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ORIGINAL ARTICLE

Phytosterols, Cholesterol Absorption and Healthy Diets

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Abstract The purpose of this review is to outline the emerging role of dietary phytosterols in human health. Dietary saturated fat, cholesterol and fiber are currently emphasized in the reduction of low-density lipoprotein cholesterol levels. However, other dietary components such as phytosterols may have equivalent or even larger effects on circulating cholesterol and need further study with respect to the potential for coronary heart disease risk reduction. Phytosterol effects were not considered in classic fat-exchange clinical trials and may account for some of the differences attributed to the food fats studied. Phytosterols reduce cholesterol absorption while being poorly absorbed themselves and the effects can be studied in human subjects in single-meal tests using stable isotopic tracers. Because phytosterols are insoluble and biologically inactive when purified, careful attention needs to be given to ensuring that commercial supplement products are rendered bioavailable by dissolution in fat or by emulsification. Recent work shows that phytosterols in natural food matrices are also bioactive. The retention of phytosterols during food manufacturing and the use of foods with high phytosterol content may constitute an alternative to the use of supplements.

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Introduction

There is a scientific consensus that reduction of low-density lipoprotein (LDL) cholesterol is important for coronary risk reduction and specific recommendations for cholesterol monitoring and treatment in the general population have been made by the United States National Cholesterol Education Program [1]. Lifestyle variables, including diet and dietary supplements, are an important part of this initiative. A better understanding of how dietary sterols may relate to LDL cholesterol can be gleaned from Fig. 1, which sketches the framework of whole body cholesterol metabolism [2]. Dietary cholesterol intake is variable but is often less than 300 mg/day and currently 200 mg/day is recommended. Biliary cholesterol is larger and is the principal component of intestinal cholesterol. Approximately 25% of the plasma cholesterol production rate is due to absorbed dietary cholesterol while 75% is accounted for by endogenously synthesized cholesterol [3]. However, dietary cholesterol appears to be quite important because it and endogenous cholesterol biosynthesis are inversely correlated, suggesting that they are coregulated [4]. In contrast to other nutrients, for which gastrointestinal absorption is nearly quantitative, cholesterol absorption averages only 56% (Fig. 2) and is variable between individuals [5]. However, when measured repeatedly in the same individual under standardized conditions, percentage cholesterol absorption is highly reproducible [6]. This indicates that there may be substantial inter-individual differences in susceptibility to dietary sterols. Since the principal route for cholesterol elimination is excretion in the stool, the efficiency of cholesterol absorption is a critical determinant of cholesterol catabolism and

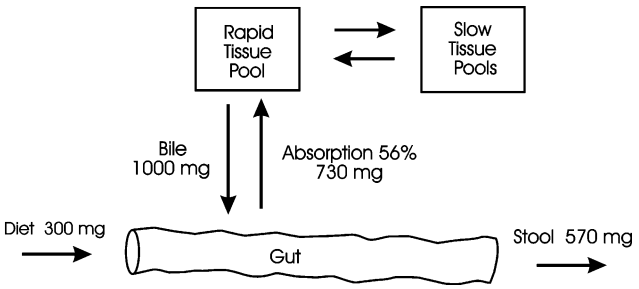

Fig. 1 A model for cholesterol absorption and metabolism. Taken from [2]

