only happened in the beginning of the study but for the rest of study there was no other complaints, this probably due to phsycological effect of the subjects because before this intervention, dringking milk is not a usual diet, this could be the typical of Indonesia's food choice, not including milk in daily diet. Previous study report that there was no side effect in consumption phytosterol and phytosterol already registered as safe food product by *Food and Drug Administration* (FDA).

Conclusion

These findings proved that giving phytosterol supplementation by 2x0,6 g/day (1,2 g/day) for 42 consecutive days and high β-carotene intakes could maintain serum β-carotene concentration, while lowering serum total cholesterol concentration.

Recommendations

There is a need to conduct further study with a gold standard design , that is randomized controlled trial (RCT) by using control group, double blind, with placebo, and bigger sample size. Education or suggestion should be given to hyper cholesterolaemic subjects who consuming food product enriched by phytosterol, to increase fruits and vegetables intakes especially food sources which are high in β-carotene (carotenoid) along with the right selection and cooking of food sources to maintain β-carotene concentration.

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