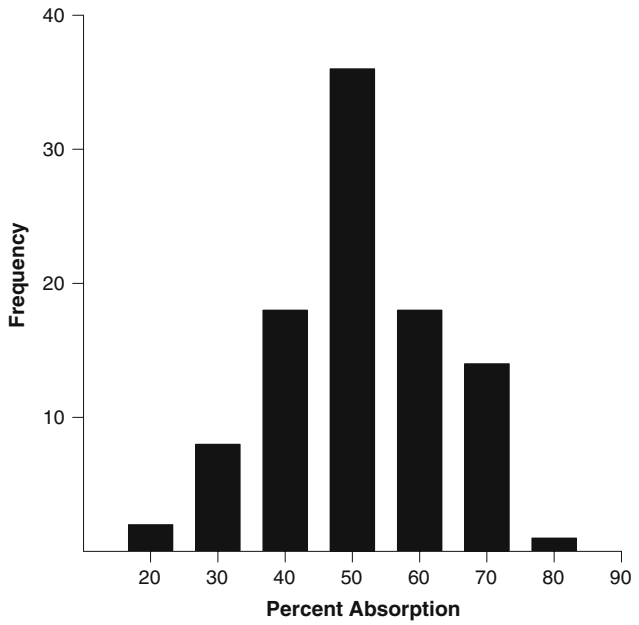

Fig. 2 Distribution of percent cholesterol absorption in normal subjects. Taken from [5]

unabsorbed dietary and biliary cholesterol are the largest terminal components of what is often termed reverse cholesterol transport [2, 7, 8]. This model underscores the potential importance of increasing cholesterol catabolism and complements the pharmaceutical emphasis on reducing cholesterol biosynthesis with statin drugs.

Types of Phytosterols

Phytosterols are structurally similar to cholesterol but have slight modifications of the aliphatic side chain [9, 10]. The principal molecular forms are sitosterol, campesterol and stigmasterol, and these may be esterified in the 3-position with fatty acids or ferulic acid. Since it is difficult to separate all the phytosterols in pure form in the amounts needed for clinical studies, there is relatively little information about the biological effects of individual phytosterols and they are usually given as mixtures in clinical trials. Phytosterols are often categorized into classes consisting of Δ^5-sterols and 5-α-reduced stanols, which appear to be equally effective in reducing LDL cholesterol [11]. Phytosterol glycosides are present in substantial quantities in many foods [12], but they are not cleaved efficiently by pancreatic enzymes in vitro so that their bioactivity has been questioned [13]. More work is needed to clarify the effectiveness of carefully purified and characterized phytosterols and their conjugates.

Mechanism of Action

Phytosterols are thought to act primarily in the intestinal lumen. As cholesterol analogs they compete for cholesterol in absorptive micelles resulting in reduced solubility of cholesterol [14–16]. The affinity of plant sterols for micelles is greater than that of cholesterol [17]. In important human physiological studies the inclusion of phytosterols in a test meal resulted in the reduction of absorbable micellar cholesterol in duodenal aspirates [18]. An intralumenal site of action fits well with the small net systemic absorption of phytosterols, which varies by chemical structure. Δ^5-Sterols such as campesterol and sitosterol have absorptions of 1.9 and 0.5% whereas stanols such as sitostanol have values as little as 0.04% [19]. A recent report of rapid and presumably transient absorption of phytosterols in an animal model [20] needs to be followed up since the low absorption figures cited above would not include those undergoing enterohepatic recycling with preferential biliary excretion. Although phytosterols have been proposed to have intracellular actions in enterocytes [21, 22], recent work has failed to show effects of phytosterols on cellular cholesterol transport proteins and the question of cell-based mechanisms remains open [23].

The reduction in cholesterol absorption by phytosterols is incomplete, with 30–40% decreases in absorption efficiency being reported even with high phytosterol doses [24, 25]. The prescription drug ezetimibe reduces cholesterol absorption by only 54% in humans [26]. These data open the possibility that there may be more than one pathway for cholesterol absorption and also suggest that more-effective methods of blocking cholesterol absorption need to be sought.

Phytosterols have a primary mechanism of action in the intestine, but these effects are mirrored in changes to circulating LDL cholesterol. Reduced delivery of absorbed cholesterol to the liver resulted in increased tissue LDL receptor expression as measured in

peripheral blood mononuclear cells [27]. In some subjects the production rate of LDL was decreased [28]. Further evidence of relative cholesterol deficiency after phytosterol treatment is the measured increase in whole body cholesterol biosynthesis of 38–53% [29]. This also demonstrates the rationale for using phytosterols with statin drugs.

Phytosterol Supplements

Phytosterols, which are inherently hydrophobic and tend to form stable crystals, must be solubilized or formulated in order to become bioavailable [24]. Figure 3 demonstrates that dried unesterified sitostanol has very little immediate solubility in artificial bile salt micelles and even after many days the results are similar. But sitostanol emulsified with lecithin or other agents dissolves in the micelles in minutes. When unesterified sitostanol was administered to humans in pure crystalline form there was no significant effect on cholesterol absorption even at a dose of 1 g, but after formulation with lecithin cholesterol absorption was reduced by 37% [24].

Sitostanol/lecithin complexes reduced cholesterol absorption by 32–38% when administered to patients in test meals in nonfat beverages or egg whites and resulted in a 14.3% reduction in LDL cholesterol when taken for several weeks [30]. Other emulsifiers are also effective. Unesterified mixed phytosterols combined with sucrose ester and dispersed in milk reduced cholesterol absorption by 32% [31]. Food matrices themselves may be used as emulsifiers as demonstrated by direct addition of mixed unesterified phytosterols to orange juice at elevated temperature and pressure to form a non-sedimenting product that reduced LDL cholesterol by 12.4% [32]. Since cholesterol needs to be in micellar form to be absorbed [33], it is likely that preparing phytosterols in micellar form would improve activity.

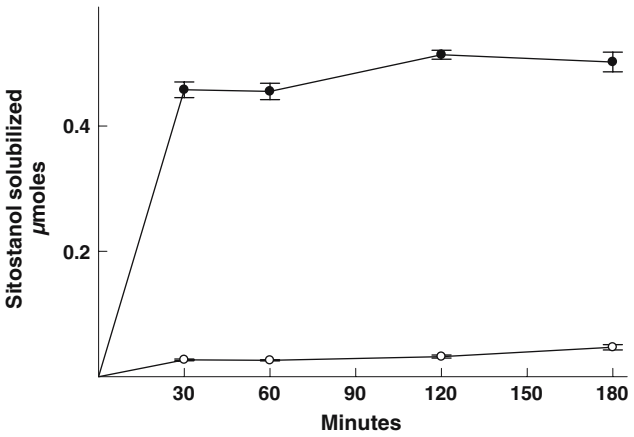

Fig. 3 Solubility of dried sitostanol in artificial bile. Sitostanol was dried in either the absence (*open circles*) or presence (*closed circles*) of lecithin and then artificial bile was added for the time indicated. Taken from [24]

A common method of formulation is solubilization in fatty foods such as oils and margarines. In order to reduce the amount of fat needed the phytosterols are usually esterified with long-chain fatty acids to increase their solubility in food oils [34]. An analysis of cholesterol-lowering clinical trials showed a dose–response relationship with phytosterol dose and a maximum effect of about 10% lowering of LDL cholesterol at a dose of 2 g/day [10, 11]. The United States National Cholesterol Education program has recommended a dose of 2 g/day as a lifestyle change for reduction of LDL cholesterol and this is the level of effectiveness that can be expected from a properly formulated material. However, the consumer may not receive acceptable products because the United States Food and Drug Administration allows health claims for phytosterol supplements without requiring any demonstration of bioactivity.

Recent work has also focused on preparation of bioactive tablet or capsule formulations. Lecithin/stanol tablets reduced LDL cholesterol by 10.4% [35] and when added to statin drug treatment resulted in a further reduction of 9.1% in LDL cholesterol [36].

Phytosterols Naturally Present in Foods

The quantity of phytosterols in natural diets is variable and values of 167–437 mg/day have been found in different populations [37–39]. Vegetable oils, breads and nuts have relatively large amounts while most fruits and vegetables have relatively little [40]. Whether or not food phytosterols would be important in achieving control of LDL cholesterol in communities is the subject of ongoing research, but existing work shows that cholesterol absorption can be significantly affected by the diet. The bioactivity of corn oil phytosterols was demonstrated by including corn oil containing 270 mg phytosterols in single-meal cholesterol absorption tests that also included labeled cholesterol [41]. Chemically purifying the corn oil triglycerides to remove phytosterols resulted in a 38% increase in cholesterol absorption and this was reduced to normal after adding the phytosterols back to purified oil. Corn oil containing as little as 150 mg phytosterols administered in a single meal had a statistically significant effect on reducing cholesterol absorption efficiency, suggesting that low levels might be more effective than previously thought. Similar results were obtained from

a study of the endogenous phytosterols of wheat germ. Cholesterol absorption was measured by including labeled cholesterol in a wheat germ muffin which contained 328 mg phytosterols [42]. Three single-meal tests were performed in the same individuals using either untreated wheat germ, selectively phytosterol-extracted wheat germ, or extracted wheat germ to which the phytosterols had been returned. Cholesterol absorption increased 42.8% when extracted wheat germ was used, and this was reduced to the original value when phytosterols were added back. These results show that phytosterols in low amounts in common foods can reduce cholesterol absorption (Fig. 4).

Many clinical trials have been performed to study the effect of food fats on serum LDL cholesterol levels. However, the phytosterol content of food oils, which is very high for vegetable oils and nil in animal fats [43], has been a confounding covariate which has not been adequately addressed in the literature [44]. For example, diets using polyunsaturated or monounsaturated fat often also contain increased amounts of phytosterols that might affect LDL cholesterol levels. Further complicating interpretation of diet studies is the lack of complete food database reference data on the phytosterol content of foods, which makes estimation of phytosterol content difficult.

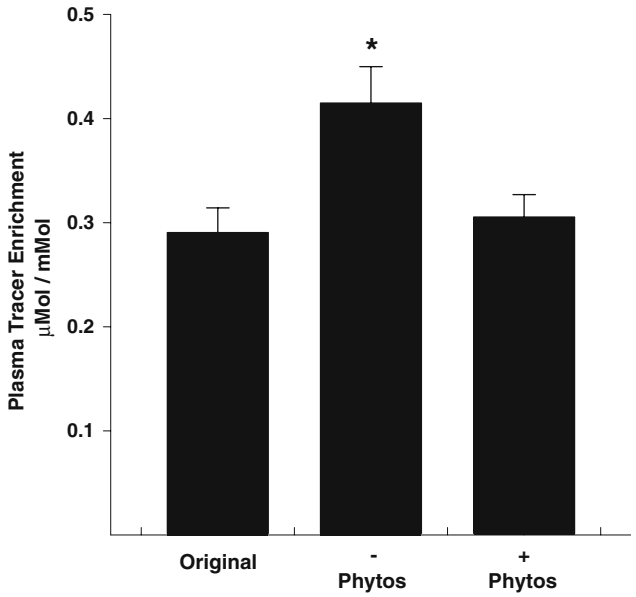

Fig. 4 Effect of endogenous wheat germ phytosterols on cholesterol absorption. Percent cholesterol absorption was measured in 10 subjects in random order on three occasions using single-meal tests consisting of a wheat germ muffin and labeled cholesterol. The wheat germ used was either the original untreated material, wheat germ from which phytosterols had been selectively extracted, or extracted wheat germ reconstituted with the original phytosterols. Plasma tracer enrichment of cholesterol reflects cholesterol absorption. *Asterisk* denotes $P < 0.01$ with respect to the original wheat germ. Taken from [42]

Conclusions

Phytosterols are recognized as an important component of healthy diets and diets designed to reduce hypercholesterolemia. The United States National Cholesterol Education Program recommends dietary phytosterol supplementation of 2 g/day as a lifestyle modification for cholesterol reduction, and this will lower LDL cholesterol by approximately 10%. Phytosterols are inactive as supplied in pure form and must be solubilized or emulsified to achieve biological activity. The principal mechanism of action appears to be competition for absorbable cholesterol in the intestine, resulting in reduced cholesterol absorption. Potentially more important than supplements are natural dietary phytosterols, which may be bioactive in their natural food matrices. Work is needed to add information about phytosterol content to existing food databases and to extend the evidence that feeding phytosterol-rich foods improves LDL cholesterol.

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